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**Editorial**

# Hypertension – A Leading Cause of Mortality

**Prof. Dr. Azhar Masud Bhatti**

Editor-in-Chief

## Introduction of Hypertension

Hypertension, or high blood pressure, means the force of blood pushing against your artery walls is too high. This makes your heart work harder to pump blood through your body. Over time, hypertension damages your arteries and heart. It can lead to serious complications, like a heart attack or stroke. Because high blood pressure usually doesn't cause symptoms, it's often known as a "silent killer."

Doctors measure blood pressure (BP) in millimeters of mercury (mmHg) and took reading as;

**Systolic blood pressure:** This is the top number. It's the pressure on your artery walls when your heart contracts.

**Diastolic blood pressure:** This is the bottom number. It's the pressure between beats when your heart relaxes. It defines as a top number (systolic BP) of 130 mmHg or higher, or a bottom number (diastolic BP) of 80 mmHg or higher.

In Europe, providers define hypertension as a top number of 140 or higher, or a bottom number of 90 or higher.

The World Health Organization has identified hypertension (high blood pressure) as the leading cause of cardiovascular mortality.

The World Hypertension League (WHL), an umbrella organization of 85 national hypertension societies and leagues, recognized that more than 50% of the hypertensive population worldwide are unaware of their condition. To address this problem, the WHL initiated a global awareness campaign on hypertension in 2005 and dedicated 17 May of each year as World Hypertension Day.<sup>1</sup>

Hypertension, also known as high blood pressure, is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure usually does not cause symptoms itself. It is, however, a major risk factor for stroke, coronary artery disease, heart failure, atrial fibrillation, peripheral arterial disease, vision loss, chronic kidney disease, and dementia. Hypertension is a major cause of premature death worldwide.<sup>2</sup>

## Types of Hypertension

There are two main types of high blood pressure, based on what's causing it:

- **Primary Hypertension:** This means your genetics, family history and age are the primary causes of your high blood pressure. Blood pressure rises with aging in societies with a western diet and lifestyle.<sup>3</sup> Depression is associated with hypertension and loneliness is also a risk factor.

Periodontal disease is also associated with high blood pressure. Arsenic exposure through drinking water is associated with elevated blood pressure. Air pollution is associated with hypertension.<sup>4</sup> Whether these associations are causal is unknown. Gout and elevated blood uric acid are associated with hypertension.

- **Secondary Hypertension:** This means there's an identifiable cause, like a medical condition, medication or addictive substance, that is making your blood pressure high. Kidney disease is the most common secondary cause of hypertension.<sup>5</sup> Hypertension can also be caused by endocrine conditions, such as Cushing's syndrome, hyperthyroidism, hypothyroidism, acromegaly, Conn's syndrome or hyperaldosteronism, renal artery stenosis (from atherosclerosis or fibromuscular dysplasia), hyperparathyroidism, and pheochromocytoma. Other causes of secondary hypertension include obesity, sleep apnea, pregnancy, coarctation of the aorta, excessive eating of liquorice, excessive drinking of alcohol, certain prescription medicines, herbal remedies, and stimulants such as cocaine and methamphetamine.<sup>6</sup>

## Stages of Hypertension

There are two main stages of hypertension:

- **Stage 1** means your top number is in the 130s, **or** your bottom number is in the 80s.
- **Stage 2** means your top number is 140 or higher, **or** your bottom number is 90 or higher.

On either end of these stages, there are other categories that providers use to guide treatment decisions:

- **Elevated blood pressure** is blood pressure that's a bit above normal but not yet diagnosed as hypertension.
- **Severe Hypertension** is dangerously high blood pressure that's not yet causing organ damage.
- **A Hypertensive Emergency** is dangerously high blood pressure that's damaging your organs. It causes symptoms and is a medical emergency.

## Signs and Symptoms of Hypertension

Hypertension is rarely accompanied by symptoms. Half of all people with hypertension are unaware that they have it.<sup>7</sup> Hypertension is usually identified as part of health screening or when seeking healthcare for an unrelated problem.

Some people with high blood pressure report headaches, as well as lightheadedness, vertigo, tinnitus (buzzing or hissing in the ears), altered vision

or fainting episodes. These symptoms, however, might be related to associated anxiety rather than the high blood pressure itself.<sup>8</sup>

Long-standing untreated hypertension can cause organ damage with signs such as changes in the optic fundus seen by ophthalmoscopy. The severity of hypertensive retinopathy correlates roughly with the duration or the severity of the hypertension. Other hypertension-caused organ damage include chronic kidney disease and thickening of the heart muscle.

Hypertension usually has no symptoms. You could have it for years without feeling any clues. In fact, the World Health Organization estimates that 46% of adults with hypertension don't know they have it.

If your blood pressure is extremely high, you may have symptoms like:

- Changes to your mental function
- Chest pain
- Dizziness
- Edema (swelling)
- Heart palpitations
- Peeing less than usual
- Seizures
- Severe headache
- Signs of stroke, like sudden facial droop, slurred speech or sudden arm/leg weakness
- Vision changes, like eye pain, vision loss or sudden blurry vision

#### **Causes of Hypertension**

Healthcare providers can't find a single cause of hypertension for most people. Instead, many factors can come together to make your blood pressure higher. These include:

- Being over age 55
- Having a history of the condition in your biological family
- Smoking or using tobacco products
- Having overweight/obesity
- Eating foods high in sodium
- Not getting enough physical activity
- Drinking too much alcohol

In some cases, providers can find a specific cause of your high blood pressure, like an underlying condition, medication or substance. Here are some examples:

- **Conditions**, like obstructive sleep apnea, renal artery stenosis, primary aldosteronism and thyroid disease
- **Medications**, like those that manage ADHD, inflammation, autoimmune disease and mental health conditions
- **Addictive substances**, like alcohol, nicotine and cocaine

#### **Pathophysiology of Hypertension**

In most people with established essential hypertension, increased resistance to blood flow (total peripheral resistance) accounts for the high pressure while cardiac

output remains normal. There is evidence that some younger people with prehypertension or 'borderline hypertension' have high cardiac output, an elevated heart rate and normal peripheral resistance, termed hyperkinetic borderline hypertension.<sup>9</sup> These individuals may develop the typical features of established essential hypertension in later life as their cardiac output falls and peripheral resistance rises with age.

It is unclear whether or not vasoconstriction of arteriolar blood vessels plays a role in hypertension. Hypertension is also associated with decreased peripheral venous compliance, which may increase venous return, increase cardiac preload and, ultimately, cause diastolic dysfunction.

Pulse pressure (the difference between systolic and diastolic blood pressure) is frequently increased in older people with hypertension.<sup>10</sup>

Excessive sodium or insufficient potassium in the diet leads to excessive intracellular sodium, which contracts vascular smooth muscle, restricting blood flow and so increases blood pressure.

#### **Epidemiology of Hypertension**

In 2024, one in three or 33% of the world population were estimated to have hypertension. Of all people with hypertension, almost half (about 44%) do not know that they have hypertension. In 1990, about 650 million people had a diagnosis of hypertension, which increased to 1.4 billion by 2024 mostly due a rise of the number of older adults in low- and middle-income countries.

Hypertension is counted as the major cause and most important factor in the development of cardiovascular diseases worldwide. However, even in the presence of efficacious antihypertensive agents and intensive research data, large numbers of patients in actual clinical practice still suffer with uncontrolled hypertension. Studies indicate that control rates vary according to various countries and geographic regions.<sup>11</sup> Even though, the rate of awareness towards hypertension is quite prominent from 62% in Australia to 72% in US, the control rates are quite discouraging as with to 24% and 35% respectively. In the South Asian region, the scenario is more threatening as China reported only 8% control rates and India with 6% in management of hypertension. At present, it is estimated that about 1 billion people worldwide have hypertension (>140/90 mmHg), and this number is expected to increase to 1.56 billion by 2025.<sup>12</sup>

A similar scenario is seen in Pakistan. The National Health Survey of Pakistan estimated that hypertension affects 18% of adults and 33% of adults above 45 years old. In another report, it was shown that 18% of people in Pakistan suffer from hypertension with every third person over the age of 40 becoming increasingly vulnerable to a wide range of diseases. It was also mentioned that only 50% of the people with hypertension were diagnosed and that only half of those

diagnosed were ever treated. Thus, only 12.5% of hypertension cases were adequately controlled.<sup>6</sup> Some remote areas like Balochistan, there is a paucity of data but the control rate is likely to get even worse.

**Complications of Hypertension**

Untreated hypertension damages your arteries and overworks your heart. Over time, it may lead to:

- **Atrial Fibrillation** : A chaotic rhythm in your heart that affects how well it can pump blood
- **Chronic kidney disease**: Problems with your kidney function that can get worse over time
- **Cognitive impairment and dementia**: Changes to your thinking, memory and personality
- **Coronary artery disease**: Narrowed or blocked arteries in your heart
- **Heart attack**: A sudden lack of blood flow to your heart
- **Heart failure**: A long-term condition that affects your heart’s pumping ability
- **Hypertensive retinopathy**: Damage to your eyes that may lead to vision loss
- **Peripheral artery disease**: Narrowed or blocked arteries in your legs or arms
- **Erectile dysfunction**: Difficulty in obtaining an erection
- **Stroke**: A sudden lack of blood flow to your brain that can cause long-term neurological changes

**Prevention of Hypertension**

Much of the disease burden of high blood pressure is experienced by people who are not labeled as hypertensive. Consequently, population strategies are required to reduce the consequences of high blood pressure and reduce the need for antihypertensive medications. Lifestyle changes are recommended to lower blood pressure.

Recommended lifestyle changes for the prevention of hypertension include:

- maintain normal body weight for adults (e.g. body mass index below 25 kg/m<sup>2</sup>)<sup>13</sup>.
- reduce dietary sodium intake to <100 mmol/day (<6 g of salt (sodium chloride) or <2.4 g of sodium per day)
- engage in regular aerobic physical activity with moderate intensity (minimum 150 minutes per week)
- limit alcohol consumption, max 1 drink for women and 2 for men per day
- consume a diet rich in whole grains, fruits, and vegetables, such as the DASH diet
- not smoking
- stress reduction and management, e.g. by meditation and yoga

**Management and Treatment of Hypertension**

Hypertension treatments include medications to lower your blood pressure and changes to your daily habits. Common medicines prescribed for high blood pressure include diuretics, beta-blockers, calcium channel

blockers, ACE inhibitors and ARBs. Your provider will recommend the right treatment plan for you based on your blood pressure readings, the cause of your high blood pressure and any other conditions you may have.

In general, changes to your habits — sometimes called “lifestyle changes” — are a key part of treatment for everyone. This is true even if you’re taking medicine. In some cases, providers recommend making changes for a while before starting medicine. It depends on your medical history and risk for a heart attack or stroke.

Changes you can make to lower your blood pressure include:

- **Keep a weight that’s healthy for you.** Your healthcare provider can give you a target range.
- **Eat nutritious foods.** A couple of examples are the DASH diet and the Mediterranean diet. These ways of eating are full of fruits, vegetables, whole grains and low-fat dairy. They’re also low in sodium and cholesterol.
- **Cut down on sodium.** Try to limit your sodium intake to no more than 1,500 milligrams (mg) per day. If this is too hard at first, start by reducing your daily intake by at least 1,000 mg.
- **Get enough potassium.** Try to get 3,500 to 5,000 milligrams per day. Ideally, this should be through foods rather than supplements. Some foods high in potassium include bananas, avocados and potatoes (with skin).
- **Get enough physical activity.** Ask your healthcare provider what’s safe for you and how to get started. In general, start slow and work your way up to 150 minutes of aerobic exercise per week. Strength training is also helpful for your heart and whole body.
- **Quit smoking.** Quitting lowers your blood pressure and has many other benefits. Your provider will help you make a plan. This may include support groups, nicotine replacement therapy and prescription medicine that can make quitting easier.
- **Limit or avoid alcohol.** If you choose to drink, do so in moderation. This means one or fewer drinks per day for females, and two or fewer per day for males. The fewer drinks, the better.
- Doing these things can also help prevent high blood pressure if your numbers are currently in the normal range.

The following fruits, vegetables, nuts and seeds are effective to reduce Hypertension (High Blood Pressure)

Fruits	Vegetable	Nuts & Seeds
Bananas	Spinach	Almonds
Oranges	Kale	Walnuts
Strawberries	Swiss chard	Pistachios
Apricots	Beetroots	Brazil Nuts
Pineapples	Broccoli	Peanuts
Melons	Cauliflower	Pumpkin Seeds

Grapefruits	Carrots	Flax Seeds
Kiwi fruits	Sweet potatoes	Chia Seeds
Papayas	Tomatoes	Sunflower Seeds
Avocados	Bell peppers	
Blueberries	Garlic	
Watermelon	Leafy greens	
Grapes		
Pomegranates		

Green tea is a popular drink that contains plant compounds linked to better heart health, lower the blood pressure and also having anti-oxidant and anti-inflammatory properties. Simple drinking water also affects blood pressure and may help lower the blood pressure. Staying well hydrated may be an important aspect of managing high blood pressure.

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# Instructional Program Toward Adolescents Attitudes of Insulin Self-Administration

Adolescents  
Attitudes of Insulin  
Self-Administration

Nawfel Fadhil Hamza<sup>1</sup> and Muna Abdulwahab Khaleel<sup>2</sup>

## ABSTRACT

**Objective:** To identify the level of attitudes adolescents have about self-administering insulin and to determine how that attitudes relates to sociodemographic characteristics

**Study Design:** A quasi-experimental study

**Place and Duration of Study:** This study was conducted at the diabetic and endocrine center of Al-Hilla city from 23<sup>rd</sup> May 2025 to 7<sup>th</sup> October 2025.

**Methods:** 120 adolescents were enrolled. The researcher uses an evaluation instrument, which consists of two sub-parts, where the first sub-part includes 7 questions related to demographic characteristics of adolescents with type I diabetes mellitus disease, the second sub-part includes 16 questions related to the attitudes of adolescents about self-injection of insulin. Each question has three options (agree, disagree and not sure). Where 3 = agree, 2 = not sure and 1 = disagree, and 3 represent the high value scale. The reliability coefficient of Cronbach Alpha, which determines the questionnaire's accuracy, is 0.86. Data collection is assessed and analyzed using SPSS-25.

**Results:** The adolescents attitudes in experimental group in posttest1 &2 is improving, while attitudes of control group is stayed in same level this indicated the instructional program was effective interventional approach to improve adolescents attitudes. Additionally, the findings point to a scientific association between the attitudes of teenagers and demographic information such as age, education level, and prior training. Repeated-measures analysis confirmed significant Time, Group, and Time × Group interaction effects with large effect sizes.

**Conclusion:** Most of the teenagers in the study samples were between the ages of 15 and 18 and resided in urban areas. Adolescent attitudes in the study group had a significant change; the mean was 23.58±13.65 in the pre-test, before to instructional program sessions; 33.41±14.96 in the post-test one, following educational program sessions; and 32.31±14.26 in the post-test two.

**Key Words:** Instructional program, Adolescents attitudes, Self-administration insulin

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## INTRODUCTION

There are three types of diabetes; type I diabetes, also referred to as insulin-dependent diabetes, is more common in children but can afflict both adults and children. The demise of pancreatic beta cells, which results in little or no insulin production, is the etiology of type I diabetes. Non-insulin-dependent diabetes mellitus (NIDDM), another name for type II diabetes, is brought on by cellular resistance to insulin production and a lack of responsiveness to insulin, which is

prevalent in adults and frequently linked to sedentary lifestyles and obesity; type III is known as gestational diabetes, which results from physiological changes in pregnant women and weight gain, which causes stress on the pancreas to produce insulin to cover that increase where this type usually disappears at birth.<sup>1</sup>

Health care officers should provide insulin to people who are taking it. A pen choice device with different length needles may be used to administer insulin; the size of the needles or syringe is decided by the healthcare officers. Alcohol swaps, continuous glucose monitors, and strips are additional supplies that are anticipated along with insulin.<sup>2</sup>

Children with DM type one need to basal insulin shots to maintain a safe range of blood glucose levels order to survive people with diabetes. To lead a healthy life and prevent numerous complications, they also need support, education, and routine blood glucose monitoring by adhering to a structured plan for clinical care for children, which includes early childhood physical activity and a healthy diet and adolescence where organized education can provide children with a lifestyle and a continuous healthy attitudes for children

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with help of their families to follow it throughout their lives.<sup>3</sup>

Insulin is one of the oldest and most significant anti-diabetic medications available, and it is the most effective way to control hyperglycemia when used at the authorized levels. The preferred treatment for individuals with type 1 diabetes mellitus is insulin given subcutaneously either continuously or in several doses. To manage the burden, patients must take insulin therapy as directed by their doctors. Patients must have a positive attitude and a firm grasp of self-insulin administration in order to significantly contribute to their care.<sup>4</sup>

Insulin injection technique is better method to control of glucose management, so there is a need to familiarize the patient with the best and latest techniques suitable for use in insulin injections. The use of short and thin needles is necessary to reduce severity of pain which the patient feels during the injection, as well as improve absorption of insulin and the response of body tissues to it. This is done through educating and training patients and their parents on the technique of insulin injection, as educate diabetic patients on safe handling and use correct injection techniques, the effectiveness contributes to improving the patient's health condition, increases patients' acceptance of injections, and reduces the risks and complications that may occur as a result of incorrect and un-modern injection methods.<sup>5</sup>

## METHODS

A quasi-experimental study is conducted at diabetic and endocrine center of Al-Hilla city from 23<sup>rd</sup> May 2025 to 7<sup>th</sup> October 2025 vide letter No. 84 dated 4<sup>th</sup> May 2025 and 120 adolescents were enrolled. The researcher uses an evaluation instrument, which consists of two sub-parts, where the first sub-part includes 7 questions related to demographic characteristics of adolescents with type I diabetes mellitus disease, the second sub-part includes 16 questions related to the attitudes of adolescents about self-injection of insulin. Each question has three options (agree, disagree and not sure). Where 3 = agree, 2 = not sure and 1 = disagree, and 3 represent the high value scale. The reliability coefficient of Cronbach Alpha, which determines the questionnaire's accuracy, is 0.86. Data collection is assessed and analyzed using SPSS-25.

## RESULTS

70.0% and 66.7% were between the ages of 15 and 18, while 30.0% of the experimental group and group of the control 33.3% were between the ages of 10 and 14. Males made up 46.7% of the study group and 43.3% of the control group, respectively, while females made up 53.3% and 56.7%. Literate; 25.0% of the experimental group and group of the control 28.3% had completed primary school, while the remaining participants (75.0% and 71.7%, respectively) had completed

secondary school. Majority of participants lived in urban areas (60.0% study, 55.0% control), with the remaining participants coming from rural areas. 63.3% of the experimental group and group of the control 66.7% had been diagnosed with diabetes for five years or less. Nearly half of each group (46.7% study, 50.0% control) said they had previously received self-insulin injection training. About 25% of people used rapid-acting insulin, 33% used slow-acting insulin, and the remaining people used both types equally. The groups were statistically comparable at baseline, according to chi-square analysis, which revealed no significant differences between groups across all sociodemographic variables ( $p > 0.05$ ) [Table 1]. The study results display that the study participants are normally distributed. So, the student researcher will go to parametric statistical measures ( $p > 0.05$ ) [Table 2].

At the pre-test, the majority of participants held negative attitudes, while only 11.7% ( $n=7$ ) had positive attitudes. Following the educational intervention, Post-test I showed a marked improvement: 81.7% ( $n=49$ ) of adolescents now held positive attitudes, and only 18.3% ( $n=11$ ) remained negative, with the mean score rising to  $33.41 \pm 14.96$ . In Post-test II, positive attitudes were largely maintained, with 80.0% ( $n=48$ ) positive and 20.0% ( $n=12$ ) negative, and the mean score slightly decreased to  $32.31 \pm 14.26$ . At the pre-test, most participants (88.3%,  $n=53$ ) held negative attitudes, with a mean total score of  $23.63 \pm 13.84$ , and only 11.7% ( $n=7$ ) held positive attitudes. In Post-test I, the distribution remained largely unchanged, with 86.7% ( $n=52$ ) negative and 13.3% ( $n=8$ ) positive, and the mean score slightly increased to  $24.56 \pm 14.68$ . At Post-test II, negative attitudes persisted in 88.3% ( $n=53$ ) of participants, with a mean score of  $24.24 \pm 13.02$ , and positive attitudes were unchanged at 11.7% ( $n=7$ ) [Table 3].

The three measurement points showed a significant change in attitudes, as evidenced by the highly significant effect of time. The study and control groups' patterns of attitude changes over time were significantly different, as evidenced by the highly significant time  $\times$  group interaction (Table 4)

The study group demonstrated higher, more positive scores as a result of the educational intervention, and the effect of Group was statistically significant (Type III SS = 1672.81,  $df = 1$ , MS = 1672.81,  $F = 7.34$ ,  $p < 0.01$ , Partial  $\eta^2 = 0.058$ ). The error term had a Type III SS of 26,990.12 with  $df = 118$  and MS = 228.84. When compared to the control group, these results demonstrate that the intervention significantly changed the attitudes of teenagers regarding self-insulin injection (Table 5).

For attitudes, large effect sizes were found between the pre-test and post-test ( $\eta^2 = 0.642$ ), indicating that the educational program had a significant immediate impact. Smaller effect sizes were seen between post-test one and post-test two, suggesting a slight decrease or stabilization over time, while overall improvements remained substantial (Table 6).

**Table No. 1: Distribution of sociodemographic variables**

Variable		Study Group (n=60)	Control Group (n=60)	Chi-square	p-value
Age (years)	10–14	18 (30.0%)	20 (33.3%)	0.267	0.967
	15–18	42 (70.0%)	40 (66.7%)		
Sex	Males	28 (46.7%)	26 (43.3%)	0.133	0.715
	Females	32 (53.3%)	34 (56.7%)		
Educational level	Able to read & write	60 (100%)	60 (100%)	0.267	0.964
	Primary school	15 (25.0%)	17 (28.3%)		
	Secondary school	45 (75.0%)	43 (71.7%)		
Residence	Urban	36 (60.0%)	33 (55.0%)	0.300	0.584
	Rural	24 (40.0%)	27 (45.0%)		
Type 1 diabetes duration	≤ 5 years	38 (63.3%)	40 (66.7%)	0.133	0.715
	> 5 years	22 (36.7%)	20 (33.3%)		
Training on Self-Insulin Injection	Yes	28 (46.7%)	30 (50.0%)	0.133	0.715
	No	32 (53.3%)	30 (50.0%)		
Type of insulin used	Rapid-acting	15 (25.0%)	17 (28.3%)	0.267	0.964
	Slow-acting	20 (33.3%)	18 (30.0%)		
	Both	25 (41.7%)	25 (41.7%)		

**Table No. 2: Normality tests**

Pre-test	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
	.215	60	.079	.850	60	.081

**Table No. 3: Overall adolescents' attitudes about self-insulin injection in study and control groups**

Weighted	Pre-test			Post-test one			Post-test two		
	No.	%	Mean±SD	No.	%	Mean±SD	No.	%	Mean±SD
<b>Study Group</b>									
Negative (≤32)	53	88.3	23.58±13.	11	18.3	33.41±14.96	12	20.0	32.31±14.26
Positive (>32)	7	11.7	65	49	81.7		48	80.0	
<b>Control group</b>									
Negative (≤32)	53	88.3	23.63±13.	52	86.7	24.56±14.68	53	88.3	24.24±13.02
Positive (>32)	7	11.7	84	8	13.7		7	11.7	

**Table No. 4: Effects of within subjects (study versus control)**

Source	Type III SS	df	MS	F	p-value	Partial η <sup>2</sup>
Time	5206.42	2	2603.21	120.43	0.000**	0.669
Time × Group	7053.28	2	3526.64	178.61	0.000**	0.758
Error (time)	1834.77	117	15.68			

**Table No. 5: Between-subjects effects**

Source	Type III SS	df	MS	F	p-value	Partial η <sup>2</sup>
Group	1672.81	1	1672.81	7.34	0.008**	0.058
Error	26990.12	118	228.84			

**Table No. 6: Effect sizes of intervention on attitudes**

Outcome	Time Point	F / t	p-value	Partial η <sup>2</sup>	Interpretation
Attitudes	Pre vs Post-test one	212.31	0.000**	0.642	Large
	Post-test one vs Post-test two	7.84	0.006**	0.062	Small
	Pre vs Post-test two	188.74	0.000**	0.616	Large

## DISCUSSION

There were no statistically significant differences ( $p>0.05$ ) in the sociodemographic variables between the experimental and control groups in this study. This reflects successful randomization and supports baseline equivalence, which is essential for attributing any later differences to the intervention rather than to

confounding factors. The sample was predominantly older adolescents (15-18 years; 70% in both groups) and gender distribution was comparable (46.7% males in the experimental group vs. group of the control 43.3%). All participants had basic literacy, and nearly three-quarters had secondary school attainment, suggesting an adequate cognitive foundation to understand insulin self-management instructions. The

dominance of older adolescents is particularly relevant because developmental maturity at this stage may enhance learning and performance of complex self-care skills such as insulin self-injection. The higher proportion of urban residents may imply differential access to diabetes services; therefore, generalizing these findings to rural adolescents should be done cautiously. Significantly, baseline gaps in knowledge and attitudes remained despite the fact that roughly half of participants reported having received prior training. This highlights the need for structured reinforcement in addition to exposure.

This study supports a study by Aldossary and Snelgrove<sup>6</sup> that emphasized family-related factors in management while highlighting similar adolescent profiles in insulin-dependent populations. Additionally, this study supports a study by Jain et al<sup>7</sup> and Ajhmed et al<sup>8</sup> showed that structured educational programs with reinforcement are the most effective way to incorporate hands-on training.

Table 3 of the study demonstrates a significant shift in the attitudes of teenagers toward self-administration of insulin over the course of the three measurement points. Just 11.7% of the study group's participants expressed positive attitudes at the pre-test stage, while the vast majority (88.3%) showed negative attitudes. This baseline pattern emphasizes the psychological resistance, fear, and ambivalence that often accompany insulin use during adolescence and reflects a significant affective barrier to effective diabetes self-management. Prior to the educational intervention, negative emotional and cognitive perceptions predominated, as further supported by the low mean attitude score ( $23.58 \pm 13.65$ ).

After test I, there was a significant reversal in attitude polarity. Positive attitudes increased dramatically to 81.7% while negative attitudes decreased to 18.3%. The mean attitude score rose to  $33.41 \pm 14.96$  in tandem with this change, indicating a 9.83-point improvement or a 41.7% reversal. Notably, there was no overlap between the pre-test and post-test I 95% confidence intervals, indicating that the observed improvement was statistically significant and unlikely to be the product of chance. From an interpretive standpoint, rather than being merely a minor improvement, this change reflects a qualitative shift in how teenagers perceive insulin self-administration. The post-test II results provide additional evidence for long-term attitude change. Overall, the attitude profile was still much better than baseline, although positive attitudes slightly decreased from 81.7% to 80%. The small 1.1-point difference between post-test 1 and post-test 2 indicates minimal attitudinal decay and good retention of positive perceptions. This pattern, which demonstrates stabilization rather than regression, supports the conclusion that the intervention produced long-lasting affective change rather than transient enthusiasm.

It is equally important that after the intervention, 18-20% of participants still have negative attitudes. Clinically speaking, this result should not be seen as a

program failure but rather as a sign of individual differences in coping mechanisms, psychological preparedness, and emotional fortitude. In addition to educational interventions, some teenagers might need extra psychosocial or counseling-based support. Further evidence that the intervention successfully addressed both cognitive and affective domains concurrently rather than focusing only on information delivery comes from the nearly parallel improvement in attitudes.<sup>9</sup>

This study supports previous research only 20.7% of Ethiopian adolescents with type I diabetes showed favorable attitudes toward insulin self-administration, according to Metu-Karl et al<sup>10</sup> and Workneh et al.<sup>11</sup> This indicates widespread affective resistance in the absence of structured education. Over 80% of participants in the current study had positive attitudes after the intervention, indicating a significantly higher degree of success. This study also supports to Mohamed et al<sup>12</sup> and Mohamed et al<sup>13</sup> found that attitudes significantly improved following structured education, direct quantitative comparison is hampered by the lack of percentage-based classifications.

The findings are also in line with a larger body of research that identifies psychological barriers specifically, anxiety and injection-related fear as significant impediments to insulin adherence. Citing Cramer and Pugh, the American Diabetes Association<sup>8</sup> highlighted that one of the biggest obstacles to using insulin is still injection-related fear. The high percentage of participants reporting positive attitudes after the intervention shows that structured, adolescent-appropriate educational interventions can effectively reduce such barriers.

According to this study, the control group showed little change in attitude over the course of the three time points, with negative attitudes continuing to be high at roughly 88%. The mean attitude scores showed modest increases that were neither clinically nor statistically significant (Table 4). From the perspective of a researcher, this stagnation draws attention to a critical flaw in conventional clinical care: in the absence of intentional educational and psychosocial intervention, adolescents' attitudes regarding insulin self-administration essentially remain unchanged.

Negative attitudes are known to predict poor adherence, insufficient glycemic control, and an increased risk of complications. The lack of improvement in the control group implies that regular clinic visits, natural disease progression, and even repeated assessment are not enough to change ingrained emotional reactions. This result is consistent with earlier studies that indicate negative attitudes toward insulin persist in the absence of focused psycho-educational strategies.<sup>14,15</sup>

This study clearly showed that structured educational interventions lead to substantial and sustained improvements in teens' attitudes toward self-administration of insulin. The difference between the experimental and control groups demonstrate that intentional, well-thought-out educational initiatives are

what lead to attitude change rather than living with diabetes mellitus. These results strongly support the integration of structured attitude-focused education into standard diabetes care for adolescents, with additional tailored support for those who continue to exhibit resistance or ambivalence despite intervention.

**CONCLUSION**

Most of the teenagers in the study sample were between the ages of 15 and 18 and resided in urban areas. Adolescent attitudes in the study group had a significant change; the mean was 23.58±13.65 in the pre-test, before to instructional program sessions; 33.41±14.96 in the post-test one, following educational program sessions; and 32.31±14.26 in the post-test two. Throughout the pre-test, post-test one, and post-test two, adolescent attitudes in the control group stayed constant. The study group's attitudes between the pre-test, post-test one, and two, where the level was greater, show that the educational program is effective.

**Recommendations:** Attending conferences and educational health programs on insulin self-injection can inspire and encourage teenagers with type I diabetes, teaching teenagers with type I diabetes the importance of adhering to health organizations' recommendations and instructions about the self-injection of insulin.

**Author's Contribution:**

Concept & Design or acquisition of analysis or interpretation of data:	Nawfel Fadhil Hamza, Muna Abdulwahab Khaleel
Drafting or Revising Critically:	Nawfel Fadhil Hamza, Muna Abdulwahab Khaleel
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# Impact of an Educational Program on Nurses' Knowledge Regarding the Care of Newborns with Respiratory Distress Syndrome in Neonatal Intensive Care Units

Nurses' Knowledge Regarding the Care of Newborns with RDS

Salim Amir Yousef and Khamees Bandar Obaid

## ABSTRACT

**Objective:** To assess the impact of an organized educational intervention on the knowledge of nurses working in the neonatal Intensive care unit on newborns with respiratory distress syndrome.

**Study Design:** A quasi-experimental study

**Place and Duration of Study:** This study was conducted at the intensive care units of Babylon Maternity and Children Teaching Hospital and Al-Noor Hospital in Al-Hilla, Babylon Province, Iraq between 1<sup>st</sup> April 2025 and 30<sup>th</sup> August 2025.

**Methods:** The study was conducted at the 50 neonatal involving 50 nurses working in Babylon Maternity and Children Teaching Hospital neonatal intensive care unit (study group n=25) and Al-Noor Hospital neonatal intensive care unit (control group n=25). The knowledge of the nurses was measured by the use of a validated questionnaire with 5 domains related to the respiratory distress syndrome at pre- and post-test.

**Findings:** The percent of high knowledge level of the study population was 80 percent as opposed to the 24 percent of the control group ( $p=0.001$ ). The effect of the study group ( $1.50\pm 0.09$  to  $1.72\pm 0.07$ ) was significant, whereas in the control group, the effect was minimal ( $1.54\pm 0.12$  to  $1.59\pm 0.12$ ). The only demographic variable that was significantly ( $p<0.05$ ) related to knowledge was educational level.

**Conclusion:** The educational intervention was effective in enhancing the understanding of respiratory distress syndrome among nurses in neonatal intensive care units. The most important factor of understanding was the educational level, which once again demonstrates the need to implement standardized and evidence-based education to enhance neonatal outcomes.

**Key Words:** Educational program, Nursing knowledge, Neonatal intensive care unit, Respiratory distress syndrome

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## INTRODUCTION

Respiratory Distress Syndrome (RDS), which is mainly brought about by a lack of surfactant in pre-term babies is one of the most prevalent and severe illnesses in the Neonatal Intensive Care Units (NICUs) across the world. RDS is a major contributor of neonatal morbidity and mortality, protracted hospital stay, escalated healthcare expenditures, and neuro-developmental complications in the long run. The World Health Organization (WHO) approximates that upto one million deaths of newborn babies world-

wide each year are caused by prematurity complications, and RDS is a significant cause of these deaths.<sup>1,2</sup>

Pathophysiology of RDS entails poor production of surfactant which causes collapse of the alveoli, reduction of the gas exchange, hypoxemia, excess carbon monoxide, and respiratory failure. Prompt implementation of evidence-based measures—including continuous positive airway pressure (CPAP), assisted mechanical ventilation, and administration of exogenous surfactant—plays a crucial role in optimizing respiratory outcomes, are needed by the management, which require advanced clinical judgment and close monitoring.<sup>3</sup>

The biggest and most stable group of medical workers in NICUs is composed of nurses. The scope of their work is more than the regular monitoring to prevent complications like pneumothorax and bronchopulmonary dysplasia, respiratory support strategies, prevention of complications, and adherence to evidence-based practice, which implies that the quality of neonatal outcomes is directly dependent on

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knowledge, skills, and adherence to the evidence-based practice by nurses.<sup>4,5</sup>

Regardless of the importance of nurses, several studies have reported a considerable gap in the knowledge of nurses regarding neonatal RDS, especially in fast emerging fields such as ventilation practices and surfactant administration.<sup>6,7</sup> some of the reasons that led to these gaps are heavy workloads, absence of organized orientation, insufficient continuing education and compliance to outdated practices.<sup>8</sup>

Formal education programs based on evidence are one of the possible ways to fill such knowledge gaps. Well-designed, multimodal, and context-specific educational interventions have been observed to increase knowledge, confidence, and clinical decision-making of nurses in complex neonatal environments. Nevertheless, there is limited empirical data on the effectiveness of these programs in enhancing RDS-related knowledge in nurses working at the NICU, especially low- and middle-income nations. Hence, the purpose of the research was to determine the impact of a structured educational initiative on the knowledge of nurses in respect to managing newborns with Respiratory Distress Syndrome in Neonatal Intensive Care Units.

**METHODS**

The location of the quasi experimental study that was carried out was Babylon Maternity and Children Teaching Hospital and Al-Noor Hospital in Al-Hilla, Babylon Province, Iraq between 1<sup>st</sup> April 2025 and 30<sup>th</sup> August 2025 in accordance with the letter No. 81 dated 24/3/2025. A total of 98 nurses were used to recruit a convenience sample of 50 NICU nurses. Participants were purposely drawn to a study group (n=25) and a control group (n=25). Age, sociodemographic data, education, NICU experience, and RDS training before recorded. Knowledge test: 30 questions of multiple choices in five domains of RDS. Scores ranged from 30

to 60. They were also confirmed by 15 nursing experts in five Iraqi universities, and it is evident that their content validity is confirmed. The reliability test gave a Cronbachs alpha of 0.811. The program was founded on the knowledge requirements, evidence-based recommendations, and practices of RDS care. It was provided to the study group alone. The analysis of data was done with the help of SPSS v23. Descriptive statistics was used to describe variables and t-tests, Mann-Whitney U, and Chi-square were used. The p value ≤0.05 was taken as significant.

**RESULTS**

The distribution of the nurses was normal as depicted by table 1. Table 2 depicts that a majority of the participants in control group and (52) in study group were between (20-25) years (mostly). When it came to level of education of nurses, (48) in the control group and (44) in the study group graduated nurses institute. In terms of training on the pediatric respiratory distress syndrome, (36) in control group and (20) in study group were engaged in training on the RDS, lastly, (56) and (64) in control and study group respectively with 5 years experience in PICU.

The mean of the control group is highest with item (causes of RDS in the babies is (except):) value of 1.88 and the least with item (endotracheal tube suctioning should be done to neonatal child). In terms of to study group, the maximum mean in study group is 1.92 with the item (characteristics of a newborn child are:) and the minimum mean is 1.08 with the item (before a newborn is fed for the first time, they must be assessed) [Table 3].

**Table No. 1: Tests of normality**

Nurse’s knowledge RSD in neonate	Shapiro-Wilk		
	Statistic	df	Sig.
	.976	100	.071

**Table No. 2: Distribution of the study samples' sociodemographic attributes**

Sociodemographic characteristics		Control		Study		P value
		No.	%	No.	%	
Age (years)	20-25	11	44.0	13	52.0	.267 N.S
	25-30	3	12.0	6	24.0	
	30-35	4	16.0	3	12.0	
	> 35	7	28.0	3	12.0	
Educational level	Nursing school graduated	4	16.0	3	12.0	.538 N.S
	Nursing institute graduated	12	48.0	11	44.0	
	College of nursing graduated	9	36.0	11	44.0	
Training regarding pediatric respiratory distress syndrome	No	16	64.0	20	80.0	.212 N.S
	Yes	9	36.0	5	20.0	
Years of experience in PICU (years)	5	14	56.0	16	64.0	.445 N.S
	6-10	4	16.0	5	20.0	
	>10	7	28.0	4	16.0	

**Table No. 3: Responses of nurses toward knowledge of respiratory distress syndrome in neonate**

Questionnaire	Control		Study	
	Mean	SD	Mean	SD
A newborn child is	1.80	.374	1.80	.408
Characteristics of a newborn child are:	1.76	.436	1.92	.277
The common problems of a newborn child are:	1.56	.507	1.76	.436
It is essential to evaluate a newborn thoroughly prior to initiating the first feeding:	1.28	.458	1.08	.277
Respiratory distress syndrome is:	1.12	.332	1.32	.476
Early signs/symptoms of RDS (Except):	1.76	.436	1.52	.510
Late signs & symptoms of RDS (Except):	1.60	.500	1.64	.490
Early feeding a newborn child is better to prevent to:	1.80	.408	1.88	.277
Causes of RDS in the babies is (Except):	1.88	.332	1.48	.510
Babies are at risk for RDS (Except):	1.24	.436	1.20	.408
The first assessed of respiratory distress by:	1.24	.436	1.20	.408
Gavage feeding in a newborn is contraindicated in the following situations:	1.72	.458	1.76	.436
An important goal of lung protective mechanical ventilation for RDS is to:	1.24	.436	1.20	.408
Occlude end of endotracheal tube, and administer surfactant as a single aliquot over:	1.48	.510	1.20	.408
A nurse is required to dispose a suction catheter	1.72	.458	1.80	.408
It is recommended to change humidifiers	1.28	.458	1.20	.408
Insertion of suction catheter into endotracheal tube	1.52	.510	1.60	.500
Dusting of respiratory and bedside equipment with antiseptic should be done	1.36	.490	1.36	.490
Head of the bed elevation should be ranging from	1.64	.490	1.44	.507
A nurse caring a ventilated neonatal child is required to wear sterile gloves during	1.48	.510	1.84	.374
Maintenance of a high nurse to neonatal child ratio in critical care setting is associated with	1.76	.436	1.76	.436
Continuous education to PICU nurses on prevention of infection is associated with	1.28	.458	1.24	.436
It is recommended to perform chest physiotherapy due to the following reason	1.48	.510	1.28	.458
Type of air way humidifier	1.52	.510	1.72	.458
Frequency of ventilator circuit changes	1.44	.507	1.40	.500
Endotracheal tube suctioning in a neonatal should be performed	1.16	.374	1.24	.436
While caring for a ventilated neonate, it is essential to ensure that endotracheal tube cuff pressure is maintained	1.72	.458	1.32	.476
Nurses knowledge about the complications of respiratory distress syndrome of the newborn	1.60	.500	1.28	.458
Respiratory distress syndrome of a newborn child may lead to:	1.36	.490	1.40	.500
Unexpected removal of the endotracheal tube can leads to:	1.80	.408	1.60	.500

**Table No. 4: Overall nurse’s knowledge toward respiratory distress syndrome in neonate**

Questionnaire		Control		Study		P value
		No.	%	No.	%	
The nurse's knowledge of the characteristics of a newborn child	Low	3	12.0	2	8.0	.143
	Moderate	12	48.0	7	28.0	
	High	10	40.0	16	64.0	
Nurse’s knowledge of physiological changes, signs and symptoms associated with respiratory distress syndrome newborn	Low	5	20.0	4	16.0	
	Moderate	8	32.0	9	36.0	
	High	12	48.0	12	48.0	
Nurses knowledge about the causes and risk factors leading to respiratory distress syndrome in newborn	Low	3	12.0	9	36.0	
	Moderate	12	48.0	10	40.0	
	High	10	40.0	6	24.0	
Nurses knowledge about treatment and nursing care for a newborn baby with respiratory distress syndrome.	Low	1	4.0	4	16.0	
	Moderate	21	84.0	20	80.0	
	High	3	12.0	1	4.0	
Nurses knowledge about the complications of respiratory distress syndrome of the newborn	Low	2	8.0	4	16.0	
	Moderate	17	68.0	17	68.0	
	High	6	24.0	4	16.0	
Overall nurses knowledge toward respiratory distress syndrome in neonate	Low	2	8.0	1	4.0	
	Moderate	17	68.0	23	92.0	
	High	6	24.0	1	4.0	
Mean ± SD		1.54±0.12		1.50±0.097		

Independent sample t-test, N.S = no significant, p-value=0.05

**Table No. 5: Overall nurse’s knowledge toward respiratory distress syndrome in neonate**

Questionnaire		Control		Study		P value
		No.	%	No.	%	
The nurse's knowledge of the characteristics of a newborn child	Low	3	12.0	-	-	.001
	Moderate	11	44.0	1	4.0	
	High	11	44.0	24	96.0	
Nurse’s knowledge of physiological changes, signs and symptoms associated with respiratory distress syndrome newborn	Low	2	8.0	1	4.0	
	Moderate	10	40.0	5	20.0	
	High	13	52.0	19	76.0	
Nurses knowledge about the causes and risk factors leading to respiratory distress syndrome in newborn	Low	3	12.0	-	-	
	Moderate	8	32.0	5	20.0	
	High	14	56.0	20	80.0	
Nurses knowledge about treatment and nursing care for a newborn baby with respiratory distress syndrome	Moderate	19	76.0	7	28.0	
	High	6	24.0	18	72.0	
Nurses knowledge about the complications of respiratory distress syndrome of the newborn	Low	2	8.0	-	-	
	Moderate	15	60.0	13	52.0	
	High	8	32.0	12	48.0	
Overall nurses knowledge toward respiratory distress syndrome in neonate	Low	2	8.0	-	-	
	Moderate	17	68.0	5	20.0	
	High	6	24.0	20	80.0	
Mean ± SD		1.59±0.12		1.72±0.07		

Independent sample t-test, N.S = no significant, p-value=0.05

**Table No. 6: An association between demographic profiles of nurses who are working in NICU and knowledge regarding respiratory distress syndrome in neonate**

Sociodemographic characteristics	Knowledge	
Age	X <sup>2</sup> Calculate	5.417
	X <sup>2</sup> table	7.82
	Sig.	N.S
Educational level	X <sup>2</sup> Calculate	7.955
	X <sup>2</sup> table	7.82
	Sig.	Sig.
Having pediatric respiratory distress syndrome training	X <sup>2</sup> Calculate	1.563
	X <sup>2</sup> table	3.84
	Sig.	N.S
Years of experience in PICU	X <sup>2</sup> Calculate	2.708
	X <sup>2</sup> table	5.99
	Sig.	N.S

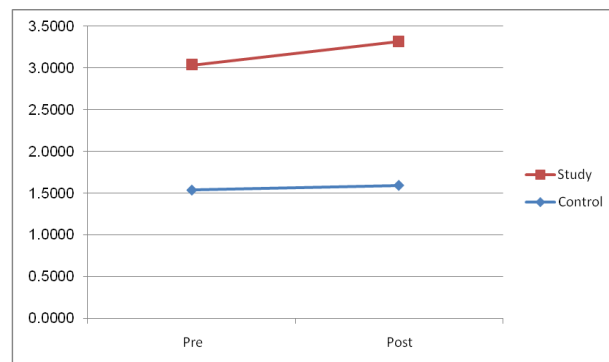
68% in control group were having a moderate knowledge towards respiratory distress syndrome in neonate and (80) in study group having a high knowledge towards respiratory distress syndrome in neonate (Table 5, Fig. 1).

There was a significant association between demographic characteristics of the working nurses in NICU and knowledge on Respiratory Distress Syndrome in Neonate at p-value =0.05 or less in relation to (Educational level) except (Age, Training, and Years of experience in PICU) which had a non-significant correlation (Table 6).

**DISCUSSION**

The results of this research evidenced that structured education program made significant progress toward enhancing the knowledge of nurses on how to attend to newborns with Respiratory Distress Syndrome in the NICUs. The high knowledge levels of 4 to 80% that have been significantly enhanced in the study group accentuate the effectiveness of the intervention. The fact that no substantial improvement of the control group was observed allows concluding that the gains observed could be explained by the educational program, and not by external factors or repeated testing. These results are consistent with other studies that have found that evidence-based interventions in the education of neonatal nurses can significantly improve their knowledge and competence.<sup>8-11</sup>

It is worth noting that the only significant predictor of knowledge was the educational level. This criticizes the years of experience as a traditional measuring rod of clinical competence and highlights the significance of formal education and ongoing professional growth.



**Figure No. 1: Differences in nurse’s knowledge toward respiratory distress syndrome in Neonate in pre-post test across the two group**

Findings like these have been demonstrated in recent publications, pointing to the fact that experience does not guarantee current knowledge in a rapidly developing clinical specialty, including neonatal care.<sup>12-15</sup>

**CONCLUSION**

The structured educational program significantly improves nurses' knowledge regarding the management of respiratory distress syndrome in neonatal intensive care units. The knowledge competence was mainly determined by the level of education instead of the number of years of experience and the use of standardized evidence-based education programs is critical to increasing the readiness of nurses and improve the outcomes of newborns with this potentially fatal condition.

**Author's Contribution:**

Concept & Design or acquisition of analysis or interpretation of data:	Salim Amir Yousef, Khamees Bandar Obaid
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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# Influence of Genetically Polymorphic GLUT4 on Insulin Remedy Response in Type 1 Diabetic Patients

Genetically Polymorphic on Insulin in Type 1 Diabetic

Mohammed Suhail Abed and Mohammed Ibrahim Rasool

## ABSTRACT

**Objective:** To investigate the association between the *SLC2A4* rs5435 (T>C) single nucleotide polymorphism and glycemic response to exogenous insulin in Iraqi patients with type 1 diabetes mellitus.

**Study Design:** A case-control study

**Place and Duration of Study:** This study was conducted at the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kerbala University, Iraq from 1<sup>st</sup> December 2023 to 31<sup>st</sup> December 2024.

**Methods:** This study was conducted involving 100 patients with type 1 diabetes and 30 healthy controls assessed fasting serum glucose and HbA1c levels and genotyped the *SLC2A4* rs5435 (T>C) polymorphism using allele-specific polymerase chain reaction. Genotype and allele frequencies were compared between groups and analyzed for associations with glycemic parameters.

**Results:** Among patients with type 1 diabetes, genotype frequencies were 89% for homozygous wild-type (TT), 6% for heterozygous mutant (TC), and 5% for homozygous mutant (CC). In the healthy control group, the corresponding frequencies were 90% (TT), 6.6% (TC), and 3.3% (CC). The *SLC2A4* rs5435 (T>C) polymorphism showed no significant differences in genotype or allele distribution between patients and controls and was not associated with FSG or HbA1c levels, indicating it does not affect response to exogenous insulin therapy.

**Conclusion:** The *SLC2A4* rs5435 (T>C) polymorphism does not affect glycemic control or response to exogenous insulin in Iraqi type 1 diabetes patients, though larger multicenter studies are needed to confirm these results and investigate genetic factors influencing insulin responsiveness.

**Key Word:** Glucose transporter type 4 (GLUT4), Polymorphism, Allele-specific polymerase chain reaction, Insulin response

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## INTRODUCTION

Glucose is a central metabolic substrate produced by photosynthesis and is essential for energy metabolism in all forms of life. In higher organisms, it also functions as a signaling molecule that maintains energy balance by regulating hormones, enzymes, gene expression, and neural activity, with its effects on lipogenic and glycolytic genes mediated by the transcription factor ChREBP.<sup>1</sup>

Through the glucosamine route, glucose flux controls transcription factor activity by encouraging O-GlcNAcylation.<sup>2</sup>

Through the generation of NAD<sup>+</sup>, glycolysis influences Sirt1 deacetylase activity, a key transcriptional regulator that has come to light.<sup>3</sup> Histone changes brought forth by glucose metabolism through the synthesis of Acetyl-CoA lead to epigenetic regulation of gene expression.<sup>4</sup> Rising glucose levels regulate glucose-sensitive neurons in the brain and insulin secretion from pancreatic  $\beta$ -cells, influencing feeding behavior, energy expenditure, and overall glucose balance. Glucose homeostasis primarily depends on cellular glucose uptake, which is controlled by glucose transporter expression on cell surfaces in most tissues (except hepatocytes and  $\beta$ -cells). The existence of multiple glucose transporter isoforms with different kinetic properties and regulated surface expression enables precise control of glucose uptake, metabolism, and signaling to maintain metabolic homeostasis.<sup>5</sup>

Since its identification as a distinct glucose transporter isoform by James et al. in the late 1980s<sup>6</sup>, GLUT4 is a well-studied glucose transporter crucial for glucose homeostasis, with extensive research highlighting its complex regulation by insulin. Since the early 1980s, insulin has been known to promote GLUT4 translocation from intracellular compartments to the cell surface in adipocytes and skeletal muscle.<sup>7-9</sup> In the three decades that have passed, a great deal has been

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discovered about this regulation's specifics.<sup>10</sup> GLUT4 is expressed in skeletal muscle, adipocytes, and cardiomyocytes and is the primary transporter responsible for insulin-stimulated glucose uptake in muscle and fat, with skeletal muscle accounting for most glucose disposal. Its expression and activity increase during exercise or after high-carbohydrate intake to meet elevated energy demands.<sup>11</sup> Insulin stimulates GLUT4 translocation through two main pathways: a PI3K-dependent pathway involving insulin receptor activation and IRS-1/IRS-2 phosphorylation, and a PI3K-independent pathway mediated by the c-Cbl/CAP complex that activates TC10 in lipid rafts. Both pathways promote GLUT4 movement to the plasma membrane in muscle and fat cells, and inhibition of the c-Cbl/CAP pathway reduces insulin-stimulated GLUT4 translocation in adipocytes.<sup>11,12</sup>

In skeletal muscle, exercise stimulates GLUT4 translocation through a PI3K-independent pathway in which muscle contraction activates AMPK, leading to increased GLUT4 movement to the plasma membrane and enhanced glucose uptake to meet elevated energy demands.<sup>11,13</sup> Insulin resistance can be assessed at the cellular level without liver involvement, and defects in insulin signaling do not always reduce glucose uptake due to compensatory regulation of SLC2A4 expression and GLUT4 translocation. Decreased glucose uptake in specific tissues may not impair whole-body glucose clearance because of tissue mass compensation. Whole-body insulin resistance depends on total GLUT4 content across tissues, with skeletal muscle as the primary site in non-obese individuals and adipose tissue playing a larger role in obesity. Many studies emphasize membrane GLUT4 alone, underscoring the need to consider total cellular GLUT4 for a complete understanding of glucose clearance.<sup>14</sup>

## METHODS

This case-control study was conducted at Department of Pharmacology and Toxicology, Faculty of Pharmacy, Kerbala University, Iraq from 1<sup>st</sup> December 2023 to 31<sup>st</sup> December 2024 vide letter No. 4545/QM/Approval/KD8399JD Date: November 11, 2023 analyzed the rs5435 SNP in 100 type 1 diabetic patients (56 females, 44 males; aged 6–18) on basal-bolus insulin and 30 age-matched healthy controls (12 females, 18 males). Venous blood samples were collected in EDTA tubes for DNA extraction and SNP analysis. Fasting serum glucose was measured using a UV-based enzymatic method, where glucose is converted to glucose-6-phosphate by hexokinase, then oxidized to gluconate-6-phosphate by glucose-6-phosphate dehydrogenase, producing NADPH, which is quantified photometrically and is proportional to glucose concentration.<sup>15</sup>

Hemoglobin A1c was measured from EDTA blood using the Cobas Integra 400+ analyzer with a TTAB-based hemolyzing reagent to prevent leukocyte

interference. The assay detected all beta-chain N-terminal glycosylated hemoglobin variants, forming soluble antigen-antibody complexes, while excess antibodies were bound by polyhapten reagents into insoluble complexes quantified by turbidimetry.<sup>15</sup>

The sample was agitated for a minimum of 10 minutes at room temperature using a rotisserie shaker. A microcentrifuge tube was prepared with 20  $\mu$ L of proteinase K (PK) solution. Then, 200  $\mu$ L of the blood sample was added to the tube containing the PK solution and briefly mixed. Next, 200  $\mu$ L of cell lysis buffer (CLB) was added, and the mixture was vortexed for at least 10 seconds. Blood samples were incubated at 56 °C by using a water bath. After taking the tube out of the water bath, 250  $\mu$ L of binding buffer (BB) was added, and the mixture was vortexed for a minimum of 10 minutes. The content of the tube was transferred to the binding column, and it was centrifuged for 1 minute at maximum speed (15000 RPM) to enhance filtration (passage of lysate from the binding tube to the collection tube). The collection tube containing the lysate was removed and discarded. The binding column was transferred to a new collection tube. 500  $\mu$ L of column wash solution (CWS) was added to the binding column, which was then centrifuged at maximum speed for 3 minutes. The flow through was discarded. Step 10 was repeated two more times, resulting in a total of three washes. The binding column was placed in a clean microcentrifuge tube. 75  $\mu$ L of nuclease-free water was added to the binding column, followed by centrifugation at maximum speed for 1 minute. The binding column was discarded, and the eluate was saved.

Polymerase chain reaction was used to amplify the SLC2A4 rs5435 gene with primers designed via Primer-BLAST and obtained from Macrogen, Korea. Lyophilized primers were dissolved to 100 pmol/ $\mu$ L stocks, diluted to 10 pmol/ $\mu$ L working solutions, and stored at –20 °C.<sup>16</sup> PCR conditions were optimized through multiple trials to determine the ideal annealing temperature, DNA and primer concentrations, and number of cycles.

**Agarose Gel Electrophoresis.**<sup>17</sup> To prepare the agarose gel, 1.5 grams of agarose powder were mixed with 70 milliliters of TBE (Tris-Borate EDTA) buffer (pH 8) and heated to boiling until the agarose was completely dissolved. The gel solution was stirred to ensure it was well mixed and free of bubbles, resulting in a clear solution. The solution was then allowed to cool to a temperature of 50-60°C. 10  $\mu$ L of red-safe nucleic acid stain was added to the gel. A comb was placed at one end of the tray to form wells for loading the PCR product samples. The agarose solution was poured into the tray and left to solidify at room temperature for 30 minutes, after which the comb was carefully removed from the gel. The gel was installed in a gel electrophoresis tank. TBE buffer was added to the tank

until it rose three to five millimeters above the gel's surface. Five microliters of DNA ladder were put into one agarose gel well, and five microliters of each PCR product were put into the remaining wells. The voltage of the electrophoresis device was adjusted to produce an electrical field of five volts for every centimeter that separated the cathode and anode. After the run was completed, a UV transilluminator set to 360 nm was used to visualize the bands. A digital camera was then used to capture an image of the gel. The data was entered and analyzed through SPSS-25.

**RESULTS**

Fasting serum glucose levels in patients varied widely (57–400 mg/dl) with a high mean of 208.28 mg/dl, whereas control participants showed a narrower range (91–118 mg/dl) and a much lower mean of 104.96 mg/dl (Fig. 1). HbA1c ranged from 8.5% to 15.1%, with a mean of 10.77% for patients while for control participants, HbA1c ranged from 4.1% to 5.7% with a mean of 4.97% (Fig. 2). The rs5435 T>C gene polymorphism produced a distinct 355-bp band, with the amplicon size confirmed by comparison to a 100–1500 bp DNA ladder (Fig. 3).

Patients were grouped into three SLC2A4 rs5435 T>C genotypes: TT, TC, and CC. Among the 100 patients, the TT genotype was predominant (89%), followed by TC (6%) and CC (5%). Control participants were categorized into TT, TC, and CC genotypes for the SLC2A4 rs5435 T>C polymorphism. As indicated in Table 8, among the 30 controls, the TT genotype was predominant (90%), followed by TC (6.6%) and CC (3.3%) [Table 1].

Table 2 displays LSD among biochemical parameters means for SLC2A4 rs5435 genotypes of study participants. Table 3 displays the HWE for the SLC2A4 rs5435 genotypes among control participants. Table 4 displays the HWE for the SLC2A4 rs5435 genotypes among the patients. Table 5 displays the odds ratio of SLC2A4 rs5435 genotypes among study participants.

**Table No. 1: Allele frequencies of SLC2A4 rs5435 T > C gene polymorphism among type 1 diabetic and healthy controls patients**

Genotype	Type t diabetic n=100		Healthy controls (n=30)	
	No.	%	No.	%
TT	89	89.0	27	90.0
TC	6	6.0	2	6.6
CC	5	5.0	1	3.4

**Table No. 2: LSD among biochemical parameter means for SLC2A4 rs5435 genotypes of study participants**

Variable	Genotypes	N	Mean	Std. Deviation	Std. Error	
HbA1c	Control	TT	27	4.96b	0.43	0.08
		TC	2	5.00b	0.14	0.10
		CC	1	5.20b	0.00	0.00
	Patients	TT	89	10.81a	1.82	0.19
		TC	6	10.80a	2.12	0.86
		CC	5	10.10a	1.44	0.64
LSD			2.56			
FSG	Control	TT	27	104.33b	7.93	1.52
		TC	2	112.50b	4.94	3.50
		CC	1	107.00b	0.00	0.00
	Patients	TT	89	209.22a	89.05	9.43
		TC	6	212.66a	82.05	33.49
		CC	5	186.20a	97.22	43.47
LSD			88.22			

**Table No. 3: HWE for SLC2A4 rs5435 genotypes among control participants**

Genotypes	TT	TC	CC	P-value
Observed	27	2	1	
Expected	26.13	3.73	0.13	0.011
HWE-freq.	87.11%	12.44%	0.44%	
Allele freq.	T=56 (93.3%)		C=4 (6.67%)	

The null hypothesis is rejected because the population is at H-W equilibrium

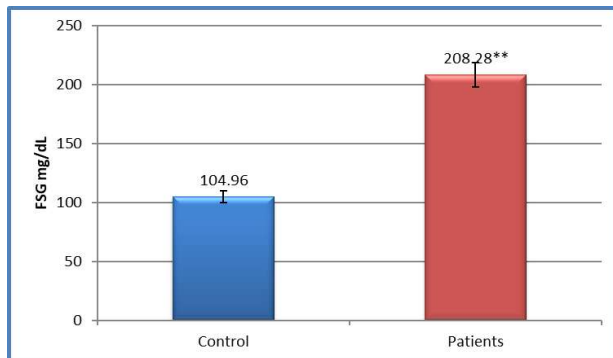
**Table No. 4: HWE for SLC2A4 rs5435 genotypes among the patients**

Genotypes	TT	TC	CC	P-value
Observed	89	6	5	
Expected	84.64	14.72	0.64	<0.0001
HWE-freq.	84.64%	14.72%	0.64%	
Allele freq.	T=184 (92%)		C=16 (8%)	

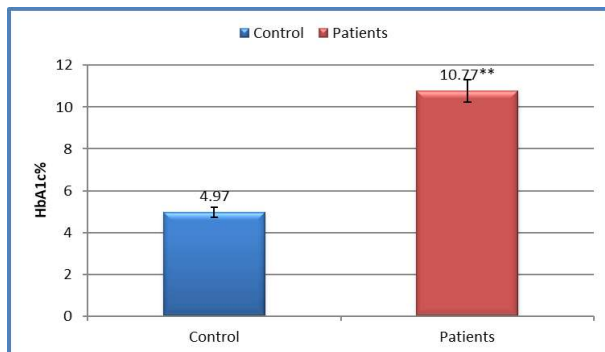
The null hypothesis is rejected because the population is at H-W equilibrium

**Table No. 5: Odds ratio of SLC2A4 rs5435 genotypes among study participants**

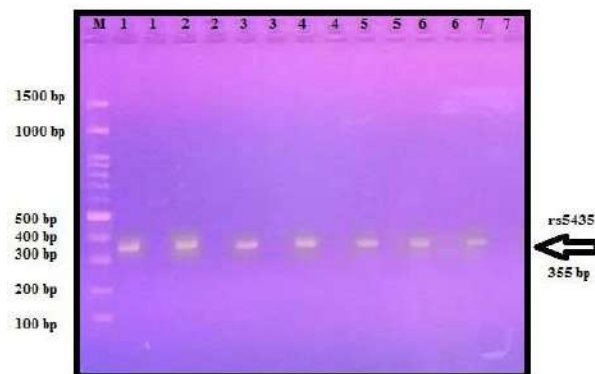
Genotypes	Control	Patient	Odds Ratio (95%CI)	P-value
TT	27	89	Reference=1	
TC	2	6	0.91 (0.17-4.77)	0.91
CC	1	5	1.52 (0.17-13.55)	0.71
Genotypes/Female	Control	Patient	Odds Ratio (95%CI)	P-value
TT	11	51	Reference=1	
TC	0	2	1.12 (0.05-24.86)	0.94
CC	1	3	0.65 (0.06-6.82)	0.71
Genotypes/male	Control	Patient	Odds Ratio (95%CI)	P-value
TT	16	38	Reference=1	
TC	2	4	0.84 (0.14-5.07)	0.85
CC	0	2	2.14 (0.10-47.13)	0.62



**Figure No. 1: Mean of FSG of patients and control samples**



**Figure No. 2: Mean of HbA1c of patients and control samples**



**Figure No. 3: Genotyping of rs5435 genetic polymorphism**

## DISCUSSION

Fasting serum glucose (FSG) and HbA1c levels were evaluated in 100 patients and 30 healthy controls. The least significant differences (LSD) between patients and controls were 46.38 for FSG and 0.96 for HbA1c. For SLC2A4 rs5435 genotypes, the LSD values were 88.22 for FSG and 2.56 for HbA1c. As shown in Table 9, the observed differences in FSG and HbA1c across all rs5435 genotypes exceeded the corresponding LSD values, indicating statistically significant differences in biochemical parameters among genotypes ( $P < 0.05$ ). These findings were similar to a previous studies.<sup>18,19</sup>

Regarding the analysis of odds ratio, for SLC2A4 rs5435 mutant genotypes for the patients; heterozygous genotype TC has an odd ratio (0.91 with a confidence interval of 0.17–4.77) lower than 1, indicating it is approximately at the same risk for increased FSG and HbA1c levels with the TT genotype.  $P = 0.91$ . Whereas homozygous genotype CC has an odd ratio (1.52 with a confidence interval of 0.17–13.55) higher than 1, indicating it is at higher risk for increased FSG and HbA1c levels than the TT genotype. However, the results revealed that the odd ratio for SLC2A4 rs5435 mutant homozygous genotype CC is at risk, but the value of the odd ratio was not significant ( $P = 0.71$ ). Odds ratio statistical test that had been employed in this study was concurrent with Kalra.<sup>20</sup>

## CONCLUSION

SLC2A4 gene polymorphism rs5435 was detected with variable frequencies and different genotypes in Iraqi patients. The SNP of the SLC2A4 gene that was detected in Iraqi type 1 diabetic patients was noted to not significantly affect the response to exogenous insulin.

### Author's Contribution:

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Drafting or Revising	Mohammed Suhail

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# Predictive Determinants of Pediatric Nursing Practices for Children Undergoing Lumbar Puncture in Oncology-Hematology Units: A Descriptive Correlational Study

Redictive  
Determents Of  
Pediatric Nursing  
Practices  
During LP

Jamal Abdunnasser Ahmed<sup>1</sup> and Zaid W. Ajil<sup>2</sup>

## ABSTRACT

**Objective:** To evaluate predictive determents of pediatric nursing practices during lumbar puncture in oncology-hematology units.

**Study Design:** Descriptive correlational study

**Place and Duration of Study:** This study was conducted at the Pediatric Oncology-Hematology Units from 8<sup>th</sup> October to 1<sup>st</sup> September 2024 to 31<sup>st</sup> December 2024.

**Methods:** 50 pediatric nurses were enrolled. The sampling includes pediatric nurse of both male and female, who works in the pediatric oncology and hematology units, and has one year of nursing experiences in these units. Conversely, the exclusion criteria comprised members who refused to participate in the study, and nurses who received more than 60% pre-test, and nurses who were included in the pilot study.

**Results:** 80% of nurses had poor nursing practices during lumbar puncture in oncology-hematology units with a total mean score of  $14.0 \pm 3.91$ . The results show a statistically significant correlation between predictive determents namely: years of nursing experiences ( $p=.028$ ); years of experiences in hematology and oncology nursing ( $p=.015$ ); and their nursing qualification ( $p=.049$ ) and pediatric nursing practices during lumbar puncture in oncology-hematology units.

**Conclusion:** Nurses' qualifications and their experiences in hematology nursing improve patient care during lumbar puncture. It emphasizes the necessities for ongoing nursingpractice development via planning strategies and suggests targeted pediatric nurses training programs to enhance their skills in pediatric patient outcomes.

**Key Words:** Children, Pediatric nursing practices, Lumbar puncture, Pediatric oncology, Hematology, Predictive determinants

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## INTRODUCTION

Lumbar Puncture (LP) refer to procedure by which a needle is inserted into the subarachnoid space of the lower back to remove cerebrospinal fluid (CSF) for diagnosis or treatment. This procedure is usually done between the third and fourth lumbar vertebrae or the fourth and fifth lumbar vertebrae (just below the end of the spinal cord).<sup>1,2</sup>

The procedure is used to diagnose conditions such as bacterial meningitis, multiple sclerosis, and subarachnoid hemorrhage, and it can also be used for intracranial teletherapy pressure management.<sup>3</sup> In pediatric oncology, LP is a systematic step in chemotherapy for children with advanced leukemia. The process begins when the disease is in remission and continues throughout the intensive treatment period, requiring regular follow-up for one to three months.<sup>4,5</sup> Repeated LP can cause significant physical and mental distress to pediatric patients, requiring effective pain management strategies and a holistic care approach to manage pain and improve the child's experience.<sup>6</sup>

Nursing staff play a key role in preparing a child for LP, ensuring proper positioning and adherence to aseptic procedures during the procedure. Correct positioning of the child is crucial as it determines the success of the procedure and reduces the risk of complications such as skull fractures after LP. Nursing staff are responsible for assessing the child's position and ensuring that the child is in constant motion to prevent neurological damage during the procedure.<sup>7</sup>

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Inadequate nursing care during a LP can have a negative adverse effect on patient outcomes and the overall success of the procedure. Lack of specialized anatomical knowledge and technical skills can lead to complications such as headache after LP, the most common side effect, and even more serious complications such as traumatic puncture of the spine and spinal cord injuries.<sup>8</sup>

Several factors influence nurses' performance variations regarding LP procedures, including practitioner experience, nurses and patient characteristics, and institutional protocols. Practitioner training and experience are major contributors to this variability.<sup>9</sup> For example, Mayo Clinic implemented a quality improvement program to enable nurses to perform LP, which physicians traditionally performed. The program was supported by a comprehensive training program to ensure nurses had the skills necessary to safely and effectively expand their scope of practice and improve patient access to care; while some nurses have a strong interest in pain management and parenting options, others may lack the skills and confidence to provide optimal support, which may negatively impact the child's experience and surgical outcome.<sup>10,11</sup>

Despite the growing interest in supporting pediatric medical procedures, few studies have specifically examined how nurses influence the clinical performance of pediatric LP procedures in oncology and hematology settings, and the knowledge gap highlights the need to study whether and how these factors influence nursing behavior and quality of care. Although some studies have shown that factors such as age, gender, and education do not directly affect nurses' performance, it is also acknowledged that demographic factors can negatively impact nurses' performance if not controlled, leading to poorer and more negative performance outcomes.<sup>4,5</sup> Therefore, the purpose was to investigate the impact of nurses' demographic factors on their nursing practices during LP procedures in pediatric oncology-hematology units.

**METHODS**

This descriptive correlational study was conducted at Pediatric Oncology-Hematology Units from 8<sup>th</sup> October to 1<sup>st</sup> September 2024 to 31<sup>st</sup> December 2024 vide letter No. 62 dated 22-8-2024. A non-probability convenience sample randomly selected from 50 nurses. The sampling includes pediatric nurse of both male and female, who works in the pediatric oncology and hematology units, and has one year of nursing experiences in these units. Conversely, the exclusion criteria comprised members who refused to participate in the study, and nurses who received more than 60% pre-test, and nurses who were included in the pilot study.

Based on a Richard Geiger equivalent population proportion of 50%, error probability of 5%, confidence

of 95%, and the standard score corresponding to the level of confidence of 1.96, the minimum required sample size would be 49 nurses. To ensure adequate statistical power for detecting significant effects, a web-based sample size calculator was employed to determine the minimum required sample size. A margin of error of 5% was preferred, which means our results would be within five percentage points of the exact population value 95% of the time. Given an estimated population size of 55 and an assumed response distribution close to 50%, the calculations revealed that a minimum sample size of 49 indicates a higher degree of representation for the sample.

The questionnaire consisted of two main parts. The first part included demographic information about the participants. The second part contains two domains. The first domain is an observational checklist containing nine items that evaluate nurses' preparation of equipment needed for LP, and the second domain is an observational checklist containing twenty items that evaluate nursing practices during the LP procedure. These sections aimed to evaluate the performance of nursing staff in managing pediatric patients undergoing LP. To assess these practices, a three-point observation rating scale was employed across three different time intervals: Never (0-0.66), Sometimes (0.67-1.33), and Always (1.34-2).

All data analyses were performed utilizing SPSS-26. The RM-ANOVA test was employed to evaluate the relationship between nurses' practices regarding the care of children Undergoing LP levels and specific demographic characteristics. Statistical significance was deemed to exist when the p-value was less than 0.05.

**RESULTS**

**Table No. 1: Sociodemographic characteristics of pediatric nurses (N=50)**

Variable	No.	%
<b>Age</b>		
20 – 25	10	20.0
26 – 30	16	32.0
31 – 35	18	36.0
36 – 40	2	4.0
41 – 45	4	8.0
<b>Gender</b>		
Male	23	46.0
Female	27	54.0
<b>Marital status</b>		
Single	21	42.0
Married	27	54.0
Divorced	-	-
Widowed	2	4.0
<b>Nursing Qualification</b>		
Nursing high school	14	28.0
Diploma	14	28.0

Bachelor	22	44.0
<b>Years of experience in hematology and oncology</b>		
1 – 5	34	68.0
6 – 10	14	28.0
11 – 15	2	4.0
<b>Years of experience in nursing</b>		
1 – 5	14	28.0
6 – 10	24	48.0
11 – 15	6	12.0
16 – 20	6	12.0

Table 1 indicates that pediatric nurses aged between 31–35 years represented (36%) and was most common age group; and female nurses represented the majority, accounting for 54% of the sample. Also, 48% of the pediatric nurses had 6-10 years of nursing experiences. Specifically, the majority of them (68%) reported having 1-5 years of experience in hematology and oncology units. Additionally, 54% were married, and (48%) held a bachelor’s degree in Nursing.

Table 2 shows that pediatric nurses had poor level of nursing practice toward LP procedures and overall inadequate performance on children with total mean

score of 14.0±3.91. Majority of pediatric nurses (80%) exhibited poor procedural competency, whereas only 12% performed at a fair level and a mere 8% attained a good level of practice (Fig. 1).

Table 3 showed that a statistically significant correlation between predictive deterrents namely: years of nursing experiences (p=.028); years of experiences in hematology and oncology nursing (p=.015); and their nursing qualification (p value=.049) and pediatric nursing practices during lumbar puncture in oncology-hematology units. However, the statistical analysis indicates no significant correlation between pediatric nurses’ practices and their age, sex, and marital status.

**Table No. 2: The level of nurses' practices on lumbar puncture procedure**

Levels of practices	No.	%	Mean±SD
Poor	20	80.0	14.0±3.91
Fair	3	12.0	
Good	2	8.0	

Poor= 0 -8, Fair= 8.1-16, Good= 16.1-24

**Table No. 3: Correlation between levels of nurses’ practices and their predictive deterrents**

Variable		Mean	Standard deviation	Relationship
Age	20 – 25	14.80	3.271	$r^s = .343$ P-value= .093
	26 – 30	16.00	3.109	
	31 – 35	12.70	3.302	
	36 – 40	10.00	.000	
	41 – 45	14.50	2.121	
Gender	Male	14.09	3.177	$r^* = .034$ P-value= .873
	Female	14.07	3.583	
Years of experience in nursing	1 – 5	14.29	2.870	$r^s = .230$ P-value= .028*
	6 – 10	15.18	3.737	
	11 – 15	11.50	2.380	
	16 – 20	13.00	3.000	
Years of experience in hematology and oncology	1 – 5	10.00	.000	$r^s = .268$ P-value= .015*
	6 – 10	13.57	4.036	
	11 – 15	14.53	3.044	
Marital status	Single	14.20	2.394	$r^s = .122$ P-value= .562
	Married	14.07	4.066	
	Widowed	13.00	.000	
Nursing Qualification	Nursing high school	12.57	2.760	$r^s = .358$ P-value= .049*
	Diploma	13.43	1.813	
	Bachelor	15.45	4.034	

$r^s$  = Spearman Correlation coefficient,  $r^*$  = Biserial correlation coefficient, P-value= 0.05.

## DISCUSSION

The current study findings shows that 36% of pediatric nurses were aged 31 to 35. This aligns with earlier research indicating that the main age group of nurses in a study on vascular access devices ranged from 33 to 37

years old.<sup>12</sup> Another study found that age distribution across nursing specialties was consistent, with most participants falling between 29 and 39 years old.<sup>13</sup> Additionally, the majority of participants were female nurses and this align with previous study found that 63.7% of nurses were females. Another study context

indicates a female majority, with 59% and 65% of the participants in the other studies being female.<sup>14-16</sup> Furthermore, the current study found that less than half of pediatric nurses had between 6-10 years of nursing experience. Recent research showed that the largest number of midwife nurses had 5-9 years of experience.<sup>17</sup> Most studies emphasize early experiences, neglecting mid-career well-being, which relates to resignation rates and strategies.<sup>18</sup>

Regarding years of experience in hematology-oncology units, most of pediatric nurses had 1-5 years, and this similar to previous study found that 72% of participants had 1 to 5 years of hematology-oncology experience, while fewer than half of the nurses in CCUs had similar experience.<sup>16,19</sup> Results also indicates that more than half of nurses were married, which aligning with a recent study of neonatal intensive care nurses with 52% of them were married. Another study found that 78% of the nurses were married, a higher percentage than in the analysis but still useful for comparison.<sup>20,21</sup> Another study showed that 44% of nurses held a bachelor's degree in nursing. The results of the current study align with the findings of another study, 41.4%.<sup>22</sup> This shows that more nursing staff were bachelor's degree graduates or have upgraded their nursing education. In contrast, another study reported that most nurses held a diploma in nursing.<sup>23</sup>

The findings of current study based on observation indicates that pediatric nurses were exhibited insufficient practice levels regarding nursing procedures while assisting children undergoing LP, signifying overall inadequate nursing performance. Further analysis illustrated that the majority of nurses demonstrated poor procedural competency, while only few of them performed at a fair level, and only less than ten percentages attained a good level of practice. These results supported by a study revealed significant deficiencies in pediatric nurses' procedures for children with meningitis, which improved following the intervention of the educational training unit.<sup>7,24</sup> Furthermore, a study conducted in Iraq revealed inadequate patient safety during LP, indicating broader nursing practice issues.<sup>25</sup>

The current study findings indicate significant correlation between nurses' practices and their years of experience in nursing. This result agrees with the previous study of hemodialysis care, which presented a strong positive correlation between nurses' practices and their years of nursing experiences in hospitals.<sup>26</sup> In contrast, another study found no significant link between nurses' years of experience and their practices.<sup>27</sup> Collectively, these studies suggest that years of nursing experience positively influence nurses' practices. Moreover, the current study results demonstrated a highly significant correlation between nurses' practices and their years of experiences in oncology-hematology units. This finding agrees with the study on nurses' practices when caring for poisoned children, which also showed a significant connection between nursing practices and their experiences in the

emergency department.<sup>28</sup> However, this result contrasts with another study that examined nurses' practices regarding chemotherapy-induced peripheral neuropathy, which reported no significant relationship between nurses' experiences and their practices in the hematology center.<sup>29</sup>

Another significant correlation was found in the current study between pediatric nurses' practices and their nursing qualifications. This finding aligns with a study conducted in Iraq among oncology nurses that reported a significant correlation between nurses' qualifications and their clinical performance.<sup>30</sup> In contrast, another study found no significant relationship between nurses' education levels and practices in specific burn care.<sup>25</sup>

## CONCLUSION

The significant relationship between nurses' experience and education level, socio-demographics, and the quality of nursing practices for children during LP were noted. Also, age, gender, and marital status had no significant impact on nurses' practice. Experienced nurses demonstrated advanced practices, emphasizing the importance of continuous education in nursing training, educational intervention programs, and procedural care for pediatric oncology-hematology.

### Author's Contribution:

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# Nurses Knowledge Regarding Advanced Cardiac Life Support

Haider B. Hami

## ABSTRACT

Nurses Knowledge  
Regarding Advanced  
Cardiac Life

**Objective:** To assess nurses' knowledge about advanced cardiac life support and the correlation between knowledge and various demographic variables.

**Study Design:** Descriptive study

**Place and Duration of Study:** This study was conducted at the Al-Nafes Teaching Hospital, Iraq from 15<sup>th</sup> August 2024 to 31<sup>st</sup> October 2024.

**Methods:** Sixty nurses from critical care units were selected. Participants received a thorough explanation of the study's goals and methods, along with reassurance that their participation would not cause any harm. By coding responses and eliminating any personal information, confidentiality and anonymity were protected. The information was safely kept and utilized only for scholarly and scientific research. Additionally, participants were made aware of their freedom to leave the research at any time without incurring any fees.

**Results:** A significant portion of those surveyed displayed moderate understandings regarding the items, eight questions reached a mean score of 1.33 or more, while seven questions ended up with scores beneath this limit. Besides, the study revealed that there is no meaningful correlation between knowledge level and selected demographic data. While the results showed up a strong association between knowledge and participation in courses.

**Conclusion:** The key weakness in their knowledge about advanced cardiac life support protocols, stress the necessity for educational programs and courses and simulation activities to develop the readiness for emergency heart situations.

**Key Words:** Assess, Nurses, Awareness, Cardiac life support

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## INTRODUCTION

The heart failure causes more than five hundred thousand deaths every year, underlining its status as a critical worldwide health concern, thus marking it as an important area for public health strategies. The survival rate for adult patients experiencing cardiac arrest have demonstrated a slight improvement over the past two decades; however, outcomes continue to be suboptimal particularly in situations where the event transpires outside of clinical environments, where the probability of surviving is estimated to be below 10%.<sup>1-3</sup>

A cardiac arrest is a severe and urgent occurrence that requires the actions of many healthcare workers to

maximize the accomplishment of cardiopulmonary resuscitation (CPR). Establishing the time inside the hospital environment introduces a major challenge, mostly because of the pressure tied to administering advanced cardiac life support (ACLS).<sup>4,5</sup>

Programs aimed at resuscitation training are designed to cut down on the occurrence of conditions and loss of life due to serious health problems through the dissemination of validated expertise and protocols. Understanding ACLS considerably reinforces the impact of ACLS team. Across the world, the perceptions and proficiency of healthcare professionals reflect significant variability. Involvement with community-orientated programs lowers the rates of mortality around the world.<sup>6,11</sup>

Under several situations, the health status of the patient can serve as a stable parameter that alters the consequences of CPR. To achieve better outcomes for patients that are performing CPR, it is crucial to improve the quality of care delivered to individuals in cardiac arrest through the application of understood expertise and clinical protocols. The skills of nurses and other healthcare practitioners in performing CPR are a fundamental determinant of their effectiveness in responding to cardiac arrest.<sup>12</sup>

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The determination of various medical emergencies, such as myocardial infarction, sudden cardiac arrest, and airway obstruction due to foreign bodies, along with the preparation and delivery of a D.C shock during CPR, makes up the foundation of Basic Life Support (BLS). The American Heart Association (AHA) highlights that the probability of victims surviving can be significantly enhanced, increasing from 49% to 75% for patients by administering CPR and D.C shock delivery within the critical initially three to five minutes after the cardiac arrest. In occurrences of unexpected cardiac arrest, the competent administration of BLS protocols can facilitate 2-3 times improvement in survival statistics.<sup>6,7,12</sup> Consequently, the awareness and proficiency in BLS among nurses are of paramount importance.

## METHODS

This descriptive study was conducted at Ibn Al-Nafes Teaching Hospital, Iraq from 15<sup>th</sup> August 2024 to 31<sup>st</sup> October 2024 vide letter No. 4545/QM/Approval/9389JFDNF dated August 11, 2024 and 60 voluntaries were to participated. Participants received a thorough explanation of the study's goals and methods, along with reassurance that their participation would not cause any harm. By coding responses and eliminating any personal information, confidentiality and anonymity were protected. The information was safely kept and utilized only for scholarly and scientific research. Additionally, participants were made aware of their freedom to leave the research at any time without incurring any fees. The evaluation was executed at the Ibn Al-Nafes Teaching Hospital, recognized for it is experienced in cardiology, thoracic and vascular surgical technique in Baghdad. A purposive sampling method was applied consisting of sixty nurses from critical care units in selected setting. To achieve the study aims, a questionnaire instrument that was developed to assess ACLS. A total of 60 participants were included. The study tool consists of two parts; the first include sociodemographic data of nurses participated in the study; the second part is concerned with nurses' knowledge regarding ACLS which is a 15 multiple choices items.

The research instrument was revised by a panel of nursing experts comprising twelve experts, each one of them have many years' experiences in nursing. The panel consists of five faculty members in College of Nursing/ University of Baghdad, four-faculty members in College of Nursing/ Al-Bayan University. Furthermore, there were three faculty members representing the AL-Russafa Health Directorate and Ibn Al-Nafes Teaching Hospital. These experts were provided with a copy of the study instruments and were solicited to critically review and evaluate the instrument concerning its content clarity and adequacy. Following a comprehensive consideration of all feedback and

recommendations, certain items were excluded while others were incorporated. The questionnaire was deemed valid subsequent to the modifications implemented in accordance with their feedback.

The questionnaire was developed utilizing a test-and-retest methodology with 5 nurses from selected hospital during 3 week period to assess the reliability of study tool. The reliability results showed that the Pearson correlation coefficient was statistically acceptable at ( $r = 0.864$ ) which is compatible with standard. The data was entered and analyzed through SPSS-25.

## RESULTS

There were 56.7% females and 40% were between age group of 20-25 years, their marital status was single (63.3%), and they also had an education level of diploma degree (56.7%), with a year of experience of 1-5 (65%), also the majority of the sample didn't get any training courses (65%) [Table 1].

The larger proportion of participants in the study exhibited responses aligned with their knowledge at a moderate level (eight questions resulting in a mean score surpassing 1.33). This is succeeded by a low level of understanding, evidenced by seven questions with mean scores falling below (1.33) [Table 2].

Table 3 suggested a notable link between the expertise that nurses hold and the training they have received, whereas no link is evident between nurses' expertise and their selected socio-demographic characteristics.

**Table No. 1: Distribution of demographic data (n=60)**

Variable	No.	%
<b>Gender</b>		
Male	26	43.3
Female	34	56.7
<b>Age (years)</b>		
20-25	24	40.0
26-30	20	33.3
31-35	9	15.0
36-40	3	5.0
41-45	4	6.7
<b>Level of education</b>		
Nursing school	10	16.7
Diploma	34	56.7
Bachelor	16	26.6
<b>Marital status</b>		
Single	38	63.3
Married	19	31.7
Others	3	5.0
<b>Years of experience (years)</b>		
1-5	39	65.0
6-10	14	23.3
11-15	4	6.7
16-20	2	3.3
21-25	1	1.7
<b>Training courses</b>		
Yes	21	35.0
No	39	65.0

**Table No. 2: Assessment of nurses' knowledge about advance cardiac life support**

Variable	Group	No.	%	MS	Assess
Abbreviation of Advanced Cardiac life Support is?	Correct	17	28.3	1.280	W
	Incorrect	43	71.7		
Initial intervention for an unresponsive patient presented in your hospital?	Correct	16	26.7	1.260	W
	Incorrect	44	73.3		
An immediate intervention if the victim has no pulse?	Correct	23	38.3	1.610	M
	Incorrect	37	61.7		
Location of chest compression?	Correct	28	46.7	1.460	M
	Incorrect	32	53.3		
Depth of chest compression for adults?	Correct	13	21.7	1.210	W
	Incorrect	47	78.3		
Adult advanced cardiac life support maximum joule for shockable rhythm	Correct	16	26.7	1.260	W
	Incorrect	44	73.3		
Non-shockable rhythms are?	Correct	22	36.7	1.360	M
	Incorrect	38	63.3		
The CPR rate of compression for adults?	Correct	30	50.0	1.500	M
	Incorrect	30	50.0		
Shockable rhythms are?	Correct	24	40.0	1.400	M
	Incorrect	36	60.0		
The reversible causes of cardiac arrest?	Correct	19	31.7	1.310	W
	Incorrect	41	68.3		
The dose and frequency of adrenaline for advanced cardiac life support?	Correct	14	23.3	1.230	W
	Incorrect	46	76.7		
The indication of amiodarone for advanced cardiac life support?	Correct	32	53.3	1.530	M
	Incorrect	28	46.7		
The dose of first dose amiodarone for advanced cardiac life support?	Correct	22	36.7	1.360	M
	Incorrect	38	63.3		
A Magnesium sulfate drug use for advanced cardiac life support?	Correct	23	38.3	1.380	M
	Incorrect	37	61.7		
What is the post-cardiac arrest care?	Correct	18	30.0	1.300	W
	Incorrect	42	70.0		

G = Good (1.67-2), M = Moderate (1.34 – 1.66), W = Weak (1 – 1.33), MS = mean score, Assess = Assessment

**Table No. 3: Association between nurses' socio-demographic characteristics and their overall knowledge**

Variables	B	Std. Error	Beta	t	Sig
Age	0.252	0.461	0.113	0.547	0.586
Gender	-0.297	0.758	-0.057	-0.392	0.697
Marital status	0.119	0.753	0.027	0.158	0.875
Education level	-0.048	0.613	-0.012	-0.079	0.938
Years of experience	-.0303	0.624	-0.103	-0.486	0.629
Training courses	- 1.113	0.944	-0.634	-2.340	0.002

B = Unstandardized coefficients; Std. Error = Standard errors; Beta = standardized coefficients; t= t-statistics; Sig. = significance

## DISCUSSION

The significant majority of the participants were females (56.7%), aligning with prevailing national and regional trends in nursing. Similar gender patterns were found in the Iraqi research conducted by Alkhaqani<sup>13</sup> (2023), where females also made up the majority group of nursing staff, reflecting the gender trends in nursing in the Middle East.

A considerable percentage of the sample was consisted of individuals within the youthful demographic

category of 20-25 years (40%), similar findings were reported in an Iranian study where nurses are in their first years of careers (2-5 years of experience).<sup>14</sup> Additionally, 56% of the respondents held a diploma qualification, confirming trends observed in regional Iraqi studies that indicate nurses with diploma-level credentials are the predominant demographic group.<sup>13</sup> A considerable of participants (65%) mentioned having about 1-5 years in their careers. This observation is consistent with prior research conducted in Rasht Teaching Hospital, were majority of nurses working in

intensive care units and emergency department had less than five years of experience, prompting apprehensions regarding their preparedness for high acuity clinical environments.<sup>14</sup>

Lastly, 65% had not participated in any instructional or practice courses is consistent with findings from Ethiopia, where over 60% of nurses had never engaged in ACLS training, thereby contributing diminished competency levels in resuscitation practices.<sup>15</sup>

Tale 2 showed that eight items were positioned within the moderate knowledge level where's seven items were categorized within the lowest level, signifying considerable deficiencies in fundamental ACLS competences. The items that are about the depth of chest compressions, the selection of joules for shockable rhythms, appropriate drug dosage (specifically epinephrine and amiodarone) and the differntiaion between shockable and non-shockable rhythms reflects trends identified in numerous regional and international investigations.

The cross-sectional study published in 2024, an analysis evaluating ACLS readiness among nursing practitioners, pointed out analogous inadequacies, particularly, regarding drug administration and rhythm determination, at last concluding that theoretical knowledge tends to decrease rapidly in the lacking of regular reinforcement.<sup>16</sup>

Moreover, Mudigoudra et al<sup>17</sup> revealed that over 90% of nursing students present only “moderate” pretraining ACLS knowledge, with almost universal challenges in advanced subjects such as drug dosing and electrical therapy, findings that align closely with inadequacies uncovered in current study.

The results in post-cardiac arrest management are distressing, notably given the international data that stresses the important effect of superior post arrest care on neurological health. A lack of knowledge regarding these protocols was also reported in a study conducted in Kuwait that assessed CPR/ACLS competencies among critical care nursing professionals.<sup>18</sup>

Table 3 demonstrates the lacking of a statistically significant association between overall ACLS knowledge and demographic variables, which include age, gender, marital status, educational levels, and years of experience. This investigation concurs with the argument documented in scientific studies that demographic factors are not reliable measures of ACLS capabilities. A survey involving nurses in Oman identified no considerable correlation between ACLS knowledge and characteristics such as age, educational qualifications and experiences, which indicates that clinical experience, cannot improve efficiency in resuscitation techniques.<sup>19</sup>

On the other hand, training programs exhibited a statistically significant correlation with knowledge (p=0.002), which suggests that systematic training is the principal aspect that influences ACLS knowledge.

This observation is strongly confirmed by recent globally research. A review in 2025 regarding simulation-based ACLS education concluded that structured training, rather than the duration of experience, appears as a more accurate predictor of knowledge retention, performance accuracy and confidence in emergent scenarios.<sup>20</sup>

Another research investigation from Ethiopia endorses this claim, indicating that nurses with ACLS training achieved considerably better outcomes in knowledge compared to their colleagues who did not have training.<sup>17</sup> Consequently the current study underlines the global agreement that steady, organized and simulation-based training is the cornerstone of ACLS proficiency, while demographic variables show minimal significance

## CONCLUSION

The results of the evaluation demonstrate that although there exists variability in the comprehension of advanced cardiac life support among nurses, considerable deficiencies are evident in essential areas such as the identification of cardiac rhythms, the execution of the correct sequence of interventions, and the administration of medications during resuscitation procedures. Patients' chances of survival could drop because of insufficient awareness or old-fashioned practices, as nurses often play the role of initial responders in urgent healthcare events. Elevating our capability and awareness about advanced cardiac life support is important to confirm that we give timely and effective resuscitation intervention.

**Recommendation:** To maintain nurses' proficiency in advanced cardiopulmonary resuscitation (CPR), ongoing training courses, simulation exercises, emergency training, and improved integration of CPR into nursing curricula are essential. Regular audits, along with constructive feedback, can help identify gaps and promote improvement. Furthermore, access to up-to-date educational resources and guidelines is beneficial.

### Author’s Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Haider B. Hami
Drafting or Revising Critically:	Haider B. Hami
Final Approval of version:	The above author
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# Role of Interactive Educational Program on Knowledge Gaps about Breast Cancer, Breast Self-Examination and Health Behaviors among Teachers and Students

Zunaira Bashir, Muhammad Saifullah and Madiha Mukhtar

## ABSTRACT

**Objective:** To assess the breast cancer knowledge and practice of breast self-examination among students and teachers of high school and to determine the impact of educational intervention on breast cancer knowledge, practice of breast self-examination and behaviour outcomes among students and teachers of high school

**Study Design:** Quasi-experimental pre- and post-intervention study

**Place and Duration of Study:** This study was conducted at the Government Girls High School Ram Ke Chattha, Hafizabad Punjab from 1<sup>st</sup> July 2025 to 30<sup>th</sup> December 2025.

**Methods:** The population included 200 school students and 15 female teachers selected through purposive sampling, ensuring participants met inclusion criteria such as age, willingness to participate, and absence of prior breast self-examination training.

**Results:** Post-intervention findings revealed statistically significant improvements in both knowledge and practice scores among students and teachers ( $p < 0.05$ ). The interactive educational workshop effectively bridged knowledge gaps, increased awareness, and promoted positive behavioral intentions regarding breast health and self-examination practices.

**Conclusion:** The educational intervention demonstrated a substantial impact in enhancing breast cancer awareness and breast self-examination practice among high school students and teachers. Integrating structured health education programs into school curricula can serve as a sustainable strategy for early detection and long-term cancer prevention.

**Key Words:** Breast Cancer, Breast self-examination, Schools health promotion

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## INTRODUCTION

Breast cancer remains one of the most commonly diagnosed and life-threatening malignancies among women worldwide, with over 2.3 million new cases reported annually.<sup>1</sup> Early detection significantly improves prognosis, with survival rates exceeding 90% when the disease is identified in its initial stages.<sup>2</sup> Despite advancements in screening technologies, a large proportion of women in low- and middle-income countries (LMICs) are still diagnosed at advanced stages due to limited awareness, sociocultural barriers, and inadequate access to screening services.<sup>3</sup>

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This highlights the urgent need for public health strategies focused on awareness, early identification, and preventive behaviors.

Globally, disparities in breast cancer incidence and mortality persist between high-income countries and LMICs. Regions such as the United States and Canada report high five-year survival rates, largely due to widespread screening and strong health systems.<sup>4</sup> However, countries in South Asia and sub-Saharan Africa continue to experience delayed diagnoses and higher mortality because of limited resources and insufficient public health education.<sup>5</sup> As populations grow and age, these gaps are expected to widen, underscoring the need for scalable, affordable, and culturally sensitive educational interventions that promote breast health literacy.

In Pakistan, breast cancer has become a major public health concern, with approximately 90,000 new cases and 40,000 deaths reported annually - the highest incidence in South Asia.<sup>6</sup> Nearly 70% of women present with late-stage disease, reflecting poor awareness and inadequate screening practices, particularly in rural districts such as Hafizabad.<sup>7,8</sup>

Awareness of breast cancer and breast self-examination (BSE) remains low among adolescents and adults, further contributing to delayed diagnosis. High school students and teachers represent a critical population for health education because they can disseminate essential knowledge within families and communities.

Educational interventions have shown promising results in improving knowledge of breast cancer and increasing the correct practice of BSE among young women, teachers, and community groups. Structured programs incorporating audiovisual sessions and hands-on demonstrations have been found to increase BSE knowledge by up to 80% and practice rates by more than 60%.<sup>9,10</sup> Teachers who participate in such interventions also demonstrate improved confidence and skill in educating students, creating a multiplier effect within the school environment.<sup>11</sup> These findings reinforce the importance of school-based initiatives as an effective platform for enhancing early detection behaviors.

Given the persistent gaps in breast cancer literacy and the absence of structured awareness programs in schools, targeted educational interventions are essential for empowering young women and educators with life-saving knowledge and skills. Enhancing BSE practice and awareness at the high school level not only promotes early detection but also aligns with national health priorities aimed at reducing breast cancer morbidity and mortality. For the nursing profession, such initiatives strengthen community engagement and highlight the vital role nurses play in preventive health education. Therefore, this study aims to assess the effects of an educational intervention on breast cancer knowledge and BSE practices among high school students and teachers in the Hafizabad District, ultimately contributing to improved public health outcomes.<sup>12,13</sup>

## METHODS

This pre and post intervention quasi-experimental research was done in Government Girls High School Ram Ke Chattha, Hafizabad, Punjab, Pakistan from 1<sup>st</sup> July 2025 to 30<sup>th</sup> December 2025 with the ethical approval granted by the Institutional Review Board of the University of Lahore (Ref. No. UOL/IREB/25/09/0022; dated 30 June 2025). There were 200 school female students and 15 female high school teachers in total, but purposive sampling of the sample was conducted based on preset inclusion criteria. The school administration gave formal consent before data collection and the teachers were given written informed consent together with parental or guardian consent to students. The data will be collected with structured questionnaires and checklists modified based on tested instruments while following the recommendations of the educational guidelines of the WHO and American Cancer Society. The data

collection tool included 3 parts: a breast cancer knowledge scale of 27 questions (correct response = 1, incorrect response = 0), a checklist on breast self-examination (BSE) practice with 11 items measuring the practice accuracy and a scale of 10 Likert-type items to measure the adherence to the preventive practices. The overall scores were classified into bad, average, good and excellent according to previously predetermined cut-off points. The first step was a baseline pre-assessment, which was aimed at assessing the demographic features of the participants, their knowledge of breast cancer, current BSE habits, and preventive measures.

A systematic educational program was administered in groups of four-45 minutes sessions following the assessment of the baseline. The activities involved breast cancer awareness, demonstration of breast anatomy and BSE technique using anatomical models, the barriers and lifestyle factors were discussed and practical training supervised by role play and interactive discussions. The post intervention test using the same questionnaire was conducted immediately after intervention to measure the knowledge and changes in practices. One month after, a follow-up evaluation was done using the preventative behavior scale in order to establish the sustainability of the behavior change.

All the data gathered were analyzed through the Statistical Package of Social Sciences (SPSS) version 25.0. Frequencies and percentages summarized the demographic characteristics and categorical variables by means of descriptive statistics. Kolmogorov-Smirnov test on students and Shapiro-Wilk test on teachers were used to test the normal distribution of data; both tests had p below 0.05, which implied that non-parametric statistics was used. Comparison of pre-intervention and post-intervention results in terms of knowledge and BSE practice was carried out with Mann-Whitney U test and McNemar test. A p-value of below 0.05 was considered statistically significant.

## RESULTS

A total of 200 students and 15 teachers participated in the study. The majority of students were aged 15–19 years (80.0%), while the remaining 20.0% were between 20–24 years. In contrast, teachers were generally older, with more than half (53.3%) aged above 30 years, 33.3% between 25–30 years, and 13.3% between 20–24 years. Regarding ethnicity, most students were Punjabi (88.0%), followed by Pakhtun (7.0%) and Sindhi (5.0%). All teachers (100.0%) were Punjabi. Family history was reported by 8.0% of students and 46.7% of teachers. Among those with a positive family history, the mother was identified as the affected family member in 37.5% of students and 28.6% of teachers. (Table 1)

Among the 200 students, baseline assessment revealed that all participants (100.0%) had poor knowledge regarding breast cancer and demonstrated poor breast self-examination (BSE) practices. Following the educational intervention, a substantial improvement was observed in both domains. Post-intervention, only 5.0% of students remained in the poor knowledge category, while 38.0% achieved average knowledge and 57.0% demonstrated good knowledge. Similarly, BSE practices improved markedly, with poor practices decreasing from 100.0% at baseline to 34.0% post-intervention. After the intervention, 42.0% of students demonstrated average practices and 24.0% achieved good practice levels. The improvements in both knowledge and practice were statistically significant ( $p < 0.001$ ), indicating a strong positive effect of the educational intervention. (Table 2)

Following the educational intervention, the post-intervention assessment of preventive health behaviors among the 200 students showed that the majority demonstrated positive behaviors. Specifically, 51.5% of students exhibited good preventive health behaviors, while 6.5% reached an excellent level. Meanwhile,

34.0% displayed poor behaviors, and 8.0% fell into the very poor category (Fig. 1).

The pre and post intervention scores were compared to show that there was a statistically significant increase in the knowledge of the teachers and students about breast cancer as well as an increased practice in breast self-examination (BSE) after the educational intervention. Median scores of teachers were significantly higher in knowledge (45.00 vs. 126.00) and BSE practice (46.50 vs. 124.50), and the results were highly significant ( $p = 0.000$ ), which showed significant improvements in awareness and practice. On the same note, there was significant improvement in the students, as median knowledge score went up to 5146.00 and the BSE practice score went up to 5704.00 after intervention compared to the situation before the intervention in 2604.00 and 2046.00 respectively. All the resulting p-values of statistical significance ( $p < 0.05$ ) prove the existence of a strong positive impact of the structured educational program on the improvement of both cognitive understanding and preventive health behaviors among the participants (Table 3).

**Table No. 1: Demographics of students and teachers**

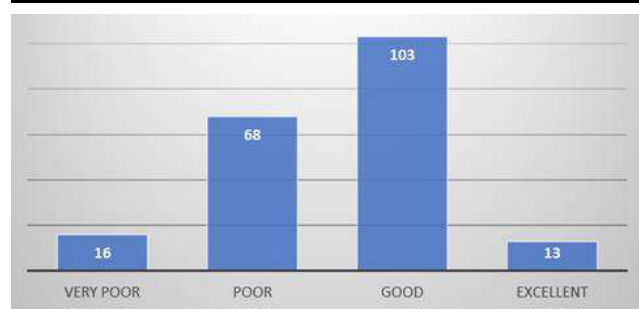
Variable	Category	Students (n = 200)	Teachers (n = 15)
Age (years)	15–19	160 (80%)	—
	20–24	40 (20%)	2 (13.3%)
	25–30	—	5 (33.3%)
	>30	—	8 (53.3%)
Ethnicity	Punjabi	176 (88%)	15 (100%)
	Pakhtun	14 (7%)	—
	Sindhi	10 (5%)	—
Family History	Yes	16 (8%)	7 (46.7%)
	No	184 (92%)	8 (53.3%)
If family history present (n=16 students; n=7 teachers)	Mother	6 (37.5%)	2 (28.6%)

**Table No. 2: Effect of Educational Intervention on Knowledge and Breast Self-Examination Practices Among Students (n = 200)**

Outcome Variable	Level	Pre-Intervention	Post-Intervention	p-value*
Knowledge regarding breast cancer	Poor	200 (100%)	10 (5%)	<0.001
	Average	-	76 (38%)	
	Good	-	114 (57%)	
Practice of breast self-examination	Poor	200 (100%)	68 (34%)	<0.001
	Average	-	84 (42%)	
	Good	-	48 (24%)	

**Table No. 3: Comparison of pre and post intervention scores among students**

Participants	Outcome Variable	Median (Pre-intervention)	Median (Post-intervention)	Mann–Whitney U	P-value
Teachers (n=15)	Knowledge towards breast cancer	45.00	126.00	0.000	0.000*
	Practice towards BSE	46.50	124.50	1.500	0.000*
Students (n=200)	Knowledge towards breast cancer	2604.00	5146.00	93.000	0.000*
	Practice towards BSE	2046.00	5704.00	651.000	0.000*



**Figure No. 1: Preventive health behaviors outcome variable among students**

## DISCUSSION

The demographic features of the current study indicate a representative school based population in Pakistan, with the majority of students being in late adolescence whilst the teachers were of a more professional mature population. The high rural rates of the participants could account for the low awareness of breast cancer and the lack of preventive behaviors at the early stage since in the past researchers have focused on the low access to health information and sociocultural hindrances in rural areas.<sup>14,15</sup>

Along with the advance in knowledge, breast self-examination among students increased significantly at the post intervention stage. There are also similar results in Ethiopia and Saudi Arabia where participatory educational strategies and demonstrations served as an important aspect of improving BSE performance in adolescents and university students aged.<sup>16-18</sup> The transformation of the universal poor practice into the enhanced engagement indicates that the training based on skills and the establishment of confidence are essential in facilitating preventive behaviors. Nevertheless, the fact that there are still some poor preventive practices points towards the possibility that a long-term follow-up and reinforcement might be required to ensure behavioral change in the long term.<sup>19,20</sup>

The stronger outcomes at the end of the intervention may be involving teachers educational background and their role as community influencers that may be seen in comparison to the outcome in case of community-based population in the future.<sup>15</sup> The teachers used in the present study also showed notable gains in knowledge, practice of breast self-examination, and preventive behavior, which enhances their position as major facilitators of health promotion in the academic setting. Similar gains have been documented among teachers in Kuwait, Nigeria, and Pakistan where organized training programs have improved awareness and actual involvement in breast health practices, both of which have been shown to be effective in these countries.<sup>16,21,22</sup>

The beneficial shift in general preventive health behavior among the students also identifies the evidence that educational interventions can redefine the

health attitudes and self-care among adolescents.<sup>23</sup> However, the existence of poor or very poor behavior students puts emphasis on incorporating the constant health education in the school systems to ensure long term compliance. The results showed a significant change in the level of knowledge among students after the educational intervention, which was consistent with earlier reports that organized awareness campaigns are effective ways of filling the gaps of breast cancer literacy knowledge in young females. Similar results have been documented in studies in Pakistan which have indicated that interactive education has a significant effect in increasing the knowledge of risk factors, symptoms and early detection practices.<sup>22,23</sup>

The substantial change in preventive health practices among teachers can be compared to the results of research held in Saudi Arabia and Turkey where it was stated that educative intervention resulted in long-term embracement of screening procedures and lifestyle changes. These results underscore the possibility of empowering teachers as health awareness propagators to increase the influence of interventions on the participants to the rest of the school.<sup>18,24</sup>

Statistical results proved the effectiveness of the structured educational programs in improving the cognitive and behavioral outcomes by showing the significant improvements in knowledge and practice scores after the intervention. Findings are congruent with the international literature that has stressed that skill-based and culturally sensitive educational approaches have the potential to enhance the gap in knowledge and facilitate preventive health practices in both adolescents and teachers.<sup>25- 27</sup> In general, the current research contributes to the existing evidence that incorporating breast health education at schools and in school curricula and teaching helps to address the issue.

## CONCLUSION

The study clearly demonstrates that interactive educational workshops play a transformative role in bridging knowledge gaps about breast cancer, breast self-examination and preventive health behaviors among both teachers and students. The intervention resulted in a remarkable improvement in awareness, understanding, and practical engagement, affirming the power of participatory and experiential learning in promoting sustainable health behaviors. By fostering an environment of open dialogue and skill-based learning, these workshops empowered teachers to become informed health advocates and students to adopt proactive attitudes toward self-care.

### Author's Contribution:

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Agreement to accountable for all aspects of work:	All the above authors

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Original Article

# Thulium Fiber Laser Versus Holmium:Yttrium Aluminium Garnet (Ho:YAG) in Flexible Ureteroscopy: A Comparative Study of Stone Dusting Outcomes

Thulium Fiber Laser and Holmium:Yttrium Aluminium Garnet Lasers for Renal Calculi

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## ABSTRACT

**Objective:** To compare the efficacy and safety of thulium fiber laser and Holmium:Yttrium Aluminium Garnet lasers during flexible ureteroscopy for renal calculi.

**Study Design:** Prospective comparative research study.

**Place and Duration of Study:** This study was conducted at the Department of Urology, Al-Kafeel Hospital, Iraq from 15<sup>th</sup> August 2024 to 31<sup>st</sup> July 2025.

**Methods:** A research including 193 cases with renal stone of 10-20 mm. The patients were divided into groups; group A (thulium fiber laser dusting group) included 95 patients and group B (Holmium:Yttrium Aluminium Garnet dusting) included 98 patients. The volumetric evaluation and maximal diameter of the stone were determined by non-contrast computed tomography. The operative time and stone free rate for four weeks after the operation were the primary outcomes. Secondary outcomes were perioperative complications, which were categorized according to the Clavien-Dindo classification.

**Results:** In Holmium:Yttrium Aluminium Garnet group, the thulium fiber laser had a considerably lower mean operating time (55±7 vs. 64±9 minutes; mean difference 9 minutes, 95% CI 7–11;  $p < 0.001$ ). At four weeks, the thulium fiber laser had a higher stone free rate (92%) than the Holmium:Yttrium Aluminium Garnet group (87%), although the difference was not statistically significant (absolute difference 5%, 95% CI –3 to 12;  $p = 0.22$ ). Only modest Clavien-Dindo grade I–II events and complication rates were similar between groups. **Conclusion:** During flexible ureteroscopy, thulium fiber laser outperforms Holmium:Yttrium Aluminium Garnet in terms of operational efficiency, safety, and a trend toward greater stone-free rates. These results call for additional validation in multicenter randomized trials and support the broader therapeutic usage of thulium fiber laser.

**Key Words:** Thulium fiber laser, Yttrium Aluminium Garnet, Stone free rate, Flexible ureteroscopy

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## INTRODUCTION

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Urolithiasis constitutes a significant and growing global health burden, with increasing prevalence and substantial implications for healthcare utilization. Advances in endourological techniques have positioned flexible ureteroscopy (fURS) as a first-line treatment for renal and proximal ureteral stones, offering high efficacy with minimal invasiveness.<sup>1</sup> Within this context, laser lithotripsy technology plays a pivotal role in determining procedural success, particularly when a dusting strategy is employed to facilitate spontaneous fragment clearance. For decades, the Holmium:YAG (Ho:YAG) laser has been regarded as the reference standard for intracorporeal lithotripsy due to its broad applicability across stone compositions and established safety profile.<sup>2</sup> However, technical limitations-including higher pulse energy requirements, increased stone retropulsion, and comparatively less efficient fine dust generation-may restrict its performance during flexible ureteroscopy, where efficient fragmentation without basket extraction is essential.<sup>3</sup>

The thulium fiber laser (TFL) has recently emerged as a novel alternative, characterized by a wavelength (1940 nm) that closely aligns with the absorption peak of water.<sup>4</sup> This property allows more efficient energy delivery, lower ablation thresholds, and the production of finer stone particles with minimal retropulsion. Furthermore, TFL enables operation at ultra-low pulse energies and very high frequencies, features that are particularly advantageous for stone dusting and may translate into improved operative efficiency and clinical outcomes.<sup>5</sup> Despite growing clinical adoption, robust comparative evidence assessing TFL against the conventional Ho:YAG laser in flexible ureteroscopy remains limited. In particular, stone dusting outcomes an increasingly favored lithotripsy strategy-have not been sufficiently evaluated in well-designed comparative studies. This gap in evidence limits the ability to draw definitive conclusions regarding the optimal laser platform for contemporary endourological practice.<sup>6</sup>

Renal calculi smaller than 20 mm can be effectively treated by FURS. The gold standard for ureteroscopic lithotripsy is still Ho:YAG. With its higher frequency, lower pulse energy, and smaller fiber diameters, the TFL, which was recently introduced, may maximize stone dusting.<sup>7</sup> According to preclinical research, TFL outperforms Ho:YAG in terms of faster ablation, decreased retropulsion, and finer dust.<sup>8</sup> With an emphasis on operational time, SFR, and safety, this study compared TFL and Ho:YAG dusting during FURS using standardized CT volumetry for stone assessment. Nephrolithiasis has risen markedly on a global scale over the past two decades.<sup>9</sup> By 2011, the morbidity rate associated with nephrolithiasis ranged from 1.2–1.4% in Europe and 1–5% in China.<sup>10</sup> The origins, diagnostic approaches, and treatment modalities for nephrolithiasis have all undergone substantial advancement. Extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL), and flexible ureteroscopy (FURS) are currently employed in its management. For stones up to 20 mm, ESWL produces favorable stone-free rates (SFR); however, the SFR attained with ESWL is obviously influenced by the size, location, and composition of the stone. When there are several kidney stones, its efficacy decreases by around 50%.<sup>11</sup> According to the 2013 European Association of Urology (EAU) guidelines<sup>12</sup>, percutaneous nephrolithotomy (PCNL) is still the recommended first-line treatment for patients with renal stones larger than 20 mm or multiple renal calculi; however, its use is restricted to those who are morbidly obese, have a coagulopathy, or are pregnant. Since Marshall's 1964 demonstration of a ureterostomy (FURS) from diagnostic to therapeutic purposes.<sup>13</sup> Despite the fact that the FURS approach is not recommended by the EAU recommendations on urolithiasis as the

conventional initial therapy for renal stones larger than 15 mm, a number of studies have presented data on the procedure and have recommended its usage due to its high success rates and low complication rates.<sup>12</sup> Accordingly, the primary aim of this study is to compare the stone dusting efficiency of thulium fiber laser and Ho:YAG laser during flexible ureteroscopy. Secondary objectives include assessment of operative time, laser energy utilization, stone-free rates, retropulsion, and perioperative safety. We hypothesize that Thulium Fiber Laser–assisted flexible ureteroscopy provides superior stone dusting efficiency and procedural performance compared to Ho:YAG laser, without compromising patient safety.

## METHODS

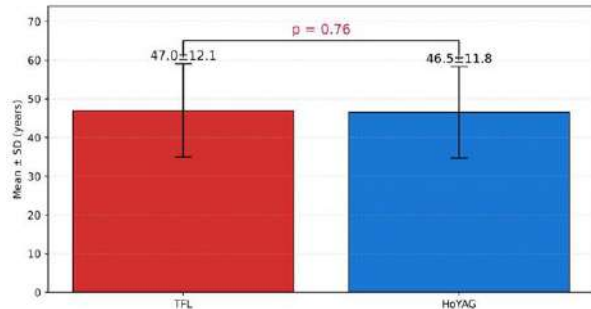
This prospective, single-center observational study was conducted at Al-Kafeel Hospital's Iraq from at Department of Urology, Al-Kafeel Hospital, Iraq from 15<sup>th</sup> August 2024 to 31<sup>st</sup> July 2025 vide letter No. 4545/QM/Approval/9389JFDNF dated 11<sup>th</sup> August, 2024. A total of 193 cases with renal stone of 10–20 mm were included. Every patient gave their informed consent, and ethical approval was secured. Adults  $\geq 18$  years old, renal stones 10–20 mm on NCCT, no history of PCNL, and normal renal architecture are the requirements for inclusion. Anatomical anomalies, strictures, coagulopathy, and pregnancy are the exclusion criteria. The patients were divided into groups; group A (thulium fiber laser dusting group) included 95 patients and group B (Ho:YAG 1 dusting) included 98 patients. The same surgical team used flexible ureteroscopes (7.5 Fr) with 200  $\mu\text{m}$  fibers to perform all procedures under general anesthesia. Ho:YAG 0.6–0.8 J, 10–20 Hz; TFL 0.15–0.2 J, 1500–2000 Hz. After surgery, DJ stents were given to every patient. Stone assessment: Blinded radiologists measured the ellipsoid volumetry ( $\pi/6 \times L \times W \times D$ ), maximal diameter, and NCCT with a slice thickness of  $\leq 3$  mm. NCCT at four weeks is the follow-up. SFR is defined as having no fragments larger than 2 mm. Clavien-Dindo graded complications. Statistics: t-test is used to evaluate continuous data, whereas  $\chi^2$  is used to study categorical data. 95% CI and effect sizes were provided. The sample size needed to detect a  $\geq 10\%$  SFR difference at  $\alpha=0.05$  was validated by power analysis.

## RESULTS

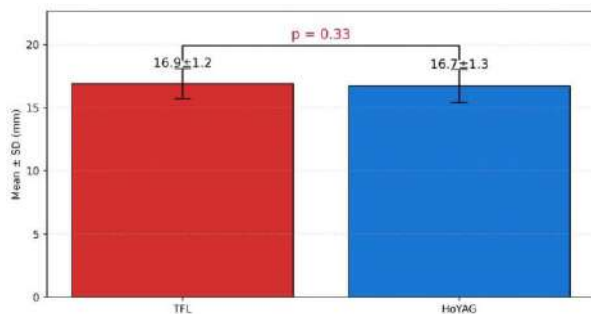
The stone size and demography of the groups were similar. Figures 1 and 2 showed the not significant differences between age and stone size, respectively in all patients. Table 1 provides a summary of detailed baseline characteristics. TFL had shorter mean operative time ( $55 \pm 7$  min) compared to Ho: YAG ( $64 \pm 9$  min,  $p < 0.001$ ).

**Table No. 1: Comparison of baseline characteristics**

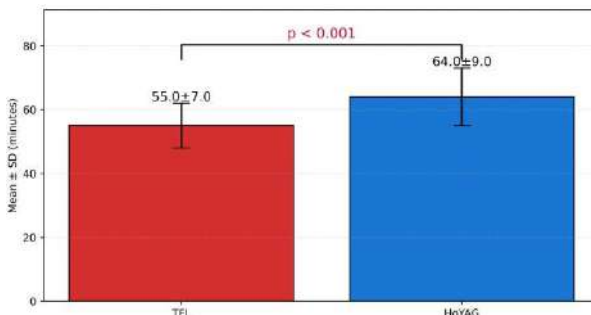
Variable	TFL (n=95)	Ho:YAG (n=98)	p-value
Age (years)	47.0±12.1	46.5±11.8	0.76
Male (%)	62%	64%	0.81
Stone size (mm)	16.9±1.2	16.7±1.3	0.33



**Figure No. 1: Comparison of age between TFL and Ho:YAG groups**



**Figure No. 2: Comparison of stone size between TFL and Ho:YAG groups**



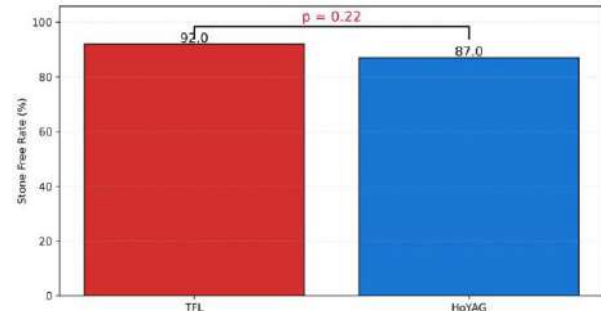
**Figure No. 3: Comparison of operative time between TFL and Ho:YAG groups**

**Table No. 2: Comparison of operative outcomes**

Variable	TFL (n=95)	Ho:YAG (n=98)	p-value
Operative time (min)	55±7	64±9	<0.001
SFR at 4 weeks	92%	87%	0.22
Complications (major)	-	-	-

Operative time differences are depicted in Figure 3, demonstrating a significant (<0.001) advantage for TFL

(Table 2). At 4 weeks, SFR was 92% in the TFL group vs 87% in the Ho: YAG group (p=0.22). The comparative SFR outcome was showing modest and non-significant advantage for TFL (Fig. 4).



**Figure No. 4: Comparison of stone free rate between TFL and Ho:YAG at 4 weeks**

## DISCUSSION

In the present study, thulium fiber laser achieves shorter operative times and slightly higher SFR compared to Ho:YAG in fURS dusting for 10-20 mm renal stones. These results are consistent with Xu et al<sup>14</sup> and with preclinical data showing superior ablation efficiency and reduced retropulsion with TFL. Although the SFR difference (5%) was not statistically significant, the trend favors TFL as compared with other studies.<sup>15,16</sup> The significant reduction in operative time is clinically important for improving workflow efficiency and patient outcomes. Complication profiles were similar, confirming both lasers as safe options for clinical use.<sup>17,18</sup> The non-significant SFR difference (Fig. 4) and significant time advantage (Fig. 3) reinforce TFL's procedural efficiency.<sup>19</sup> Baseline comparability ensures unbiased comparison. Strengths include prospective design, standardized CT volumetry, and homogeneous groups (Figs. 1-2, Table 1). Specifically, TFL was associated with a statistically significant reduction in operative time and a non-significant but clinically relevant improvement in SFR at four weeks.

This study showed that observed reduction in operative time with TFL is consistent with its distinct physical and technical properties. The higher water absorption coefficient of TFL at a wavelength of 1940 nm allows more efficient energy transfer to the stone fluid interface, resulting in faster ablation rates and finer dust production at lower pulse energies. This mechanism has been repeatedly demonstrated in preclinical and in vitro studies, which reported superior ablation efficiency and markedly reduced retropulsion compared with Ho:YAG lasers.<sup>20</sup> Our clinical findings corroborate these experimental observations and align with prior prospective and multicenter studies reporting shorter operative times when TFL is employed for dusting strategies.

In the present study, the difference in SFR between the two groups did not reach statistical significance, the

higher SFR observed in the TFL group (92% vs. 87%) suggests a favorable trend toward improved stone clearance. This finding may be attributed to the generation of finer dust particles with TFL, which facilitates spontaneous evacuation and reduces dependence on basket extraction.<sup>21,22</sup> From a clinical perspective, even modest improvements in SFR may translate into fewer ancillary procedures, reduced patient morbidity, and lower healthcare costs, particularly in high-volume endourology centers.

Importantly, both laser modalities demonstrated comparable safety profiles, with only minor Clavien Dindo grade III complications observed and no major adverse events in either group. This confirms that the enhanced efficiency of TFL does not come at the expense of increased perioperative risk. Reduced retropulsion associated with TFL may further contribute to procedural safety by minimizing unintended stone migration and decreasing the need for prolonged manipulation within the collecting system.

The use of standardized non-contrast CT volumetry and uniform follow-up protocols strengthens the validity of our findings. Accurate stone measurement and consistent SFR definitions are essential for meaningful comparison between laser technologies, as emphasized in contemporary endourological reporting standards. Baseline equivalence between groups in terms of stone size, patient demographics, and operative technique further supports the reliability of the comparative outcomes observed.

Despite these strengths, certain limitations warrant consideration. The single-center design may limit the generalizability of the results, and laser allocation based on availability rather than randomization introduces the potential for selection bias. Additionally, the follow-up period was limited to four weeks; longer follow-up may be necessary to assess late fragment passage and recurrence rates. Future multicenter randomized controlled trials with extended follow-up and cost-effectiveness analyses are therefore required to fully define the role of TFL in routine clinical practice. Overall, the findings of this study support the growing body of evidence suggesting that TFL represents a meaningful technological advancement in ureteroscopic lithotripsy. Its superior operative efficiency, combined with comparable safety and a trend toward improved stone clearance, positions TFL as a compelling alternative to Ho:YAG laser for dusting-based fURS, particularly in patients with renal stones up to 20 mm.

## CONCLUSION

In patients with renal stones measuring 10-20 mm undergoing flexible ureteroscopy, Thulium Fiber Laser assisted dusting demonstrated superior operative efficiency compared with the conventional Ho:YAG laser, as evidenced by significantly shorter operative times. While stone-free rates were comparable between

the two modalities, a consistent trend toward higher stone clearance was observed with TFL, without an associated increase in perioperative complications. These findings support the safety and clinical effectiveness of TFL and highlight its potential advantages in optimizing procedural performance. Larger multicenter randomized studies with longer follow-up are warranted to further validate these results and to establish the role of TFL as a preferred platform in contemporary endourological stone management. TFL dusting during fURS demonstrated superior operative efficiency and comparable safety relative to Ho:YAG.

### Author's Contribution:

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Agreement to accountable for all aspects of work:	All the above authors

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# Frequency of Perforation and Spillage of Gallstones During Laparoscopic Cholecystectomy and Their Outcome

Imran Mazhar<sup>1</sup>, Fahd Mudassar Hameed<sup>2</sup>, Nauman Anwar Rana<sup>2</sup> and Khadija Aurangzeb Abbasi<sup>1</sup>

Perforation and Spillage of Gallstones During Laparoscopic Cholecystectomy

## ABSTRACT

**Objective:** This study aims to evaluate the frequency of gallbladder perforation and gallstone spillage during LC and assess their impact on postoperative complications and recovery.

**Study Design:** Cross-sectional study

**Place and Duration of Study:** This study was conducted at the Department of General Surgery, PAF Hospital, Islamabad from 19th July 2025 to 19<sup>th</sup> October 2025.

**Methods:** A total of 177 patients undergoing LC were enrolled using stratified random sampling. Data on patient demographics, gallbladder size, number of stones, and surgical outcomes were collected. Descriptive statistics were used to summarize continuous variables such as age, BMI, duration of surgery, and length of hospital stay, presented as mean  $\pm$  standard deviation or median (interquartile range) depending on the data distribution. Categorical variables, such as gender, presence of gallbladder perforation, gallstone spillage, and postoperative complications, were presented as frequencies and percentages.

**Results:** Gallbladder perforation occurred in 14 (7.9%) patients, while gallstone spillage was observed in 18 (10.2%) patients. Patients with gallstone spillage had comparatively higher rates of postoperative complications, including port-site infection (44.4%), intra-abdominal abscess (27.8%), and reoperation (27.8%). The mean length of hospital stay for the spillage group was  $5.2 \pm 2.1$  days, comparatively longer than the  $3.4 \pm 1.2$  days for the no-spillage group.

**Conclusion:** Gallbladder perforation and gallstone spillage, though infrequent, are associated with significantly worse postoperative outcomes, including higher complication rates, longer hospital stays, and increased surgery durations.

**Key Words:** Laparoscopic cholecystectomy, gallbladder perforation, gallstone spillage, postoperative complications

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## INTRODUCTION

Cholelithiasis is estimated to be present in 15% of the adult population<sup>1</sup>; for symptomatic cases, laparoscopic cholecystectomy (LC) has attained the status of most preferred treatment modality in recent decades due to the advantages of reduced length of hospital stay and shorter recovery time as compared to traditional open cholecystectomy (OC)<sup>2</sup>.

Laparoscopic cholecystectomy is a technique in which the gall bladder is removed via ports that are inserted with minimal manipulation thus improving the time of

recovery and markedly decreasing the complications like wound infection<sup>3</sup>. The laparoscopic cholecystectomy complications comprise primary or late complications. Primary complications include port entrance complications, intestinal trauma, bleeding, and late complications, including gallstone spillage, biliary leakage, and biliary trauma. Gallstone spillage is common during laparoscopic cholecystectomy. Gallbladder perforation with stone spillage into the peritoneal cavity is more frequent with laparoscopic cholecystectomy as compared with open cholecystectomy. Early reports on laparoscopic cholecystectomy stated that stones left in the peritoneal cavity had no deleterious effect. Although the incidence of split gallstones and their complications are low, they are of a large variety. The incidence rate of spillage of gallstones secondary to perforation was reported 16%. In addition, to 50% of the spilled stones remained unretrieved. They may migrate in different regions and the reported complication rate varies from 0.08 to 0.3%. However, most recent evidence reported that the incidence rate of complications of spilled gallstones may range from 0.04 to 19%<sup>5,6</sup>. Abdlhakim MA et al

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evaluated the different short-term complications of bile and gallstones spillage during laparoscopic cholecystectomy. Perforation Group was significantly associated with Bile and stone spillage, abdominal collection and re operate to treat complications, regardless of conversion to open surgery and Port site infection they were also associated with Perforation Group but not significantly. Outcome showed that bile and stone spillage was 10.0% conversion to open surgery was 8.0% and port site infection 18.0%<sup>7</sup>. Because of lack of consensus recommendations and guidelines, the management of spilled gallstones varies widely between institutions and individual surgeons. Therefore, there is need to accumulate evidence so that recommendations can be made to treat spilled gallstones in our population to reduce morbidity.

## METHODS

This Cross-Sectional Study was conducted at Department of General Surgery, PAF Hospital, Islamabad from 19<sup>th</sup> July 2025 to 19<sup>th</sup> October 2025. The sample size was calculated using the WHO sample size calculator with the following parameters:

- Confidence level = 95%
- Anticipated proportion = 8%
- Absolute precision = 4%

Using these values, the calculated sample size was 177.

Stratified random sampling was employed to select participants from the general surgery department. Patients between the ages of 18 and 60 years with an American Society of Anesthesiologists (ASA) physical status score of II to III were included in the study. Patients who developed complications other than gallbladder perforation or gallstone spillage were excluded. Additional exclusion criteria included the presence of a gallbladder abscess, acute pancreatitis, abnormal blood clotting profiles, or common bile duct stones requiring surgery.

Data collection began after ethical approval was obtained from the hospital's ethical review committee. Written informed consent was taken from all patients before enrollment in the study. Demographic details, including the patient's MR number, age, sex, and BMI, were recorded. All patients underwent an ultrasonogram to gather clinical details such as gallbladder size, the number of stones, gallbladder wall thickness, and the size and site of the stones. Follow-up assessments were performed at one-week post-surgery to record the occurrence of gallstone spillage, conversion to open surgery, and any port-site infections, as per the operational definitions. All data were systematically entered into a predesigned proforma for accuracy and consistency. Data collected from the study were entered and analyzed using SPSS Version 25. Descriptive and inferential statistics were applied to evaluate the results. Descriptive statistics

were used to summarize continuous variables such as age, BMI, duration of surgery, and length of hospital stay, presented as mean  $\pm$  standard deviation or median (interquartile range) depending on the data distribution. Categorical variables, such as gender, presence of gallbladder perforation, gallstone spillage, and postoperative complications, were presented as frequencies and percentages.

## RESULTS

Table 1 shows that the mean age of all 177 patients. The mean age was  $45.87 \pm 8.30$  years, indicating that most participants were middle-aged adults. The average BMI was  $26.06 \pm 4.13$  kg/m<sup>2</sup>, suggesting that the majority fell in the overweight range, which is typical for gallstone disease patients. The average duration of surgery was  $54.50 \pm 14.54$  minutes, reflecting uncomplicated laparoscopic procedures for most cases. Patients stayed in the hospital for an average of  $3.75 \pm 1.59$  days, showing a relatively quick postoperative recovery. When stratifying age by perforation status, patients with perforation had a slightly higher mean age ( $48.08 \pm 8.19$  years) compared to those without perforation ( $45.68 \pm 8.31$  years), though the difference was subtle.

**Table No. 1. Baseline Demographic Characteristics of Patients (n = 177)**

Variable	Mean $\pm$ SD
Age (years)	45.87 $\pm$ 8.30
BMI (kg/m <sup>2</sup> )	26.06 $\pm$ 4.13
Duration of Surgery (minutes)	54.50 $\pm$ 14.54
Length of Hospital Stay (days)	3.75 $\pm$ 1.59
<b>Perforation Status</b>	
No perforation (n=163)	45.68 $\pm$ 8.31
Perforation (n=14)	48.08 $\pm$ 8.19
<b>Total</b>	45.87 $\pm$ 8.30

Gallbladder perforation occurred in 14 patients (7.9%), while gallstone spillage was observed in 18 patients (10.2%). Postoperative complications were relatively uncommon: port-site infection occurred in 10 patients (5.6%), intra-abdominal abscess in only 3 patients (1.7%), and reoperation was needed in 5 patients (2.8%).

**Table No.2: Frequency of Intraoperative and post-operative Events (n = 177)**

Event	Frequency (n)	%
Gallbladder Perforation	14	7.9
Gallstone Spillage	18	10.2
Post-operative Complication	Frequency (n)	%
Port-site infection	10	5.6
Intra-abdominal abscess	3	1.7
Reoperation required	5	2.8

Perforation occurred in 8.4% of males and 7.3% of females, showing no meaningful difference. Spillage

rates were also similar between sexes, with 9.5% in males and 11.0% in females. Port-site infection appeared slightly more common in males (7.4%)

compared to females (3.7%), but again the differences were small.

**Table No.3: Crosstab of Gender with Perforation, Spillage, and Port-site Infection**

Gender	Perforation n (%)	Spillage n (%)	Port-site Infection n (%)
Male (n=95)	8 (8.4%)	9 (9.5%)	7 (7.4%)
Female (n=82)	6 (7.3%)	9 (11.0%)	3 (3.7%)

**Table No. 4: Postoperative Complications and Recovery Metrics (n = 177)**

Parameter	Category / Statistic	Value
Port-site infection	n (%)	10 (5.6%)
Intra-abdominal abscess	n (%)	3 (1.7%)
Reoperation required	n (%)	5 (2.8%)
Length of hospital stay (days)	Mean ± SD	3.75 ± 1.59
	Minimum – Maximum	1.0 – 10.2
Duration of surgery (minutes)	Mean ± SD	54.50 ± 14.54
	Minimum – Maximum	21.2 – 105.7

**Table No. 5: Spillage Frequency and Overall Postoperative Complications (n = 177)**

Variable	Category / Statistic	Value
Gallstone Spillage	n (%)	18 (10.2%)
Port-site Infection (overall)	n (%)	10 (5.6%)
Intra-abdominal Abscess (overall)	n (%)	3 (1.7%)
Reoperation Required (overall)	n (%)	5 (2.8%)
Length of Stay (days)	Mean ± SD	3.75 ± 1.59
Duration of Surgery (minutes)	Mean ± SD	54.50 ± 14.54

Port-site infections (5.6%), intra-abdominal abscesses (1.7%), and reoperations (2.8%) were all infrequent, highlighting the generally safe postoperative course. The recovery profile shows that hospital stay ranged from 1 to 10 days, averaging 3.75 ± 1.59 days. The duration of surgery ranged widely from 21.2 to 105.7 minutes, with a mean of 54.50 ± 14.54 minutes, reflecting variability in operative difficulty.

Spillage was documented in 18 patients (10.2%). When viewed alongside the overall complication burden port-site infection (5.6%), intra-abdominal abscess (1.7%), and reoperation (2.8%) it suggests that although spillage occurred in a minority of cases, major postoperative morbidity remained low across the cohort. Average length of stay (3.75 ± 1.59 days) and surgery duration (54.50 ± 14.54 minutes) remained consistent, indicating that spillage did not prolong operative time or recovery when looking at overall means.

## DISCUSSION

The findings of this study provide valuable insights into the frequency of gallbladder perforation and gallstone spillage during laparoscopic cholecystectomy (LC), as well as their association with postoperative complications and recovery outcomes. While both gallbladder perforation and gallstone spillage are relatively infrequent events during LC, they are associated with significantly worse postoperative

outcomes, including higher complication rates, longer hospital stays, and increased surgery duration. The incidence of gallbladder perforation (7.9%) and gallstone spillage (10.2%) in our study is consistent with findings from previous studies, where perforation and spillage were observed in a range of 6% to 40% of cases. Gallstone spillage occurred in 10.2% of the patients in this study, which is within the reported range, and we found that the majority of the spills were single events. Interestingly, the co-occurrence of both perforation and spillage was observed in 2.8% of the patients, emphasizing the need for caution during the procedure, especially when dealing with difficult-to-access stones<sup>8-10</sup>.

Our results show that patients with gallstone spillage experienced a significantly higher rate of postoperative complications compared to those without spillage. Specifically, 44.4% of the spillage group had port-site infections, and 27.8% had intra-abdominal abscesses, compared to only 0.6% and 1.3%, respectively, in the no spillage group. This finding aligns with previous research suggesting that gallstone spillage can lead to increased risk of postoperative infections, especially when stones are not retrieved or properly managed. The incidence of reoperation in the spillage group (27.8%) further highlights the serious impact of spillage on surgical outcomes, requiring further interventions in some cases to address complications such as bile leakage or abscess formation<sup>11</sup>. Patients with gallstone spillage had a significantly longer length of hospital

stay ( $5.2 \pm 2.1$  days) compared to those without spillage ( $3.4 \pm 1.2$  days), which is consistent with the increased risk of complications observed in this group. The prolonged hospital stay is likely due to the need for additional treatment and monitoring for infections or abscess formation. Furthermore, the duration of surgery was also longer in patients with spillage ( $65.3 \pm 15.4$  minutes) compared to those without spillage ( $54.7 \pm 12.9$  minutes), which could be attributed to the time spent managing the spillage and performing additional steps to ensure proper retrieval of spilled stones<sup>12</sup>. The chi-square test revealed a significant association between gallstone spillage and the occurrence of postoperative complications, including port-site infections, intra-abdominal abscesses, and reoperation. This suggests that gallstone spillage is not just a benign event but a potential cause of significant postoperative morbidity. The use of independent t-tests further confirmed that patients with spillage had longer surgery durations and hospital stay, reinforcing the clinical significance of this complication<sup>13-15</sup>. This study underscores the importance of careful technique during laparoscopic cholecystectomy, particularly in managing gallstones. Surgeons should be vigilant for signs of perforation or spillage and take appropriate measures to retrieve spilled stones when possible. Adequate lavage of the peritoneal cavity and the use of prophylactic antibiotics may reduce the risk of postoperative complications. Furthermore, patients with gallstone spillage may require closer monitoring postoperatively to identify and manage complications promptly, which may help to minimize the need for reoperation or extended hospital stays. While this study provides valuable insights, it has limitations. The cross-sectional design limits our ability to establish causality between gallstone spillage and postoperative complications. Additionally, the study was conducted at a single center, which may affect the generalizability of the results. A larger, multicenter study would be beneficial to confirm these findings and further explore the impact of gallstone spillage on long-term outcomes.

## CONCLUSION

It is concluded that gallbladder perforation and gallstone spillage, though relatively infrequent during laparoscopic cholecystectomy, are significant risk factors for postoperative complications. The occurrence of these events is strongly associated with higher rates of port-site infections, intra-abdominal abscesses, and the need for reoperation. Furthermore, patients with gallstone spillage had longer hospital stays and increased surgery durations, highlighting the additional burden these complications impose on both the healthcare system and patients. Given the association between spillage and worse outcomes, it is crucial for surgeons to adopt meticulous techniques to prevent and manage perforation and spillage. Effective management

strategies, including thorough lavage, retrieval of spilled stones, and prophylactic measures, may reduce the likelihood of postoperative complications and improve overall patient outcomes.

### Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Imran Mazhar, Fahd Mudassar Hameed
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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# Investigating the Relationship Between Chorionic Villous Sampling and Pregnancy Complications

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## ABSTRACT

**Objective:** To determine the relationship between chorionic villous sampling (CVS) and pregnancy complications by evaluating immediate procedural, antenatal, delivery, and neonatal outcomes among women undergoing first-trimester prenatal diagnosis.

**Study Design:** Prospective observational study

**Place and Duration of Study:** This study was conducted at the Department of Obstetrics and Gynaecology, Jinnah Postgraduate Medical Centre, Karachi, from September 2025 till January 2026.

**Methods:** Pregnant women between 10 and 13+6 weeks of gestation undergoing CVS for prenatal genetic diagnosis were enrolled using non-probability consecutive sampling. Singleton pregnancies with complete follow-up until delivery were included. Baseline demographic and obstetric variables were recorded. CVS was performed via transcervical or transabdominal approaches under ultrasound guidance.

**Results:** A total of 73 women were included with a mean age of  $32.18 \pm 7.32$  years and mean gestational age of  $11.03 \pm 0.87$  weeks. Transcervical CVS was performed in 43 (58.9%) cases. Immediate complications were absent in 49 (67.1%) participants, with minor symptoms such as uterine contractions in 10 (13.7%) and spotting in 14 (19.2%). Ongoing pregnancy was observed in 50 (68.5%) cases, while 37 (50.7%) remained free of antenatal complications. Vaginal delivery occurred in 39 (53.4%) women. Neonatal survival was noted in 68 (93.2%) cases, with 56 (76.7%) neonates appearing clinically normal.

**Conclusion:** CVS is a relatively safe and effective first-trimester diagnostic procedure with low rates of maternal and fetal complications. When performed by experienced operators with appropriate follow-up, it provides substantial diagnostic benefit with acceptable risk.

**Key Words:** Chorionic villous sampling, prenatal diagnosis, pregnancy complications, miscarriage, invasive procedures.

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## INTRODUCTION

Chorionic villus sampling (CVS) is an invasive first-trimester prenatal test in which invasive testing is conducted to take samples of cells known as chorionic villi, which develop in the placenta in week 10 to week 13+6 of a pregnant woman<sup>1</sup>. It is used to obtain a sample of placental villi via transabdominal or transcervical procedures for genetic, chromosomal, and molecular testing.

The use of CVS is implied in cases where the fetus is at high risk of aneuploidy, an abnormality of the first-

trimester screening test, or where the parents of the fetus have a family history of a genetic abnormality or one of the parents carries a chromosome abnormality<sup>2</sup>. Although CVS offers earlier diagnosis than amniocentesis, its safety profile has been questioned. Complications in pregnancies that occur during miscarriage, Vaginal bleeding or premature rupture of membranes (PROM) or preterm labor, preterm birth, and pregnancy (including limb defect of a fetus), have also been discussed in various articles<sup>3</sup>. A recent systematic review estimated the risk of pregnancy loss associated with CVS at 0.22, which overlaps with the background risk<sup>4</sup>. Through other research, the rate of miscarriage/after CVS is between 0.5 and 1.0 per cent of all the pregnancies that take place. Other complications include vaginal bleeding in 10% of 10% of the cases, PROM in 710 per cent, fetal growth restriction (FGR) in 5%, and preeclampsia in about 6 per cent<sup>5</sup>. An article about a 2023 retrospective cohort study, published in the Korean journal, informed that women who had CVS were at a higher risk of having a shortened cervix requiring cerclage compared to women who had amniocentesis (adjusted OR 3.17)<sup>6,7</sup>.

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The defects associated with the limb reduction are associated with the operations that take place within the first 10 weeks of pregnancy, but are mainly rare (0.05-0.2)<sup>7,8</sup>. A 2020 study of 468 CVS by Turkish researchers (n=468) with a five-year follow-up in 2015 demonstrated that the rates of complications were low and the procedure is safe when conducted by any specialist, who has trained to perform CVS procedure 2020 However, the article by one more multicentric study predicted the importance of pre-procedure counseling, risk stratification and expertise of the operator in the reduction of risks<sup>9</sup>. The post-procedural effects were also recorded in terms of transient uterine reversal and focal placental hemorrhage, as shown by recent evidence<sup>10,11</sup>.

## METHODS

This prospective observational study was conducted at the Department of Obstetrics and Gynecology, Jinnah Postgraduate Medical Centre, Karachi, a tertiary care teaching hospital, from September 2025 till January 2026. The study included pregnant women with a singleton pregnancy who were between 10 and 13+6 weeks of gestation and experience CVS. Women who had provided written informed consent and had follow-up visits through delivery were recruited solely to facilitate full outcome evaluation. Females with an increased number of gestations, observed abnormalities of the uterine structure, or those with a prior history of cervical insufficiency were taken out because of their own risk of pregnancy complications. Also, patients with a chronic systemic disease like uncontrolled diabetes mellitus, kidney disease, or autoimmune disorders were excluded to eliminate the risks of confounding pregnancy outcomes. Patients who were either lost to follow-up before delivery or whose medical records were incomplete were also omitted from the final analysis. A non-probability consecutive sampling method was used. The World Health Organization formula on prevalence studies was used to calculate the sample size. Using an anticipated prevalence rate of pregnancy complications following CVS of 14%, a 95% confidence level ( $Z = 1.96$ ), and an 8% margin of error, the required minimum was estimated at 73 subjects. It was sufficient to provide a good level of statistical power to identify significant relationships between CVS and adverse pregnancy outcomes.

**Data Collection:** After Institutional Review Board approval was obtained, potential respondents were contacted and informed consent was obtained prior to participation. A previously developed pro forma was used to capture baseline demographic and obstetric data, including maternal age, parity, gestational age at the time of CVS, the reason for the procedure, and other pertinent medical and obstetric history. CVS was conducted in 10-13+6 gestational weeks under real-

time ultrasound guidance of experienced fetal medicine consultants, either using the transabdominal or the transcervical method under aseptic, stringent conditions. The early complications, such as vaginal bleeding, abdominal pain, uterine contractions, or indications of infection, were monitored immediately after the procedure. They were thereafter managed through regular antenatal clinic appointments until delivery. Clinical examination, ultrasound, hospital records, and delivery note data were used to record pregnancy outcomes such as miscarriage (pregnancy loss < 20 weeks), preterm birth (before 37 weeks), premature rupture of membranes, fetal growth restriction, and hypertensive disorders of pregnancy. All collected data were stored in encrypted electronic files to ensure confidentiality, and incomplete or missing records were excluded.

**Data Analysis:** Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 26.0. Continuous variables such as maternal age and gestational age were summarized as means and standard deviations, while categorical variables, including miscarriage, preterm birth, PROM, fetal growth restriction, and hypertensive disorders, were expressed as frequencies and percentages. Associations between CVS and pregnancy complications were evaluated using the Chi-square test. A p-value of 0.05 or less was considered statistically significant.

## RESULTS

Data were collected from 73 patients; the mean maternal age was  $32.18 \pm 7.32$  years, and the mean gestational age at the time of CVS was  $11.03 \pm 0.87$  weeks, indicating early first-trimester testing. The average gravida and para were  $2.92 \pm 1.47$  and  $1.84 \pm 1.09$ , respectively, reflecting moderate multiparity. The transcervical approach was used in 43 (58.9%) cases, whereas 30 (41.1%) underwent transabdominal or other techniques. Indications included thalassemia risk in 29 (39.7%) and other genetic or aneuploidy concerns in 44 (60.3%). Immediately post-procedure, 49 (67.1%) experienced no complications, whereas uterine contractions occurred in 10 (13.7%) and spotting or pain in 14 (19.2%); amniotic fluid leakage and other minor events were each seen in 8 (11.0%), with 57 (78.0%) reporting no specific adverse event.

**Table No.1: Maternal Characteristics, Procedure Details, and Pregnancy Outcomes (n = 73)**

Variable	Category	Value
<b>Baseline Characteristics</b>		
Age (years)	Mean $\pm$ SD	$32.18 \pm 7.32$
Gestational age at CVS (weeks)	Mean $\pm$ SD	$11.03 \pm 0.87$
Gravida	Mean $\pm$ SD	$2.92 \pm 1.47$
Para	Mean $\pm$ SD	$1.84 \pm 1.09$

Procedure Type		
	Transcervical	43 (58.9%)
	Transabdominal/ Other	30 (41.1%)
Clinical Indication		
	Thalassemia major risk	29 (39.7%)
	Other genetic/ aneuploidy risks	44 (60.3%)
Immediate Post-Procedure Status		
	No complication	49 (67.1%)
	Uterine contractions	10 (13.7%)
	Spotting/leakage/ pain	14 (19.2%)
Specific Procedure Events		
	Amniotic fluid leak	8 (11.0%)
	Other minor events	8 (11.0%)
	None recorded	57 (78.0%)
Pregnancy Course		
	Ongoing pregnancy	50 (68.5%)
	Loss/termination	23 (31.5%)
Antenatal Complications		
	None	37 (50.7%)
	Hypertensive disorders	11 (15.1%)
	Other obstetric/medical issues	25 (34.2%)
Mode of Delivery		
	Normal vaginal delivery	39 (53.4%)
	LSCS/operative delivery	34 (46.6%)
Delivery Complications		
	None	60 (82.2%)
	Infection	5 (6.8%)
	Other complications	8 (11.0%)

Neonatal outcomes were generally favorable. Survival was documented in 68 (93.2%) neonates, while 5 (6.8%) represented perinatal loss or other adverse outcomes. Clinical assessment at birth showed that 56 (76.7%) neonates appeared normal, whereas 17 (23.3%) exhibited mild abnormalities.

**Table No. 2. Neonatal Outcomes (n = 73)**

Variable	Category	Value
Survival status	Alive	68 (93.2%)
	Perinatal loss/other	5 (6.8%)
Clinical appearance	Normal	56 (76.7%)
	Mild abnormality	17 (23.3%)

Baseline demographic and obstetric characteristics were comparable between the transcervical (n = 43) and other approach (n = 30) groups. The mean maternal age was 31.84 ± 7.28 years versus 32.67 ± 7.47 years (p = 0.639), and the mean gestational age was 11.12 ± 0.82 weeks versus 10.90 ± 0.92 weeks (p = 0.307). Similarly, gravida averaged 2.74 ± 1.53 compared with 3.17 ± 1.37 (p = 0.220), and para was 1.86 ± 1.10 versus 1.80 ± 1.10 (p = 0.818).

**Table No. 3. Baseline Characteristics by Procedure Type**

Variable	Trans-cervical (n = 43)	Other Approach (n = 30)	p-value
Age (years), Mean ± SD	31.84 ± 7.28	32.67 ± 7.47	0.639
Gestational age (weeks), Mean ± SD	11.12 ± 0.82	10.90 ± 0.92	0.307
Gravida, Mean ± SD	2.74 ± 1.53	3.17 ± 1.37	0.220
Para, Mean ± SD	1.86 ± 1.10	1.80 ± 1.10	0.818

No complications were reported in 29 (67.4%) women in the transcervical group and 20 (66.7%) in the other approach group, totaling 49 (67.1%). Any complication occurred in 14 (32.6%) transcervical and 10 (33.3%) other approach cases, totaling 24 (32.9%).

**Table No. 4. Immediate Procedure Complications by Procedure Type**

Complication Status	Transcervical	Other Approach	Total	p-value
No complication	29 (67.4%)	20 (66.7%)	49 (67.1%)	0.109
Any complication	14 (32.6%)	10 (33.3%)	24 (32.9%)	0.109

**Table No. 5. Pregnancy and Delivery Outcomes by Procedure Type**

Outcome	Transcervical	Other Approach	Total	p-value
Ongoing pregnancy	30 (69.8%)	20 (66.7%)	50 (68.5%)	0.781
Pregnancy loss/termination	13 (30.2%)	10 (33.3%)	23 (31.5%)	0.781
Vaginal delivery	24 (55.8%)	15 (50.0%)	39 (53.4%)	0.612
LSCS/operative	19 (44.2%)	15 (50.0%)	34 (46.6%)	0.612
Any delivery complication	7 (16.3%)	6 (20.0%)	13 (17.8%)	0.674

Ongoing pregnancy was observed in 30 (69.8%) transcervical and 20 (66.7%) other approach participants, while pregnancy loss or termination occurred in 13 (30.2%) and 10 (33.3%), respectively ( $p = 0.781$ ). Vaginal delivery occurred in 24 (55.8%) transcervical and 15 (50.0%) other approach cases, whereas LSCS or operative delivery was required in 19 (44.2%) and 15 (50.0%) women ( $p = 0.612$ ). Delivery complications were noted in 7 (16.3%) and 6 (20.0%) cases, respectively ( $p = 0.674$ ).

## DISCUSSION

This is a prospective observational study carried out in the Department of Obstetrics and Gynaecology, Jinnah Postgraduate Medical Centre that assessed the relationship of chorionic villous sampling (CVS) and subsequent pregnancy complications on a cohort of 73 women undergoing first-trimester invasive prenatal diagnosis. All in all, the results indicate that CVS had a positive safety profile, characterized by minimal cases of immediate procedural complications and overall positive maternal and neonatal outcomes. The study population baseline data revealed a mean maternal age of 32.18 -1 -7.32 years and a mean gestation age of 11.03 -1 -0.87 weeks at the time of the procedure, which suggests that the majority of the study population had CVS in the most appropriate timeframe of the first trimester. The moderate multiparity was noted and it indicates that the population under consideration were common patients who were brought to genetic screening because of previous obstetric history or of having an increased risk factor<sup>12</sup>. These adults are in line with the past studies where CVS was mostly used among women whose pregnancy was at higher risk or those who have experienced some negative outcomes in the past, which contributes to the representativeness of the study sample. Short-term complications with the procedure were also rare and 67.1 percent of women did not have any negative effects. The commonest ones were minor including uterine contractions and spotting whereas the serious ones like amniotic fluid leakage were of rare occurrence. This is in line with the past studies that have shown that CVS, when done under the guidance of ultrasound by skilled operators, has low complication rate<sup>13</sup>. In practice, the process was more of a managed outpatient intervention, as opposed to a high-risk or dramatic one, and this is reassuring to both clinicians and patients. In terms of the progression of the pregnancy, over two-thirds of the pregnancies proceeded normally, and about half of the individuals did not have any antenatal problems at all. Despite the fact that the pregnancy was lost or terminated in 31.5 percent of the cases, this needs to be understood with caution as some of the losses could have been as a result of underlying fetus abnormalities as detected by the use of CVS and not as a result of the procedure itself<sup>14,15</sup>. The other past studies also remark that

focusing on attribution of miscarriage to the CVS alone may overestimate the riskiness of the procedure since most of the high-risk pregnancies already have an inherent probability of poor outcome<sup>16-18</sup>. Hence, causality may not be assumed out of context. Hypertensive disorders became the commonest antenatal complication<sup>15,16</sup>. The conditions are, however, multifactorial and are often observed in obstetric populations, regardless of invasive testing. Previous studies have documented similar prevalence rates across age groups among mothers, which makes CVS unlikely to be a direct causative agent. The lack of a precise procedural connection also provides evidence of the technique's relative safety<sup>19</sup>.

The delivery results were also positive. More than 50 percent of the women gave birth naturally, and most of them had no complications associated with delivery. There were also expected clinical ranges in operational delivery rates in tertiary care settings. The most promising results were observed in neonatal outcomes, with 93.2% surviving, and the vast majority were clinically normal at birth<sup>20</sup>. These results support the conclusion that CVS in the first trimester did not adversely affect fetal viability or perinatal health in the majority of cases. This research has several shortcomings that should be considered when interpreting the outcomes. First, the statistical power is relatively low due to the relatively small sample size of 73 participants and might restrict the relevance of the findings. Second, as a single-centre study at Jinnah Postgraduate Medical Centre, the results may not be generalizable to other healthcare settings, as they may reflect local practice and operator competencies. Third, the non-CVS comparison group does not reduce it to an observational design, and thus it becomes hard to make a direct causal relationship between CVS and pregnancy complications since some of the adverse outcomes can be caused by maternal or fetal risk factors, but not the procedure itself. Additionally, minor complications may have been underreported due to reliance on clinical records and patient follow-up, potentially introducing information bias.

## CONCLUSION

It is concluded that chorionic villous sampling is a safe and effective first-trimester diagnostic procedure with a low incidence of immediate and subsequent pregnancy complications. This prospective study conducted at Jinnah Postgraduate Medical Centre found that most women experienced no adverse events related to the procedure, most pregnancies were uncomplicated, and infant outcomes were generally positive. Minor ones like uterine contractions and spotting were noted sporadically, and severe complications were rare. There were also no major differences between transcervical and alternative methods of procedure, suggesting similar safety profiles among the methods. Generally,

CVS has a significant diagnostic advantage and a reasonable risk when performed by qualified professionals during ultrasound-guided procedures, and with proper patient selection and follow-up, it can be recommended to continue the practice of CVS in tertiary care units as a means of early prenatal diagnosis of genetic disorders.

#### Author's Contribution:

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# Comparison of Dexmedetomidine Versus Quetiapine for Management of Delirium in ICU Patients

Dexmedetomidine  
Versus  
Quetiapine for  
Management of  
Delirium in ICU

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Khadija Zubair<sup>4</sup> and Laiba Fayyaz<sup>4</sup>

## ABSTRACT

**Objective:** To compare the efficacy and safety of dexmedetomidine versus quetiapine for the treatment of delirium among critically ill patients admitted to the Intensive Care Unit (ICU).

**Study Design:** Randomized Controlled trial study

**Place and Duration of Study:** This study was conducted at the Combined Military Hospital, Lahore from October 2025 to December 2025.

**Methods:** A randomized controlled trial was conducted on 70 ICU patients diagnosed with delirium, who were allocated to receive either dexmedetomidine or quetiapine. Primary outcomes included time to delirium resolution, ICU length of stay, number of delirium days, and mortality. Secondary outcomes assessed adverse events and cost-effectiveness.

**Results:** Dexmedetomidine demonstrated superior clinical performance, with faster delirium resolution, reduced ICU and hospital stay, and lower mortality compared to quetiapine. It also showed a more favorable safety profile with fewer adverse events, particularly lower rates of hypotension and excessive sedation.

**Conclusion:** Dexmedetomidine appears to be a more effective and safer option than quetiapine for managing delirium in ICU patients. Further multi-center trials are recommended to validate these findings and assess long-term outcomes.

**Key Words:** Delirium, Dexmedetomidine, Quetiapine, Intensive Care Unit, Randomized Controlled Trial.

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## INTRODUCTION

Delirium is a neurocognitive disorder, which occurs at a quick and unstable fluctuation of attention, awareness and cognition<sup>1</sup>. It has also been noted to cause 10-40 per cent of the patients admitted in hospitals<sup>2</sup>, especially in patients in critical-care, about 80 per cent of the patients under mechanical ventilation<sup>3</sup>. Delirium has three subtypes: the most frequent-type of delirium amongst patients of intensive care unit (ICU) is referred to as the hypoactive type since it manifests in

An inactive and calm manner; the other two types of delirium are the hyperactive and the mixed type where patients are usually reversible and hyperactive respectively<sup>4,5</sup>. Delirium patients may also be hallucinated, delusional and confused and disoriented<sup>6</sup>. In addition to the fear that delirium causes to patients, it has also been linked to some dire results in the form of longer ICU stay and mechanical ventilation, delayed discharge, multiple organ failure besides increased mortality<sup>7,8</sup>.

Past research has explored the pharmacological interventions as a mode of treatment of delirium<sup>6-9</sup> and the recent emphasis has been placed on the approach after the discovery of the risk factors of delirium. Antipsychotic drugs are also used in treating delirium symptoms in clinical practice<sup>10</sup>. In fact, according to the guidelines provided by the National Institute of Health and Care Excellence (NICE)<sup>11</sup>, underlying or reversible causes of delirium should be identified and managed and short-term administration of antipsychotic drugs are also possible when the distressing symptoms persist<sup>12</sup>. Antipsychotic drugs have since been widely used in treatment of delirium in hospitalized patients since then. In one of the studies, an assessment carried out showed that about one in every three delirium hospital admissions was given antipsychotics<sup>13</sup>.

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## METHODS

The study was a comparative randomized controlled trial (RCT), conducted at Combined Military Hospital, Lahore from October 2025 to December 2025. The patients in the two groups of treatments were randomly selected to provide random sampling. The total sample size of the study was 70 ICU patients with 35 patients each in each treatment group:

- Dexmedetomidine group (n=35)
- Quetiapine group (n=35)

**Inclusion Criteria:** The participants of the study were ICU patients who satisfied the following conditions:

- Adult age group (no specific age range is given).
- Patient diagnosed with delirium or at high risk of delirium (via either the CAM-ICU or ICDCS scale).
- Patients under the care of mechanical ventilation or non-ventilated treatment were considered to depict a true ICU population.
- APACHE II scores and GCS at admission measured the severity of illness and neurological status respectively.

**Exclusion Criteria:** Patients that were left out of the study were those who had:

- Cognitive impairments or another neurological impairment that may interfere with delirium assessment or treatment evaluation.
- Contraindication to either therapy (Dexmedetomidine or Quetiapine) including severe hypotension (in the case of Dexmedetomidine) or increased QTc (in the case of Quetiapine).

**Data collection:** Data were collected through systematic clinical evaluations and continuous monitoring throughout the patients' ICU stay. Baseline assessments included demographic information such as age, gender, and BMI, along with illness severity measured by the APACHE II score and neurological status assessed using the Glasgow Coma Scale (GCS) on admission. Delirium was evaluated using standardized tools, including the CAM-ICU and ICDCS

scales. Treatment administration followed predefined protocols, with dexmedetomidine given at a dosage of  $0.7 \pm 0.1$  mg/day for 5 days (range 3–7 days), while quetiapine was administered at  $100 + 25$  mg/day for 7 days (range 5–9 days). Throughout the treatment course, delirium recovery was regularly monitored using CAM-ICU and ICDCS, and sedation levels were assessed using the RASS score. Patients were randomly allocated to either the dexmedetomidine or quetiapine treatment group, and all interventions were carried out according to protocol. Outcomes such as delirium resolution, ICU stay, and mortality were measured at predetermined intervals to assess treatment efficacy. Data analysis involved descriptive statistics to summarize baseline characteristics and compare both groups. A multivariate Cox regression model, adjusted for potential confounders including age and APACHE II score, was used to determine treatment effects. Sensitivity analyses were performed to ensure the robustness of findings, and p-values for different outcomes were reported.

## RESULTS

The baseline characteristics of the participants from both the Dexmedetomidine and Quetiapine groups are included in Table 1. The data show that there were no significant differences in age, gender, BMI, severity of illness (i.e., APACHE II score) and the level of consciousness (i.e., GCS on admission). The differences in the characteristics of the groups are minor and are statistically insignificant.

Based on the data presented in the table, there were no significant differences in the clinical parameters of the two groups when admitted to ICUs. The APACHE II score, GCS, or ventilation status of the groups did not have any significant differences either. Table 2.

The treatment details indicate that the dose and duration of treatment with Dexmedetomidine was markedly less than that of Quetiapine, implying that Dexmedetomidine may have more rapid-acting properties. Table 3.

**Table No. 1: Baseline Characteristics of Patients**

Characteristic	Dexmedetomidine Group (n=35)	Quetiapine Group (n=35)	p-value
Age (years)	55 ± 12 / 53 (45-60)	54 ± 11 / 52 (44-59)	0.062
Gender (Male/Female, %)	60% Male, 40% Female	58% Male, 42% Female	
BMI (kg/m <sup>2</sup> )	27.5 ± 4.3 / 26 (23-30)	28.1 ± 5.1 / 27 (24-31)	
APACHE II Score	21 ± 5 / 22 (17-25)	20 ± 4 / 21 (16-24)	
GCS on Admission	10 ± 2 / 9 (8-12)	11 ± 3 / 10 (9-13)	

**Table No. 2: ICU Admission Clinical Parameters**

Parameter	Dexmedetomidine Group (n=35)	Quetiapine Group (n=35)	p-value
APACHE II Score	21 ± 5 / 22 (17-25)	20 ± 4 / 21 (16-24)	0.062
GCS on Admission	10 ± 2 / 9 (8-12)	11 ± 3 / 10 (9-13)	0.084
Ventilator Status (Yes/No)	60% Ventilated, 40% Non-Ventilated	70% Ventilated, 30% Non-Ventilated	0.048

**Table No. 3: Treatment Details**

Parameter	Dexmedetomidine Group (n=35)	Quetiapine Group (n=35)	p-value
Type of Medication	Dexmedetomidine	Quetiapine	0.01
Dosage (mg)	0.7 ± 0.1 / 0.6 (0.5-0.8)	100 ± 25 / 90 (75-125)	0.02
Duration of Treatment (days)	5 (3-7)	7 (5-9)	0.03
Sedation Level (RASS)	3 ± 1 / 3 (2-4)	4 ± 1 / 4 (3-5)	0.02

## DISCUSSION

The study gives useful data on the effectiveness and safety of dexmedetomidine over quetiapine in treating confusion in intensive care unit patients. The main results of the study were faster orienting recovery, lesser loss of lives, and reduced critical care unit admissions. Dexmedetomidine would be the best intervention to use in this kind of population based on these findings. It was determined that the Dexmedetomidine group recovered consciousness to disorientation much quicker than the Quetiapine group. It was also not 48 hours but 24 hours circle as compared to Quetiapine group. The observation has been also correlated with other past studies who have reported that Dexmedetomidine had a potential of reducing the duration and incidence of delirium in the ICU since the ordeal of untreated delirium could lead to prolonged hospitalization, increased costs, and poor patient outcomes. The beneficiaries are the healthcare system and the patients of the fast resolution of the delirium as experienced in the case of using Dexmedetomidine<sup>14</sup>. Also, the increase in the length of stay of ICUs in both groups, i.e. the Dexmedetomidine group (6 days) and the Quetiapine group (8 days) can be accounted by the results found by a researcher during the implementation of Dexmedetomidine in ICU patients<sup>15</sup>. This insufficient extended length of stay at the ICU is not only indicative of better patient outcomes; it also indicates possible cost-saving since the ICU beds are scarce and costly assets. Moreover, the study has found that the length of stay in the hospital decreased in the Dexmedetomidine group and this is also the sign of the positive effect of the latter on the general recovery of the patient<sup>16</sup>. Moreover, the study sample was adequate to indicate significant differences across the two treatments, yet larger studies with a more diverse group of patients would be needed to conclude whether the research results can be applicable in other ICUs settings<sup>17,18</sup>. It should also be supplemented by further research that focuses on investigating the cost-effectiveness of Dexmedetomidine in a broader clinical context that would also include a more profound analysis of the healthcare resource usage and the economic benefits that might be obtained as a result of the improved delirium outcomes<sup>19</sup>. Ultimately, the findings of this study justify a high rating that Dexmedetomidine over Quetiapine is a better and safer alternative in the management of delirium in the patients within the ICU.

Since the delirium-resolution time of Dexmedetomidine is shorter, its safety profile is better, and its effects on the duration of stay in the ICU and the hospital are amazing, its application in patients within the critical care environment is more desirable. The results indicate that Dexmedetomidine is to be considered as an initial treatment intervention of ICU delirium, especially in patients with a modicum to more severe illness.

## CONCLUSION

This research indicates that dexmedetomidine is safer and effective as compared to quetiapine in the treatment of delirium in the ICU. More to the point, patients receiving dexmedetomidine had a shorter time of delirium and better outcomes, such as a shorter duration of ICU and hospital stays, mortality, etc., compared to quetiapine. In addition, dexmedetomidine patients had a higher number of delirium-free days and a reduced number of adverse events, especially the risk of sedation and hypotension, which were eminent in these patients in critical condition.

### Author's Contribution:

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# Technological Optimization of Sintering Protocols for Dental Zirconia: In Vitro Evaluation of Structural and Mechanical Properties

Optimization of Sintering for Dental Zirconia Properties

Khalid Dhafer Alhendi

## ABSTRACT

**Objective:** This study compared the effects of a conventional sintering cycle (1500°C for 2h, total 11h) versus a short cycle (1500°C for 2h, total 4h) on the properties of a commercial 3Y-TZP (Upscera YZ HT).

**Study Design:** Experimental analytical study

**Place and Duration of Study:** This study was conducted at the Department of Prosthetic Dental Science, College of Dentistry, University of Najran, Saudi Arabia, from 1<sup>st</sup> June 2025 to 30<sup>th</sup> November 2025.

**Methods:** Thirty bar-shaped specimens (n=15/group) were milled, sintered, and analyzed for flexural strength, grain size, and monoclinic phase content. Intergroup comparisons were performed using Student's t-test and Mann-Whitney U-test. A p-value < 0.05 was considered statistically significant.

**Results:** Results showed no statistically significant differences between groups: flexural strength (Conventional: 1309.42 ± 329.88 MPa; Short: 1187.23 ± 326.35 MPa; p=0.208), monoclinic phase content (Conventional: 47.92 ± 12.18%; Short: 50.42 ± 8.03%; p=0.84), or grain size (Conventional: 0.64 ± 0.18 µm; Short: 0.46 ± 0.08 µm; p=0.11). Correlations among these parameters were also non-significant.

**Conclusion:** Within the study's limitations, the short sintering cycle yielded zirconia with comparable mechanical properties, phase stability, and a clinically acceptable microstructure to the conventional cycle. This supports accelerated sintering as a viable, technology-driven, time-efficient alternative for dental laboratories without compromising core material performance.

**Key Words:** Yttria-stabilized zirconia, Dental ceramics, Sintering protocols, Flexural strength, Digital dental technology

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## INTRODUCTION

Tooth loss, predominantly resulting from dental caries and periodontal disease, continues to represent a significant global oral health concern.<sup>1</sup> Although the incidence of complete edentulism is gradually declining, the prevalence of partial edentulism is on the rise.<sup>2,3</sup> Conventional treatment modalities encompass removable partial dentures, fixed dental prostheses (FDPs), and implant-supported crowns.<sup>4</sup>

Among the various FDP options, metal-ceramic restorations, characterized by a durable metallic

framework overlaid with a veneering ceramic layer, have long been favored due to their mechanical strength and resistance to fracture.<sup>5,6</sup> Nevertheless, longitudinal clinical data indicate that veneering ceramic fractures occur in approximately 5–10% of cases within ten years.<sup>7</sup> Increasing patient demands for superior esthetics, heightened awareness of potential hypersensitivity to metal components, and the preference for more conservative tooth preparations have collectively driven the development and adoption of metal-free alternatives, thereby contributing to the growing popularity of all-ceramic restorations.<sup>8,9</sup>

Dental ceramics are generally classified into three main categories: glassy ceramics (e.g., feldspathic porcelain), reinforced glass-ceramics, and polycrystalline ceramics.<sup>10</sup> While glass-ceramics offer excellent aesthetics, their limited strength led to the development of high-strength oxides like alumina and zirconia.<sup>11,12</sup> Among these, 3Y-TZP zirconia is widely used for frameworks due to its superior mechanical properties, biocompatibility, and chemical stability.<sup>13</sup>

Pure zirconia exhibits polymorphism, existing in monoclinic (room temperature to 1170°C), tetragonal (1170°C to 2370°C), and cubic (>2370°C) phases.<sup>14</sup>

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The incorporation of 3–6 mol% yttria ( $Y_2O_3$ ) stabilizes the tetragonal phase at room temperature in a metastable form.<sup>15</sup> This metastability enables transformation toughening—a stress-induced phase shift from tetragonal to monoclinic, accompanied by ~4% volumetric expansion.<sup>15</sup> The resulting compressive stresses around cracks inhibit their propagation,<sup>16</sup> enhancing fracture toughness (5–10 MPa√m) and flexural strength (800–1300 MPa). These properties support the use of Y-TZP in crowns, FDPs, implant abutments, and endodontic posts.<sup>11,15</sup>

The sintering densifies a ceramic compact, critical for zirconia frameworks, as it transforms the porous, granular structure into a dense, cohesive polycrystalline solid.<sup>17</sup> Conventional sintering typically involves prolonged heating (8–12 hours) between 1350°C and 1550°C.<sup>18</sup> The sintering parameters such as temperature, duration, and heating/cooling rates profoundly influence the final microstructure (grain size, density) and phase composition of Y-TZP, directly dictating its mechanical performance and long-term stability.<sup>19</sup> Grain size is particularly crucial as larger grains facilitate the stress-induced t→m transformation, enhancing toughness, but grains exceeding a critical size can undergo spontaneous transformation during aging (e.g., in the oral environment), potentially leading to low-temperature degradation (LTD) characterized by surface roughening, microcracking, and strength reduction.<sup>20</sup>

While Y-TZP offers significant advantages, optimizing its processing, particularly sintering, is essential for maximizing clinical performance, cost-effectiveness, and sustainability. The use of accelerated sintering technology, characterized by shortened heating durations and precise thermal control, offers significant workflow and energy-efficiency benefits, especially in digital dentistry and same-day fabrication protocols. However, concerns remain regarding the potential compromise of mechanical properties and phase stability. Despite the widespread adoption of CAD/CAM systems and high-temperature furnaces in dental technology, studies evaluating the effect of short-cycle sintering protocols as a technological innovation are limited and sometimes contradictory.<sup>20</sup> Understanding these relationships is crucial for integrating advanced processing technologies into routine clinical and laboratory workflows.

Therefore, this study aimed to evaluate and compare the effect of a conventional sintering cycle versus a commercially available short sintering cycle on the flexural strength, grain size, and phase transformation behavior of a widely used yttria-stabilized presintered zirconia (3Y-TZP). The null hypothesis was that the sintering cycle (short vs. conventional) would have no significant effect on the flexural strength, grain size, or monoclinic phase content of the material.

## METHODS

This study was conducted at the Department of Prosthetic Dental Science, College of Dentistry, University of Najran, Saudi Arabia, from 1st June 2025 to 30th November 2025. Zirconia blanks (Upcera YZ HT; Shenzhen Upcera Dental Technology, China) were used for specimen fabrication. The blanks primarily comprised zirconium oxide (94–96 wt%), yttrium oxide (4–6 wt%), and trace amounts of hafnium oxide (1–2 wt%), alumina, and silica (0–0.1 wt%). 30 bar-shaped specimens (25 mm × 4 mm × 1.2 mm) were designed using CAD software and milled from the pre-sintered zirconia blanks using a CAD/CAM system (MC XL, Dentsply Sirona, Germany). The STL file was digitally transferred to the milling unit, illustrating the integration of computer-aided design and manufacturing (CAD/CAM) technology in dental prosthesis production. All specimens were randomly divided into two groups (n = 15 per group) based on sintering protocol: Group 1 (Conventional sintering): 1500 °C for 2 h with a total cycle time of 11 h. Group 2 (Short sintering): 1500 °C for 2 h with a total cycle time of 4 h.

**Sintering:** Sintering was performed in a high-temperature furnace according to the protocols specified. In both groups, the heating rate was 10 °C/min, with a holding temperature of 1500 °C. Samples were cooled to room temperature at a controlled rate.

**Flexural Strength Testing:** All specimens were subjected to three-point bending tests using a universal testing machine (model no. 3369, Instron, Canton, MI, USA) at a crosshead speed of 1 mm/min. The flexural strength ( $\sigma$ ) was calculated using the formula:

$$\sigma = 3NI/2bd^2$$

where N is the fracture load (N), I is the span length (mm), b is the width (mm), and d is the thickness (mm) of the specimen.

**Grain Size Analysis:** Five samples from each group were polished up to 1 μm using diamond suspension, ultrasonically cleaned in isopropanol, and sputter-coated with gold. Surface morphology and grain size were examined using a Scanning Electron Microscope (JEOL JSM-6490, Tokyo, Japan) at 30,000× magnification.

**Phase Transformation Analysis:** X-ray diffraction (XRD) analysis was conducted on five samples per group using Ultima IV diffractometer (Rigaku Corporation, Tokyo, Japan) with Ni-filtered  $CuK\alpha$  radiation. Scans were performed in the 2θ range of 25°–35° with a step size of 0.01° at 0.5 s/step. The monoclinic phase fraction ( $X_m$ ) was calculated using the Garvie and Nicholson formula:

**Statistical Analysis:** Data were analyzed using SPSS version 11.0. Descriptive statistics (mean ± standard deviation) were calculated for all variables. Intergroup

comparisons were performed using Student’s t-test or Mann–Whitney U-test, depending on data normality. A p-value < 0.05 was considered statistically significant.

**RESULTS**

The table presents the flexural strength of the study groups subjected to different sintering cycles. Group I exhibited a higher mean flexural strength (1309.42 ± 329.88 MPa) compared to Group II, which had a mean of 1187.23 ± 326.35 MPa. Although Group I showed a higher median value (1382 MPa) and narrower range, the statistical comparison between the two groups yielded a non-significant p-value of 0.208. The 95% confidence interval for the mean difference ranged from 1125.6 – 1372.1 MPa, suggesting overlapping variability.

The mean phase transformation percentages for the study groups are summarized in Table 2. Group I exhibited a mean of 47.92 ± 12.18%, while Group II

(conventional sintering) demonstrated a mean of 50.42 ± 8.03%. Median values remained comparable, and the minimum and maximum values in both groups showed overlapping distributions. The 95% confidence interval for the comparison (32.85–59.76%) reflects the observed variability. Statistical analysis showed no significant difference between the groups (p = 0.84).

Table 3 and Figure 1 illustrate the comparison of grain size (µm) between zirconia samples subjected to short and conventional sintering protocols. Group I (short sintering) demonstrated a lower mean grain size (0.46 ± 0.08 µm) in contrast to Group II (conventional sintering), which exhibited a mean of 0.64 ± 0.18 µm. The medians and observed range further support the trend of finer-grain structure in the short sintering group. The 95% confidence interval for the comparison (0.36–0.87 µm) indicates partial overlap between the two groups. The statistical comparison showed a non-significant difference (p = 0.11).

**Table No. 1: Comparison of Flexural Strength (MPa) Between Short and Conventional Sintering Cycles of Zirconia Cores**

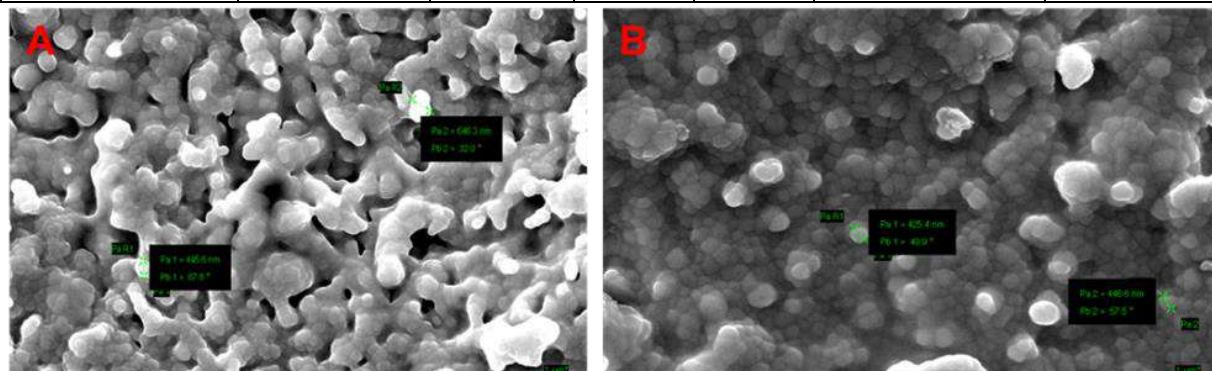
Group	Mean (MPa) ± SD	Median (MPa)	Min	Max	95% CI (Lower–Upper)	Significance
Group I	1309.42 ± 329.88	1382	822	1956		0.208 (Non-significant)
Group II	1187.23 ± 326.35	1094	912	2280		
Group I vs II					1125.6 – 1372.1	

**Table No. 2: Comparison of Phase Transformation Percentages Between Short and Conventional Sintering Cycles of Zirconia Cores**

Group	Mean ± SD (%)	Median (%)	Min (%)	Max (%)	95% CI (Lower–Upper)	Significance
Group I	47.92 ± 12.18	49.1	31.24	60.85		0.84 (Non-significant)
Group II	50.42 ± 8.03	50.31	38.22	60.12		
Group I vs II					32.85 – 59.76	

**Table No. 3: Comparison of Grain Size (µm) Between Zirconia Cores Subjected to Short and Conventional Sintering Cycles**

Group	Mean ± SD (µm)	Median (µm)	Min (µm)	Max (µm)	95% CI (Lower–Upper)	Significance
Group I	0.46 ± 0.08	0.45	0.37	0.58		0.11 (Non-significant)
Group II	0.64 ± 0.18	0.66	0.43	0.85		
Group I vs II					0.36 – 0.87	



**Figure No. 1: SEM images (30,000× magnification) showing the surface microstructure of zirconia specimens: (A) Specimen from the short sintering group showing comparatively smaller and regular grain morphology, while (B) shows a specimen from the conventional sintering group with larger and more irregular grain morphology**

**Table No. 4: Spearman's correlation coefficients ( $\rho$ ) and significance levels for relationships among flexural strength, grain size (SEM), and monoclinic phase fraction (XRD) in zirconia cores subjected to short and conventional sintering cycles**

Group	Comparison	$\rho$ (rho)	p-value	Significance
<b>Group I (Short Sintering)</b>	SEM vs Flexural Strength	0.382	0.269	Non-significant
	SEM vs XRD	-0.231	0.502	Non-significant
	Flexural Strength vs XRD	-0.041	0.912	Non-significant
<b>Group II (Conventional Sintering)</b>	SEM vs Flexural Strength	0.096	0.882	Non-significant
	SEM vs XRD	-0.387	0.518	Non-significant
	Flexural Strength vs XRD	0.057	0.927	Non-significant

Table 4 summarizes Spearman's correlation analysis. No statistically significant associations were found between flexural strength, grain size, and monoclinic phase content in either sintering group. In Group I (short sintering), a weak positive correlation was observed between SEM and flexural strength ( $\rho = 0.382$ ), while a weak negative correlation existed between SEM and XRD ( $\rho = -0.231$ ). Similarly, Group II (conventional sintering) demonstrated negligible to weak correlations, with the most notable being a weak negative correlation between SEM and XRD ( $\rho = -0.387$ ). However, all p-values remained  $> 0.05$ , confirming the absence of statistically significant relationships.

## DISCUSSION

Sintering critically influences the mechanical properties of yttria-stabilized tetragonal zirconia polycrystals (3Y-TZP), the most commonly employed form of dental zirconia.<sup>10,17</sup> While manufacturing, design, and milling also affect its properties, the sintering temperature and duration directly impact the grain size and phase composition.<sup>19</sup> Kulyk et al. investigated the effects of sintering temperature and yttria content on 3Y- and 6Y-doped zirconia ceramics, reporting that specimens sintered at 1550 °C for 2 h exhibited significantly larger average grain sizes ( $\sim 0.5\text{--}0.8\ \mu\text{m}$ ) and higher flexural strengths ( $\sim 1600\ \text{MPa}$ ), compared to those sintered at 1450 °C with finer grains ( $\sim 0.1\text{--}0.4\ \mu\text{m}$ ) and lower strength ( $\sim 1080\ \text{MPa}$ ),<sup>21</sup> while Casellas et al. reported reduced grain size correlating with increased flexural strength; though both effects were statistically insignificant. Optimizing the sintering cycle is therefore essential for enhancing YTZP's performance.<sup>22</sup>

Phase transformation behavior was assessed using XRD, revealing no significant difference in monoclinic phase content between the two groups ( $p = 0.854$ ). While controlled tetragonal-to-monoclinic transformation remains a critical mechanism for enhancing fracture toughness, commonly referred to as transformation toughening, excessive transformation can compromise long-term integrity. Recent study affirms that the stress-induced  $t \rightarrow m$  transformation, with its associated  $\sim 4\text{--}5\%$  volumetric expansion, generates compressive stresses around crack tips that impede crack propagation, thereby increasing

toughness.<sup>23</sup> The comparable monoclinic content observed in this study suggests that both sintering protocols preserved phase stability in 3Y-TZP zirconia without risking detrimental over-transformation

Grain size analysis indicated a marginally larger grain size in the conventional sintering group ( $0.64\ \mu\text{m}$ ) relative to the short-sintering group ( $0.46\ \mu\text{m}$ ), with the difference lacking statistical significance ( $p = 0.11$ ). This trend aligns with recent findings by Shahmiri et al., who demonstrated that prolonged sintering durations and higher temperatures in 3Y-TZP lead to increased grain growth, while careful control of thermal profiles maintains microstructures within optimal size ranges ( $< 0.8\ \mu\text{m}$ ) conducive to phase stability and mechanical integrity.<sup>24</sup> Both groups remained well within the clinically acceptable grain size threshold as defined by ISO 13356:2008, and below the critical limit ( $\sim 1\ \mu\text{m}$ ) where transformation-induced degradation becomes more likely. Additionally, another study reported average grain sizes around  $0.65\ \mu\text{m}$  for specimens sintered at 1475 °C for 2 h, consistent with the dimensions observed in our study, further confirming the adequacy of our sintering protocols.<sup>25</sup>

The short sintering cycle employed a rapid heating rate ( $70^\circ\text{C}/\text{min}$ ) without dwell time and reached the sintering temperature of 1500 °C in about 4 hours and 20 minutes, whereas the conventional cycle required approximately 11 hours with a total 4-hour dwell time. Despite these differences, mechanical and microstructural outcomes were comparable. A study by Luo and Pan shows that fast heating quickly surpasses the temperature range where surface diffusion dominates. Consequently, densification proceeds via grain boundary diffusion much earlier, contributing to efficient neck formation and densification.<sup>26</sup> While Pan et al., reported that even minimal dwell time could yield favorable outcomes, corroborating the present findings that effective sintering is possible without prolonged thermal holding.<sup>27</sup> These findings reinforce the potential of advanced sintering furnaces equipped with precise thermal control to optimize processing protocols in dental technology applications.

Cooling time was standardized to 4 hours in both groups, ensuring gradual thermal reduction and minimizing the risk of residual stresses. The previous studies emphasized the importance of controlled

cooling in preserving strength and reducing crack propagation in zirconia, highlighting the relevance of uniform cooling in the present methodology.<sup>17,19</sup>

Although the correlation between flexural strength, phase transformation, and grain size was statistically non-significant, some trends were noted. The group subjected to short sintering exhibited slightly higher flexural strength, accompanied by reduced grain size and lower monoclinic phase content. These trends align with a study reporting that flexural strength initially rises with grain size before declining beyond a critical limit.<sup>28</sup>

This in vitro study did not evaluate long-term aging, bonding behavior, or clinical performance. Only flexural strength was assessed, excluding other key mechanical properties like fracture toughness or fatigue resistance. Findings are limited to one zirconia type and sintering furnace. Future studies should incorporate thermocycling, aging protocols, and more comprehensive mechanical tests. Evaluating different zirconia grades and their bonding potential after varied sintering cycles is recommended. Clinical trials and cost-benefit analyses will help validate the practicality of rapid sintering protocols.

### CONCLUSION

Within the limitations of this in vitro study, it can be concluded that the short sintering cycles produced yttria-stabilized zirconia with mechanical and microstructural properties comparable to conventional sintering. Hence the null hypothesis is accepted. However, time-efficient sintering protocols can be adopted in clinical and laboratory workflows without compromising material performance. As zirconia continues to play a central role in restorative dentistry, optimizing processing parameters through the use of digital design and controlled thermal technologies can significantly enhance productivity, cost-effectiveness, and consistency in laboratory workflows.

**Author Contributions:** The author solely conceived, designed, and conducted the study; collected, analyzed, and interpreted the data; and wrote and approved the final manuscript.

**Ethical Approval:** This study was based on the synthesis and testing of dental materials; ethical approval was not required in accordance with institutional and national research ethics guidelines.

**Author’s Contribution:**

Concept & Design or acquisition of analysis or interpretation of data:	Khalid Dhafer Alhendi
Drafting or Revising Critically:	Khalid Dhafer Alhendi
Final Approval of version:	The above author
Agreement to accountable for all aspects of work:	The above author

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# Outcome of Intra-Articular Triamcinolone Acetonide Injection in Osteoarthritis of Knee

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Intra-Articular  
Triamcinolone  
Acetonide  
Injection in  
Osteoarthritis of  
Knee

## ABSTRACT

**Objective:** To evaluate the outcome of intra-articular triamcinolone acetonide injection in patients with osteoarthritis of the knee.

**Study Design:** Cross-sectional analytical study

**Place and Duration of Study:** This study was conducted at the Department of Orthopedic surgery, National Hospital & Medical Center Lahore from 15<sup>th</sup> October 2025 till 15<sup>th</sup> of January 2026.

**Methods:** This cross-sectional analytical study, including 85 patients with clinically and radiologically diagnosed knee osteoarthritis. All patients received intra-articular triamcinolone acetonide injection. Pain and functional outcomes were assessed using the Visual Analog Scale (VAS) and WOMAC score before and after treatment.

**Results:** The mean age was  $58.6 \pm 9.4$  years, with a female predominance (57.6%). The mean BMI was  $28.9 \pm 3.8$  kg/m<sup>2</sup>. Significant improvement was observed in pain and function, with VAS scores decreasing from  $7.8 \pm 1.2$  to  $3.9 \pm 1.5$  and total WOMAC scores improving from  $63.3 \pm 11.4$  to  $35.5 \pm 10.2$  ( $p < 0.001$ ). Overall, 72.9% of patients showed good to excellent improvement. Better outcomes were associated with younger age, lower BMI, shorter symptom duration, and less severe disease.

**Conclusion:** Intra-articular triamcinolone acetonide injection provides significant short-term pain relief and functional improvement in knee osteoarthritis. It is an effective treatment option, particularly in patients with early-stage disease and fewer risk factors.

**Key Words:** Osteoarthritis knee; Triamcinolone acetonide; Intra-articular injection; VAS; WOMAC; Pain relief

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## INTRODUCTION

Knee osteoarthritis is one of the most common degenerative joint diseases and is a major cause of chronic pain, stiffness, and loss of functional ability in adults in the global population<sup>1</sup>. It is defined by the progressive erosion of cartilage, subchondral bone, development of osteophytes and differing levels of synovial inflammation, which eventually result in the loss of mobility and quality of life<sup>2</sup>. Knee osteoarthritis keeps on increasing its burden because of the prolonged life span, sedentary living and escalating cases of obesity<sup>3</sup>. There is no clear-cut curative treatment of knee osteoarthritis thus the treatment is mostly symptomatic<sup>4</sup>.

The common treatment methods are lifestyle change, weight loss, physiotherapy, and medications like nonsteroidal anti-inflammatory drugs<sup>5</sup>. Nevertheless, numerous patients get poor improvements using conservative treatments, which requires them to take intra-articular interventions<sup>6</sup>. The reason behind the use of intra-articular corticosteroid injections is the fact that they act quickly and are effective in alleviating pain associated with inflammation<sup>7</sup>. One of the corticosteroids that are mostly used in this case due to its strong anti-inflammatory effects and rather long-lasting intra-articular action is triamcinolone acetonide<sup>8</sup>. Its mechanism of action is by inhibiting the inflammatory mediators in the joint and subduing synovitis and enhancing the joint functioning<sup>9</sup>.

A number of studies have also revealed that intra-articular injections of triamcinolone can bring a lot of short-term pain and functional improvement especially in patients with moderate to severe symptoms<sup>10</sup>. But the length of benefit is not fixed and there is some data to indicate that repeated use could be linked to structural alterations including cartilage loss which causes concern over long term safety<sup>11</sup>. Although these issues have been raised, corticosteroid injections are still a valuable treatment tool, particularly in patients who need to have a temporary symptom relief to continue with their daily functions and be able to take part in the rehabilitation process<sup>12</sup>. The effect of intra-articular

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corticosteroid injections can be different with regard to the severity of the disease, starting levels of pain, and personal characteristics of the patient<sup>13</sup>. As such, assessments of treatment outcomes among certain groups of people are necessary to maximize clinical decision-making<sup>14</sup>. In Pakistan, there is little local information on the efficacy of intra-articular injection of triamcinolone acetonide in knee osteoarthritis<sup>15</sup>. Creating this evidence is significant to inform practice in resource-constrained environments<sup>16</sup>.

Therefore, the purpose of the study is to determine the result of intra-articular triamcinolone acetonide injection as a treatment to patients with knee osteoarthritis.

**METHODS**

This was an analytical cross-sectional study conducted at Department of Orthopedic surgery, National Hospital & Medical Center Lahore from 15<sup>th</sup> October 2025 till 15<sup>th</sup> of January 2026, including 85 patients diagnosed with osteoarthritis of the knee.

**Inclusion Criteria**

- Patients aged  $\geq 40$  years diagnosed with osteoarthritis of the knee
- Patients with symptomatic knee pain not adequately controlled with conservative therapy
- Patients willing to receive intra-articular triamcinolone acetonide injection
- Patients providing informed consent

**Exclusion Criteria**

- Patients with inflammatory arthritis (e.g., rheumatoid arthritis, gout)
- Patients with septic arthritis or active joint infection
- Patients with recent knee trauma or surgery
- Patients with contraindications to corticosteroid injection
- Patients with incomplete follow-up data

**Data Collection:** After institutional approval, data were collected using a structured proforma. Baseline variables included age, gender, body mass index, duration of symptoms, and severity of osteoarthritis based on clinical and radiographic findings. All patients received a standardized intra-articular injection of triamcinolone acetonide under aseptic conditions. Outcomes were assessed using pain and functional scores such as the Visual Analog Scale (VAS) for pain and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) at baseline and follow-

up visits. Any adverse effects following injection were also recorded.

**Statistical Analysis:** Data were entered and analyzed using SPSS version 26.0. Continuous variables were expressed as mean  $\pm$  standard deviation, while categorical variables were presented as frequency and percentage. Pre- and post-injection outcomes were compared using paired t-test. A p-value of  $\leq 0.05$  was considered statistically significant.

**RESULTS**

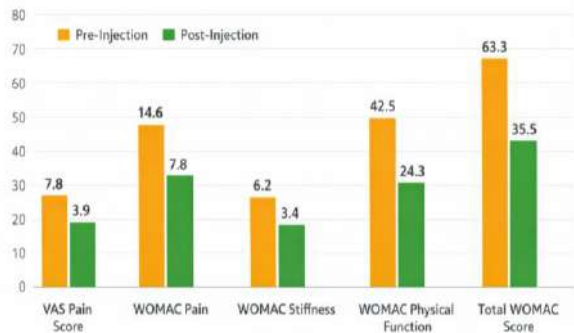
The baseline characteristics show that most patients were older adults, with a mean age of  $58.6 \pm 9.4$  years, and a higher proportion above 60 years (41.2%), which aligns with the typical age distribution of knee osteoarthritis. Females were slightly more affected (57.6%), reflecting the known higher prevalence in women. The mean BMI was  $28.9 \pm 3.8$  kg/m<sup>2</sup>, with the majority being overweight or obese, highlighting obesity as a key contributing factor. Most patients had symptoms for more than one year, and a large proportion had moderate to severe disease (Grade III–IV), indicating that the study population largely consisted of patients with established and clinically significant osteoarthritis.

**Table No. 1. Baseline Demographic and Clinical Characteristics of Patients (N = 85)**

Variable	Category	Overall (N=85)
Age (years)	Mean $\pm$ SD	58.6 $\pm$ 9.4
Age Group	40–50 years	18 (21.2%)
	51–60 years	32 (37.6%)
	>60 years	35 (41.2%)
Gender	Male	36 (42.4%)
	Female	49 (57.6%)
BMI (kg/m <sup>2</sup> )	Mean $\pm$ SD	28.9 $\pm$ 3.8
BMI Category	Normal	14 (16.5%)
	Overweight	38 (44.7%)
	Obese	33 (38.8%)
Duration of Symptoms	<1 year	26 (30.6%)
	1–3 years	34 (40.0%)
	>3 years	25 (29.4%)
Kellgren-Lawrence Grade	Grade II	28 (32.9%)
	Grade III	36 (42.4%)
	Grade IV	21 (24.7%)

**Table No. 2. Pre- and Post-Injection Pain and Functional Outcomes (N = 85)**

Variable	Pre-Injection Mean $\pm$ SD	Post-Injection Mean $\pm$ SD	Mean Difference	p-value
VAS Pain Score	7.8 $\pm$ 1.2	3.9 $\pm$ 1.5	-3.9 $\pm$ 1.3	<0.001
WOMAC Pain	14.6 $\pm$ 3.2	7.8 $\pm$ 2.9	-6.8 $\pm$ 2.4	<0.001
WOMAC Stiffness	6.2 $\pm$ 1.8	3.4 $\pm$ 1.5	-2.8 $\pm$ 1.2	<0.001
WOMAC Physical Function	42.5 $\pm$ 8.6	24.3 $\pm$ 7.9	-18.2 $\pm$ 6.7	<0.001
Total WOMAC Score	63.3 $\pm$ 11.4	35.5 $\pm$ 10.2	-27.8 $\pm$ 9.1	<0.001



**Figure No.1: Comparison of Pre- and Post-Injection Pain and Functional Outcomes in Patients with Knee Osteoarthritis (N = 85)**

The mean VAS score significantly reduced from  $7.8 \pm 1.2$  to  $3.9 \pm 1.5$ , indicating substantial pain relief. Similarly, all WOMAC domains showed significant improvement, with total WOMAC scores decreasing from  $63.3 \pm 11.4$  to  $35.5 \pm 10.2$  ( $p < 0.001$ ). The largest improvement was observed in physical function, reflecting enhanced mobility and daily activity. Outcome stratification revealed that the majority of patients achieved favorable results, with 32.9%

showing excellent and 40.0% showing good improvement. Only a small proportion had moderate (17.6%) or poor (9.4%) outcomes, indicating that most patients benefited significantly from the intervention.

**Table No.3: Outcome Stratification Based on Clinical Improvement (N = 85)**

Outcome Category	Criteria (VAS Reduction)	n (%)
Excellent	$\geq 70\%$ reduction	28 (32.9%)
Good	40–69% reduction	34 (40.0%)
Moderate	20–39% reduction	15 (17.6%)
Poor	$< 20\%$ reduction	8 (9.4%)

Clinical factors such as younger age, lower BMI, shorter duration of symptoms, and less severe radiographic disease were associated with better outcomes. Patients with good or excellent outcomes had a lower mean age ( $57.1 \pm 8.9$  vs  $62.3 \pm 10.1$  years) and BMI ( $28.1 \pm 3.5$  vs  $30.7 \pm 4.1$  kg/m<sup>2</sup>). Higher disease severity (KL Grade III–IV) and longer symptom duration were more common in those with poorer outcomes.

**Table No. 4. Association of Clinical Factors with Treatment Outcome (N = 85)**

Variable	Good/Excellent Outcome (n=62)	Moderate/Poor Outcome (n=23)	p-value
Age (years) Mean $\pm$ SD	$57.1 \pm 8.9$	$62.3 \pm 10.1$	0.021
Gender (Female)	34 (54.8%)	15 (65.2%)	0.381
BMI (kg/m <sup>2</sup> ) Mean $\pm$ SD	$28.1 \pm 3.5$	$30.7 \pm 4.1$	0.008
KL Grade III–IV	38 (61.3%)	19 (82.6%)	0.049
Duration >3 years	14 (22.6%)	11 (47.8%)	0.018
Baseline VAS	$7.6 \pm 1.1$	$8.2 \pm 1.3$	0.047

**DISCUSSION**

This paper assessed the efficacy of intra-articular injection of triamcinolone acetonide on patients with knee osteoarthritis and reported a significant reduction in the level of pain and functional status. The population of the study was primarily older adults with a mean age of  $58.6 \pm 9.4$  years with a greater percentage of females, which is aligned to the known epidemiology of osteoarthritis. Mechanical and metabolic factors in the development of the disease are also emphasized by the high prevalence of overweight and obesity (mean BMI  $28.9 \pm 3.8$  kg/m<sup>2</sup>). The same tendencies have been mentioned in the past studies, with age, female gender, and BMI increasing among the significant factors of knee osteoarthritis<sup>17,18</sup>. The major conclusion of the research was that there was a substantial post-injection of triamcinolone reduction of pain and enhancement of function. The VAS score decreased from  $7.8 \pm 1.2$  to  $3.9 \pm 1.5$ , while the total WOMAC score improved from  $63.3 \pm 11.4$  to  $35.5 \pm 10.2$  ( $p < 0.001$ ), indicating substantial symptomatic relief. All domains of WOMAC were reported to have

improved, especially physical functioning, indicating a better mobility and activity. These effects of pain and functional outcomes are similar to those of the intra-articular corticosteroid injections reported as having a consistent positive effect (particularly in the short-term) in previous studies<sup>19</sup>.

Outcome stratification was further used to support the efficacy of the intervention where most patients recorded good (40.0) or excellent (32.9) improvement. The percentage of moderate and poor results was also low, which means that intra-articular triamcinolone is useful to most patients. Similar results have been noted in other studies where a large percentage of patients have shown meaningful clinical improvement following corticosteroid injections. The analysis of related factors showed that the positive results were noted in younger patients, patients with lower BMI, shorter symptoms, and less intensive radiographic disease. Patients who had worse outcomes tended to be older and have advanced osteoarthritis, and had longer periods with the symptoms, indicating that severity of the disease determines response to treatment. These are similar to other studies done previously, which have demonstrated

that early disease and reduced mechanical load correlate with improved response to intra-articular treatments<sup>20</sup>. On the whole, the results of this research indicate that intra-articular triamcinolone acetonide injection is a well-founded short-term management tool that can be used to alleviate pain and improve the functionality of knee osteoarthritis. The findings correlate with the past studies and underline the role of the patient selection since the results can be different in case of the variation of the baseline features and the severity of the disease.

**CONCLUSION**

It is concluded that intra-articular triamcinolone acetonide injection is an effective treatment modality for providing significant short-term relief of pain and improvement in functional outcomes in patients with osteoarthritis of the knee. The majority of patients achieved good to excellent clinical improvement following the intervention.

**Author’s Contribution:**

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Drafting or Revising Critically:	Lavinia Kamla Lincoln, M.A Wajid
Final Approval of version:	All the above authors
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**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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**Ethical Approval:** No.NHMC/IRB/264 Dated 11.10.2025

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# The Khash khash Paradox: Mystery of Poppy Seed Consumption Can Trigger False-Positive Opiate Test

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## ABSTRACT

**Objective:** To identify the knowledge and perceptions of participants regarding the poppy seeds (khash-khash) consumption and false-positive opioid urine test results and related consequences

**Study Design:** Cross-sectional study

**Place and Duration of Study:** This study was carried out in major urban cities of Pakistan from August to November 2025.

**Methods:** A structured questionnaire based at Google Forms was shared with 384 participants. The questionnaire contained 3 sections that measured demographic variables, knowledge and perceptions of participants regarding poppy seed (khash-khash) consumption, false-positive opioid urine test and related consequences. Subsequently, twenty participants of different age groups were selected who regularly consumed poppy seeds provided urine samples at 6 and 24 hours after ingestion. Samples were first screened for opioids using enzyme immunoassay (EIA).

**Results:** The results demonstrate that (89.84%) participants reported widespread consumption of poppy seeds. Nonetheless, awareness of the risk of false-positive results was extremely low. A large proportion of the respondents (77.6%) lacked the awareness that poppy seeds lead to a false positive urine test for opioids. Moreover, 51.2% of the participants felt that the current urine drug tests are not adequate to distinguish between the use of opiates and consumption of poppy seeds. Majority of the participants were of the opinion that this false positive opioid drug can result in dismissal, emotional distress, legal actions and loss of reputation. All participants who regularly consumed poppy seeds were confirmed to have false positive urine test for opioids. Urine tests of opioids were detected to be false positive using enzyme immunoassay (EIA) with a cutoff of 300 ng/mL, and positive results were confirmed using LC-MS/MS.

**Conclusion:** This study confirms a significant awareness gap and validates poppy seed-induced false-positive opiate tests. Targeted education and policy-level improvements in drug testing protocols are urgently needed to ensure fairness and accuracy.

**Key Words:** Drug testing, Urine analysis, false positive, Khash-khash, Opioids, Pakistan, Perceptions, Poppy seed

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## INTRODUCTION

Poppy seeds, referred to as "khash khash" in Pakistan, have been utilized for centuries in both traditional

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medicine and culinary traditions.<sup>1</sup> The renowned Persian physician Avicenna, in his influential work "The Canon of Medicine," documented the medicinal properties of poppy seeds, in treating various conditions.<sup>2</sup> Opium poppy seeds have been used in the culinary applications of a variety of world cuisines. In Pakistan, they are used in many different types of food: in curry dishes, deserts and drinks, and are appreciated for their nutty flavor and overall cultural significance. Poppy seeds offer numerous benefits, including a rich nutritional profile. In fact, they are an excellent source of essential minerals, including calcium, iron, and zinc, as well as, dietary fiber and healthy fats.<sup>3</sup> Moreover, poppy seeds are associated with potential health benefits, such as improved digestion, enhanced bone health, and reduced inflammation.<sup>4</sup> They contain substantial amounts of minerals necessary for the human body, calcium, magnesium, potassium, and iron. As well as carbohydrates, vitamins (B and E), Trace

elements (selenium, copper) and also fiber and substances for flavor and fragrance.<sup>5</sup> Their versatility in culinary applications has made them a staple ingredient in many Pakistani households. Poppies (*Papaver somniferum*) represent a major industrial crop grown around the world (Turkey, China, India, Czech Republic, Pakistan), for food (oil-rich seeds) and for medicinal use chiefly because of opium since ancient times<sup>6</sup>. These seeds, whether raw or cooked, contain opiate alkaloids, primarily morphine and codeine, along with smaller amounts of thebaine, noscapine, and papaverine.<sup>7</sup>

Despite the fact the seeds do not inherently possess opiates, they can become contaminated with these substances during the harvesting process due to the presence of poppy latex.<sup>8</sup> In many social contexts, such as clinical diagnoses, forensic investigations, and employment screening, drug testing is essential. The accuracy of these tests is paramount, false positives can have adverse effects ranging from legal issues to job loss wrongful accusations to poor medical management.<sup>9-11</sup> This is especially important where certain dietary staples, such as poppy seeds, contain naturally occurring opiates that can trigger false positive results in drug screens.<sup>12</sup> The "khash khash paradox" is a phenomenon that poses a substantial challenge to the interpretation and reliability of drug test results in Pakistan. The present study was designed to investigate the knowledge and perceptions of participants regarding the poppy seeds (khash-khash) consumption and false-positive opioid urine test results and the possible consequences.

## METHODS

The present cross-sectional study was conducted nation-wide in Pakistan from August to November 2025. The survey targeted participants residing in the major Pakistani cities of Karachi, Lahore, and Islamabad. The data was collected using a structured

survey form consisting of eighteen questions, divided into three sections.

This study was reviewed and approved by the Institutional Review Board (IRB) of Bahria University Health Science Karachi (BUHSK) reference number (BUHS-IRB 176/25). The participants were briefed on the purpose of the research and provided consent form at the beginning of the survey. First section contained basic demographics, including gender, age group, current employment status, level of education, and questions regarding dietary behaviors, including consumption of poppy seed. However, section two included questions about poppy seed consumption and knowledge of false positive urine testing for opium due to poppy seed consumption. Section three included questions about consequences of a false positive result of an individual who had never used or been exposed to using drugs.

For the analysis of false positive opiate test, 20 participants (five from each group age) who ingested poppy seeds on daily basis were selected for urine analysis. These participants were used to obtain urine samples at two time points after 6 hours and 24 hours of poppy seeds ingestion. The initial screening was based on opiate screening by enzyme immunoassay (EIA) and was then confirmed by liquid chromatography and mass spectrometry (LC -MS/MS). The qualitative limit was <300 ng/mL above which the result was considered opiate positive in EIA screening.

Statistical analyses were done by using SPSS version 23. Descriptive data were described as frequencies and percentages. The chi-square test was applied with statistical significance  $p < 0.005$ .

## RESULTS

Table -1 shows the demographic data of 384 participants who completed the survey. The sample was predominantly female, at 80.6%, while the majority of participants were between 20 and 30 years of age, accounting for 86.3%, showing a young adult population.

**Table No.1: Demographic characteristics and poppy seed consumption of participants (n=384)**

S.no	Variables	Response type	Frequency (n)	Percentage(%)
1.	Gender	Female	282	80.6%
		Male	68	19.4%
2.	Age	20-30	302	86.3%
		31-40	18	5.1%
		41-50	24	6.9%
		51-60	9	1.7%
3.	Current Occupation	Student	264	75.4%
		Government/public sector employee	28	8%
		Healthcare professional	32	9.1%
		Support service worker	4	1.1%
		Employed	16	4.6%
		Home maker	2	0.6%
4.	Highest level of education	High school	142	41.3%

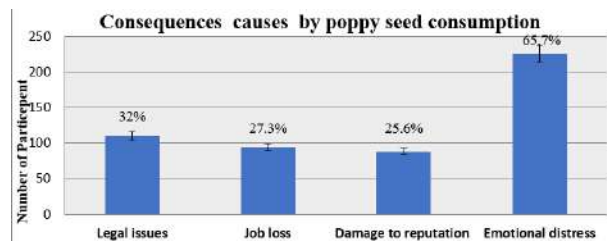
		Bachelors	138	40.1%
		Masters	28	6.4%
		Doctorate PHD	24	7%
		Vocational/technical	6	1.7%
		Others	12	3.5%
5.	Do you consume khash khash (poppy seed) in your diet	Yes	345	89.84%
		No	39	10.15%
6	Do you know that poppy seeds lead to a false positive result on a drug test due to opiates?	Yes	296	77.6%
		No	88	22.9%
	Total (n)		384	

**Table No.2: Participants’ Perceptions Regarding the Sufficiency of Current Urine Drug Testing Methods in Differentiating Poppy Seed Consumption from Illicit Opiate Use**

Response Category	Frequency (n)	Percentage (%)
Not sufficient	216	56.2
Moderately sufficient	148	43.0
Completely sufficient	10	2.9
Disagree	6	1.7
Strongly agree	4	1.2
Total	384	

Perceptions regarding forensic drug testing accuracy revealed substantial concern, with 56.2% of participants considering current urine drug tests insufficient to reliably distinguish poppy seed consumption from illicit opiate use, while only 2.9% perceived them as completely sufficient. Overall, findings demonstrate significant gaps in public awareness and confidence in existing drug testing practices.

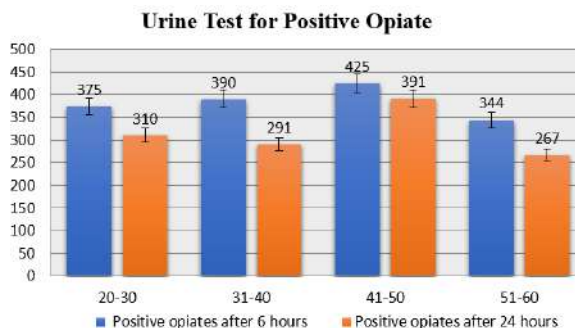
The final section of the questionnaire assessed participants’ opinions and concerns regarding false-positive opiate test results associated with poppy seed consumption. Most participants perceived poppy seed-related false-positive opiate results as a significant concern, with emotional distress, legal consequences, and employment-related risks identified as the most common adverse outcomes as shown in Figure-1.



**Figure No.1: Numerous Consequences causes by poppy seed consumption**

All specimens’ samples showed positive results by enzyme immunoassay (EIA) at 4- 6 hour time point

however opiates remained positive in samples after 20-24 hours. These results suggest that a realistic dose of poppy seeds in the diet may lead to the appearance of opiate in the urine up to 24 hours and may lead to false positive results in the standard urine drug screening tests.



**Figure No.2: Urine Test for Opiate Positive after 6 and 24 hours**

Figure-2 shows that dietary poppy seeds can produce false positive test and detected opiate levels after 6 and 24 hours. The results of the urine opiate concentrations after consuming poppy seeds showed a definite time-dependent decrease in all age groups (Figure 2). At 6 hours of ingestion, mean opiate levels exceeded the screening cutoff (300 ng/mL) across all the age groups. The participants aged 41-50 years had the highest concentrations. These results demonstrate that poppy seed consumption elevates urinary opiate concentrations above immunoassay thresholds for up to 24 hours, effectively invalidating the screening result as a sole indicator of illicit drug use.

## DISCUSSION

The increasing demand in food science and technology has been associated with the rising need of healthy products and food stuffs. Recent studies have highlighted previously overlooked ingredients, such as the poppy plant, which belongs to the Papaveraceae family<sup>13</sup>. The poppy plant, which is traditionally grown to produce oil and opium, has also provided the source of alkaloid that can be used in pharmaceutical

productions.<sup>14</sup> Poppy plant seeds have been used as very important culinary ingredients in savory and sweet dishes such as curry, baked goods, confectionaries, and dessert<sup>15</sup>

The study revealed a widespread lack of public awareness about the fact that consuming poppy seeds can cause false positive results in opiate drug tests, where most of the respondents were unaware of the so called khash khash paradox, despite poppy seeds being commonly part of public dietary practices. This lack of awareness highlights a serious public health and forensic issue, as poppy seeds contain morphine and codeine, which are easily excreted in urine after eating. Interestingly opiates concentrations in the urine may exceed the standard cut-off level of 300 ng/mL.<sup>16</sup> Subsequent intakes of poppy seeds could make it difficult to distinguish between the use of opiates (codeine, heroin, morphine) and the ingestion poppy seeds in the urine drug tests.<sup>17</sup> This is a serious issue and particularly when such results are misinterpreted in areas such as employment checks and forensic investigations where misinterpretation of such results may have some devastating social and legal implications to people in Pakistan.

The protein and oil content in poppy seeds exhibit considerable variation, with protein levels ranging from 21.5% to 23.5% and oil content from 46.2% to 49.4%. Poppy seed oil has a high content of polyunsaturated fatty acids and a relatively high level of  $\beta$ -tocopherol (239-450 mg/kg).<sup>18</sup>

In many areas of the society, drug testing is a crucial part of the employment process, forensic analysis, or even medical determination. High precision of such tests is critical as the false positive test can have extremely grave implications including false incrimination, loss of employment and wrong medical treatment.<sup>19</sup> This is especially relevant in those areas where some food products, e.g. poppy seeds, are known to have naturally occurring opiates that can lead to false positive results on drug.<sup>20</sup> The common presence of poppy seeds in Pakistani cuisine creates a significant hurdle for accurate drug testing. The data suggests that a more compassionate and accurate interpretation of these tests is necessary one that balances chemical evidence with an understanding of local food traditions to protect individuals from being wrongly accused. In particular, the fact that morphine and codeine can be further absorbed into poppy seeds despite the usual culinary treatment and can then be detected in biological samples, which, under the conditions of a highly-stigmatized society against drug misuse, is a legal offense, makes it particularly difficult to balance the socio-economic impact of a false-positive drug test result with the extra confirmatory testing or contextual evidence.<sup>21, 22</sup>

The current result confirms that the consumption of the poppy seed may lead to the production of urine opiate levels that are higher than the typical immunoassay screening cutoff, and especially during the first 6 hours of intake. Despite an overall decrease in concentrations

at 24 hours, some age groups still showed concentrations above the cutoff, as an illustration of inter-individual variation in metabolism and elimination. The greatest and most unremitting levels were found amongst the age group of 41-50 years, indicating the likelihood of being susceptible to prolonged presumptive positivity. Such findings affirm the limitations of immunoassay-based screening as a single method and the need to verify the outcome with highly specific analytical methods such as LC-MS/MS as a possible cause of false-positive opiate urine test results and must be considered when interpreting screening results.<sup>23</sup> False-positive opiate test is potentially devastating to a person breaking their hopes and self-esteem, not only influencing their family and social status.<sup>24,25</sup> Therefore, it is vital to comprehend the widespread nature of this problem and how the population is informed of the fact that the intake of poppy seeds may affect the results of a drug test, hence, the need to develop effective strategies related to the populations and advance the fairness of the legal regulations. It is also indicative of the need to develop improved, more precise testing regimens and clearer protocols on results interpretation, especially in those situations where experimentation on dietary aspects might be involved. Besides, it emphasizes the significance of teaching the masses and those who deal with drug testing in general, on the possibility of false positives and why they should be retested with a confirmatory test before coming up with life-altering decisions based on the initial results.

## CONCLUSION

This study reveals a critical lack of awareness among participants and confirms analytically that poppy seed consumption may yield false-positive opiate urine test results. Immediate implementation of targeted educational programs and policy-level refinement of drug testing practices is essential to ensure accurate interpretation and equitable outcomes.

### Author's Contribution:

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Drafting or Revising Critically:	Najmul Sahar Ilyas, Uzma Shabbir and Attique Hussain Khan.
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# Outcome of Vinegar Versus Normal Saline Dressing in Diabetic Foot Ulcers

Vinegar VS  
Normal Saline  
Dressing in  
Diabetic Foot  
Ulcers

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## ABSTRACT

**Objective:** To evaluate the outcome of vinegar compared to normal saline dressings in diabetic foot ulcers in terms of rate of negative cultures, change in necrotic material amount and change in wound surface area.

**Study Design:** Randomized clinical trial study

**Place and Duration of Study:** This study was conducted at the Department of Surgery, Jinnah Hospital Lahore from 1<sup>st</sup> July 2025 to 31<sup>st</sup> December 2025.

**Methods:** Eighty-four patients aged 20 to 65 years of both genders having Wagner grade 3 and 4 diabetic foot ulcers were randomized through lottery method for one of treatment. In Group A normal saline dressings were started in wet to dry manner and in Group B vinegar dressings were done in wet to dry manner. Data was entered in SPSS version 22.0 and analyzed. Frequency and percentages were calculated for qualitative variables like gender, rate of negative of wound cultures.

**Results:** The baseline characteristics of patients were comparable in both groups. The wound size was decrease lesser in normal saline group to  $2.76 \pm 0.83$  while in vinegar group it decreased to  $1.36 \pm 0.84$ . The necrotic wound size was decrease lesser in normal saline group to  $1.09 \pm 0.60$  while in vinegar group it decreased to  $0.25 \pm 0.35$ . The results were statistically significant ( $p < 0.001$ ). Almost 95% of infections got cleared by vinegar while 76% infections were cleared by normal saline at 8 weeks ( $p = 0.013$ ).

**Conclusion:** Vinegar dressing is better than normal saline dressing for diabetic foot ulcers for decreasing size of wound, necrotic material as well as infection rate.

**Key Words:** Vinegar dressing, Normal saline dressings, Diabetic foot ulcers

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## INTRODUCTION

The physical barrier for body to protect against bacterial invasion is the skin, which may be destroyed either surgically or by trauma, sometimes leading to sepsis and even death.<sup>1</sup> These wounds also have physical, nutritional, vocational, financial, psychological and social toll on the patient.<sup>2</sup> One type of these wounds are diabetic foot ulcers (DFU) which are a common, highly morbid consequence of longstanding and poorly managed diabetes and one of the most difficultly treated wounds. Of the estimated 537 million people worldwide who have diabetes, 19% to 34% will develop a DFU in their lifetime.<sup>3</sup>

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DFU is associated with numerous risk factors and has complex mechanisms and insignificant clinical manifestations. Its pathogenesis is roughly categorized into peripheral neuropathy, Peripheral arterial disease and infection.<sup>4</sup> Out of these, infection is treatable with adequate debridement, antibiotics and proper dressing. The different materials used in the past include honey, vinegar, wine and spider webs. Modern types of dressings made their appearance in the twentieth century which included gauze, lint and plasters.<sup>5</sup>

In a retrospective study comparing normal saline and vinegar therapy, the size of necrotic tissue in wound decreased by 4.1 cm by vinegar therapy while there was no change with normal saline dressings ( $p < 0.02$ ). The vinegar therapy was 70% effective in making culture negative as compared to 40% of saline therapy at end of 3 weeks ( $p < 0.001$ ). The mean decrease in size of wound was  $2.67 \pm 1.0$  cm in vinegar group as compared to  $1.55 \pm 0.4$  cm in saline group ( $p = 0.001$ ).<sup>6</sup> Another quasi-experimental study shows that there is significant reduction in stage of diabetic foot ulcer with use of vinegar.<sup>7</sup> In some older studies it has been shown to be effective against pseudomonas destroying later in 14 out of 16 patients with two weeks treatment.<sup>8</sup>

The rationale of this study is that healthy wound healing is a very important part of treating the patients

with DFU. It helps to reduce anxiety of patient, hospital stay, healthcare burden and other patient problems. There are a number of dressings available with variable effectiveness for DFU but are also costly. The two types of dressings under consideration are cheap, easily accessible and easy to use. Also despite the effectiveness, locally there is very less utilization of this kind of dressing. But keeping the need of the hour under consideration, the study is undertaken to compare the outcome of vinegar compared to normal saline dressings in diabetic foot ulcers in terms of rate of negative cultures, change in necrotic material amount, changes in wound surface area in our setting to evaluate technique that has better outcome and reduce morbidity for patients.

## METHODS

This randomized clinical research was conducted at the Department of Surgery, Jinnah Hospital, Lahore, Pakistan, from 1<sup>st</sup> July 2025 to 31<sup>st</sup> December 2025. The study was approved by College of Physicians and Surgeons vide letter number CPSP/REU/SGR-2022-055-14063 dated 1<sup>st</sup> January 2025 and also registered with clinical trials numbered NCT07373327. Eighty-four patients were selected by non-probability consecutive sampling. The sample size was calculated keeping confidence level at 95%, power of study at 80% and assuming the treatment efficacy by culture at end of 3<sup>rd</sup> week of saline and vinegar were 40% and 70% respectively.<sup>6</sup> The patients aged 20 to 65 years of both genders having Wagner grade 3 and 4 diabetic foot ulcers were included. The patients having Wagner grade 1, 2, 5 and 6 diabetic foot, received radiotherapy, chemotherapy, steroids, immunosuppressive drugs or any allergies to vinegar were excluded. Diabetic foot ulcers are ulcers which occur on feet of diabetic patients. They may occur on the sole or any of the toes of foot. They were classified according to Wagner classification in this study. Grade 3 and 4 included severe ulcer without and with bone involvement respectively. The outcome of the study was measured in terms of negative culture rate, change in amount of necrotic material as well as change in size of wound. The bacteriological cultures were assessed in coordination with pathology department. The swabs were taken weekly and reports recorded up till the wound was culture free. The change in amount of necrotic material, measured in centimeters was the non-viable tissue in wound; assessed using manual planimetry weekly up till complete healing. The change

in wound size in centimeters was measured by planimetry from start day of dressings. The wound outcomes were recorded on baseline, 1<sup>st</sup> to 8 weeks on weekly bases and then fortnightly till complete healing. Complete epithelialization of wound was considered as healing.

Informed consent was taken from each subject. Subjects were randomized through lottery method for one of treatment. In Group A normal saline dressings were started in wet to dry manner and in Group B vinegar dressings were done in wet to dry manner. The vinegar solution was prepared by adding a tablespoon of plain white vinegar to a cup of normal saline. Then, soak clean gauze with vinegar solution just so much that it's wet and not drips. The soaked gauze was applied to wound, and this was covered by dry gauzes. This process was done at least twice daily. The normal saline dressing was done by simply washing wound with normal saline and doing wet to dry saline-soaked dressing twice daily. The diabetic control was strictly observed. Data was entered in SPSS-22.0 and analyzed. The Chi-square test was applied for qualitative variables while student t-test was applied for quantitative variables. A  $p < 0.05$  was taken as statistical significance.

## RESULTS

The baseline characteristics of patients were comparable in both groups. These characteristics included age (years), gender and duration of disease, grades of diabetic ulcers, co-morbidities and presenting complaints (Table 1). The wound size was similar in both groups at baseline,  $6.14 \pm 0.83$  in vinegar and  $6.27 \pm 0.62$  ( $p = 0.420$ ). The necrotic wound size was, however,  $3.60 \pm 0.64$  in vinegar and  $3.76 \pm 0.58$  in normal saline group ( $p = 0.250$ ). The size of both these started to decrease from the first week onwards. The wound sizes were decrease lesser in normal saline group to  $2.76 \pm 0.83$  while in vinegar group it decreased to  $1.36 \pm 0.84$  ( $p < 0.001$ ). The necrotic wound sizes were decrease lesser in normal saline group to  $1.09 \pm 0.60$  while in vinegar group it decreased to  $0.25 \pm 0.35$  ( $p < 0.001$ ). The results were statistically significant (Table 2). Almost 95% of infections got cleared by vinegar while 76% infections were cleared by normal saline at 8 weeks ( $p = 0.013$ ) (Table 3). The decrease in wound size was more significant in 46 to 60 and more than 60 years of age ( $p < 0.001$ ) (Table 4). Figures 1 and 2 showed graphical representation of decrease in size of wound as well as infected wound size.

**Table No.1: Baseline characteristics of patients (n=84)**

Variable	Vinegar	Normal Saline	P value
Age (years)	$53.31 \pm 7.25$	$53.88 \pm 6.93$	0.713 <sup>(f)</sup>
<b>Gender</b>			
Male	23 (54.8%)	20 (47.6%)	0.513 <sup>(c)</sup>
Female	19 (45.2%)	22 (52.4%)	

Duration (weeks)	12.10±6.03	11.45±5.17	0.602 <sup>(t)</sup>
<b>Wagner Grade</b>			
Grade-3	30 (71.4%)	30 (71.4%)	-
Grade-4	12 (28.6%)	12 (28.6%)	
Hypertension	20 (47.6%)	19 (45.2%)	0.827 <sup>(c)</sup>
IHD	11 (26.2%)	6 (14.3%)	0.277 <sup>(c)</sup>
Debridment done	42 (100%)	42 (100%)	-
HbA1C	8.95±1.09	8.95±1.12	0.992 <sup>(t)</sup>
<b>Presenting Complaint</b>			
Discharge with fever	4(9.5%)	6(14.3%)	0.617 <sup>(c)</sup>
Foul smell and discharge	9(21.4%)	4(9.5%)	
Foul smell with necrotic tissue	11(26.2%)	10(23.8%)	
Pain and discharge	5(11.9%)	9(21.4%)	
Pain with difficulty walking	7(16.7%)	6(14.3%)	
Swelling & pain	6(14.3%)	7(16.7%)	

(t): Independent sample t-test, (c): Chi Square test

**Table No. 2: Infected wound necrotic size and wound size in groups (n=84)**

Week	Infected Wound Necrotic size (cm)			Wound Size		
	Vinegar	Normal Saline	p-value	Vinegar	Normal Saline	p-value
Baseline	3.60±0.64	3.76±0.58	0.250	6.14±0.83	6.27±0.62	0.420
1 <sup>st</sup>	2.89±0.52	3.48±0.61	<0.001*	5.38±0.72	5.86±0.71	0.003*
2 <sup>nd</sup>	1.89±0.56	3.03±0.58	<0.001*	4.29±0.77	5.18±0.68	<0.001*
3 <sup>rd</sup>	1.04±0.53	2.45±0.61	<0.001*	3.22±0.74	4.43±0.74	<0.001*
4 <sup>th</sup>	0.47±0.40	1.89±0.56	<0.001*	2.32±0.84	3.88±0.68	<0.001*
8 <sup>th</sup>	0.25±0.35	1.09±0.60	<0.001*	1.36±0.84	2.76±0.83	<0.001*

\*p-value <0.05

**Table No.3: Infection clearance in study groups**

Variable		Vinegar	Normal Saline	p-value
Infection Clearance	Baseline	-	-	-
	Week-1	4 (9.5%)	4 (9.5%)	-
	Week-2	21 (50%)	15 (35.7%)	0.186 <sup>(c)</sup>
	Week-3	32 (76.2%)	21 (50%)	0.013 <sup>(c)</sup>
	Week-4	34 (81%)	25 (59.5%)	0.032 <sup>(c)</sup>
	Week-8	40 (95.2%)	32 (76.2%)	0.013 <sup>(c)</sup>

(c): Chi Square test

**Table No.4: Comparison of Wound Size and Infected wound necrotic size at 8th week post treatment stratified for patient’s characteristics**

Variables	Group	Wound Size		p-value	Infected Wound Necrotic size		p-value
		N	Mean±SD		N	Mean±SD	
29-45 Years	Vinegar	7	1.68±1.15	0.499	7	0.51±0.44	0.026*
	Normal Saline	4	2.17±1.00		4	1.21±0.36	
46-60 Years	Vinegar	29	1.30±0.84	<0.001	29	0.19±0.31	<0.001*
	Normal Saline	33	2.72±0.75		33	1.04±0.60	
>60 Years	Vinegar	6	1.25±0.38	0.001	6	0.25±0.33	0.028*
	Normal Saline	5	3.42±0.96		5	1.33±0.74	
<b>Gender</b>							
Male	Vinegar	23	1.46±0.82	<0.001*	23	0.27±0.30	<0.001*
	Normal Saline	20	2.89±0.75		20	1.14±0.67	
Female	Vinegar	19	1.23±0.88	<0.001*	19	0.23±0.39	<0.001*
	Normal Saline	22	2.63±0.89		22	1.05±0.52	
	Normal Saline	24	2.79±0.99		24	1.21±0.67	

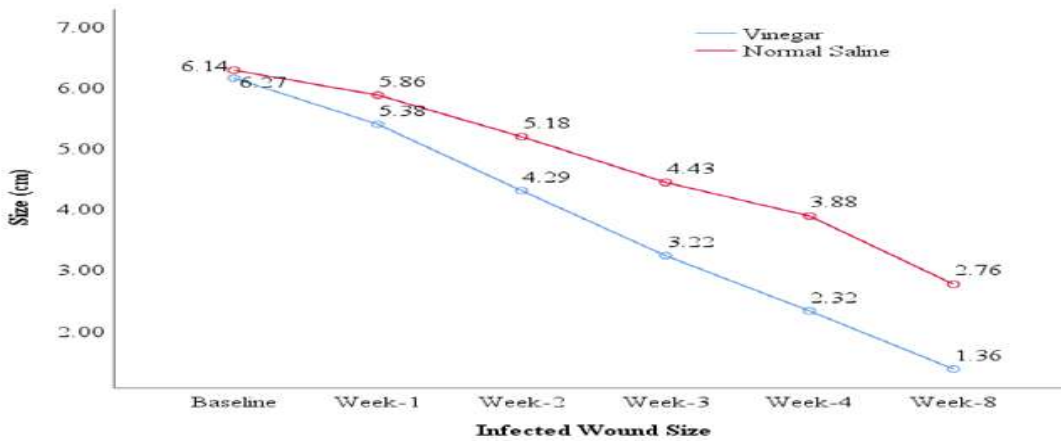


Figure No. 1: Infected wound necrotic size at different follow up interval



Figure No. 2: Wound size at different follow up interval

## DISCUSSION

The severe complications of diabetes include foot ulcer as well as gangrene.<sup>9</sup> Almost 85% of amputations in non-trauma patients result from diabetic foot ulcers (DFU). DFU are associated with extensive infections which results in gangrene.<sup>10</sup> Diabetic foot ulcers have multifactorial etiology. Almost 45 to 60% of these are neuropathic in nature while rest 45% have mixed ischemic as well as neuropathic components.<sup>11</sup> The management of wound is very significant in these cases as chronic wounds like DFU can add to economic burden in the form of expensive treatments and medical visits along with possibility of complications including infections, hospitalizations or amputations.<sup>12</sup> The cleansing of wound and appropriate dressings are very essential in this regard.<sup>13</sup> Hippocrates in ancient Greece used vinegar to treat wounds from 460 to 370.<sup>14</sup> Our study is the first comparative study for comparing vinegar therapy with conventional saline soaked dressings. It is, to the best of our knowledge, the first randomized trial to compare both.

Acetic acid present in vinegar acts as antibiotic at low concentrations including killing Gram-negative and Gram-positive pathogens which are opportunistic and act as biofilms. Other compounds in vinegar enhance the effect of acetic acid to accelerate its effect on

bacteria.<sup>15</sup> Using vinegar soaks for 15 minutes a day effectively decrease the wound pH and reduce bacterial load. This has been also shown to be effective for *Staphylococcus aureus* and other gram-negative rods in previous case series.<sup>16</sup>

The wound and necrotic wound size started to decrease from first week onwards in our study, however, decrease was lesser in normal saline group to  $2.76 \pm 0.83$  while in vinegar group it decreased to  $1.36 \pm 0.84$  ( $p < 0.001$ ). The necrotic wound sizes decrease lesser in normal saline group to  $1.09 \pm 0.60$  while in vinegar group it decreased to  $0.25 \pm 0.35$  ( $p < 0.001$ ). The results were statistically significant (Table 2). Almost 95% of infections got cleared by vinegar while 76% infections were cleared by normal saline at 8 weeks ( $p = 0.013$ ) [Table 3]. The decrease in wound size was more significant in 46 to 60 and more than 60 years of age ( $p < 0.001$ ) [Table 4]. Fana et al<sup>17</sup> also showed that vinegar use effectively debrided the non-healing diabetic foot ulcers. They showed that there was rapid decrease in the size of wound, growth of bacteria and improvement in the granulation tissue. They showed that wound healing was complete within three weeks' time with vinegar in comparison to 0% with conventional therapy. Other dressing materials like honey, olive oil and papaya have been more under consideration in the past. AlSaleh et al<sup>18</sup> concluded that

85% of patients treated with honey had complete ulcer healing in the same time period as 55% had complete healing with conventional dressings. The acetic acid soaks have also shown improvements in literature. Nayak et al<sup>19</sup> showed that the wound decreased from  $41.05 \pm 4.77$  to  $24.6 \pm 12.87$  ( $P < 0.001$ ) with acetic acid soaks while no significant change was observed in the control group ( $P=0.94$ ).

## CONCLUSION

Vinegar dressings are more effective than conventional normal saline dressings in treatment of diabetic foot ulcers for decreasing wound size and reducing infection of wound.

### Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mian Muhammad Bilal, Khushbakht Ali Khan, Masooma Bukhari
Drafting or Revising Critically:	Masooma Bukhari, Ghufuran Akbar, Farhan Javed
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# Vascular Injury in Carpal Tunnel Surgery: Evaluating the Impact of Scalpel versus Scissors in Flexor Retinaculum Release

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and Asim Zia<sup>4</sup>

## ABSTRACT

**Objective:** To evaluate the frequency of vascular injuries in patients undergoing carpal tunnel release (CTR), with a specific comparison between scalpel and scissors techniques for flexor retinaculum division, and to assess the association with demographic and clinical variables.

**Study Design:** Cross sectional study.

**Place and Duration of Study:** This study was conducted at the emergency wards of DHQ Teaching Hospital and Mufti Mehmood Memorial Teaching Hospital from January 2024 to January 2025.

**Methods:** A total of 290 patients who underwent CTR were retrospectively analyzed. Patients with clinically diagnosed carpal tunnel syndrome (CTS), confirmed by nerve conduction studies (NCS) and/or electromyography (EMG), were included. Exclusion criteria comprised incomplete surgical records, previous CTR, concomitant hand surgeries, and preexisting vascular disorders unrelated to CTS. Patients were categorized based on the surgical instrument used for flexor retinaculum release (scalpel vs. scissors). The relationship between vascular injuries and patient age, gender, symptom duration, and severity was assessed. Statistical analysis was performed using SPSS version 26.0.

**Results:** Among the 290 patients, the overall incidence of vascular injury was 11.7%. A significantly higher rate of vascular injury was observed in the scissors group (25.6%) compared to the scalpel group (5.5%) ( $p < 0.05$ ). No statistically significant association was found between vascular injuries and demographic variables (age, gender) or clinical characteristics (symptom duration and severity).

**Conclusion:** The findings demonstrate a significant difference in safety profiles between surgical techniques for CTR. The use of scissors for flexor retinaculum division is associated with a markedly higher risk of vascular injury compared to the scalpel technique, suggesting that instrument choice is a critical factor in minimizing intraoperative complications.

**Key Words:** Carpal tunnel syndrome, carpal tunnel release, vascular injury, surgical outcomes

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## INTRODUCTION

Carpal tunnel syndrome is a widely observed neuropathic disorder distinguished by the median nerve becoming compressed as it passes through the carpal tunnel in the wrist<sup>1</sup>.

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Frequently accompanied by symptoms including pain, tingling and numbness in the hand and digits, this condition can have a substantial negative impact on daily activities and overall well-being.

Contributing elements to CTS comprise anatomical variations, repetitive wrist movements, systemic conditions such as diabetes and rheumatoid arthritis and anatomical variations<sup>2-3</sup>. When patients fail to respond to conservative management, surgical intervention, particularly CTR, is frequently utilized as a treatment option<sup>4</sup>.

The primary objective of CTR surgery is to relieve pressure on the median nerve through the expansion of the carpal tunnel via transection of the transverse carpal ligament. Although this procedure is widely regarded as effective and risk-free, it is not devoid of potential hazards<sup>5</sup>. Vascular injury is a potential complication of CTR surgery that, despite being uncommon, can have severe repercussions for the patient<sup>6</sup>. Vascular structures situated in close proximity to the carpal tunnel, including the radial and ulnar

arteries, are susceptible to potential harm throughout the course of the procedure. Such injuries may result in the formation of hematomas, impaired circulation and in extreme circumstances, necessitate additional surgical procedures to mend the impaired blood vessels<sup>7-8</sup>.

The reported rates of vascular injury during CTR surgery vary between 0.1% and 1.2%, according to different investigations. Variations in study designs, patient populations and surgical methodologies utilized account for these disparities<sup>9-10</sup>. One example of a procedure that may present a higher risk of vascular injury is ECTR, which is characterized by a longer learning curve than the conventional OCTR. Further, the probability of vascular complications occurring during surgery may be impacted by patient-specific variables, including anatomical dissimilarities and preexisting vascular disorders<sup>11</sup>.

Notwithstanding the potential gravity of vascular injuries, comprehensive data regarding their incidence and consequences in patients enduring CTR surgery are scarce<sup>12</sup>. For the purpose of optimizing surgical techniques, enhancing patient safety and directing informed consent discussions, a comprehensive understanding of these hazards is vital. The purpose of this research was to evaluate the prevalence of vascular injury among patients who underwent the surgery for CTS to determine and improve the clinical approach to managing this prevalent condition.

## METHODS

**Study Design and Setting:** This cross-sectional study was conducted at Emergency ward of DHQ Teaching Hospital and Mufti Mehmood Memorial Teaching Hospital, Dera Ismail Khan from January 2024 to January 2025. The objective of this investigation was to determine the incidence of vascular injury among patients undergoing surgery for CTS (Figure 1).

**Study Population:** All patients diagnosed with carpal tunnel syndrome who underwent carpal tunnel release surgery at DHQ Hospital within the designated timeframe were enrolled in the study. The sample of the research comprised 290 patients, comprising a diverse range of ages and both male and female participants.

**Sample Size Calculation:** The sample size of 290 patients was calculated using a power analysis as follows:

### 1. Parameters:

- **Effect Size:** Estimated difference in vascular injury rates between the Scissors (25.6%) and Scalpel (5.5%) techniques.
- **Significance Level ( $\alpha$ ):** Set at 0.05 to control the Type I error rate.
- **Power (1 -  $\beta$ ):** Desired power of 95% to detect a true difference.

### 2. Estimate Proportions:

- **Injury Rates:** Estimated proportions of vascular injuries for each technique were used (25.6% for Scissors and 5.5% for Scalpel).

### 3. Calculate Sample Size:

- Using the formula for comparing two proportions:

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \times (p_1(1-p_1) + p_2(1-p_2))}{(p_1 - p_2)^2}$$

where:

- $Z_{\alpha/2} = 1.96$  (for  $\alpha = 0.05$ )
- $Z_{\beta} = 1.645$  (for 95% power)
- $p_1 = 0.256$  (injury rate for Scissors)
- $p_2 = 0.055$  (injury rate for Scalpel)

### 4. Adjust for Dropouts:

- The calculated sample size was rounded and adjusted to 290 to account for potential dropouts and ensure reliable results.

## Inclusion and Exclusion Criteria

### Inclusion Criteria:

- Patients who have been clinically diagnosed with CTS and have had nerve conduction studies (NCS) or electromyography (EMG) to corroborate the diagnosis.
- Individuals who underwent CTR surgery at DHQ Hospital during the study period through open carpal tunnel release (OCTR).

### Exclusion Criteria:

- Patients whose surgical outcomes were not fully documented in their medical records.
- Patients who had previously undergone CTR surgery or who were undergoing other concurrent hand surgeries.
- Unrelated to CTS patients who had preexisting vascular conditions.

### Data Collection

The information listed below was extracted:

- Demographic information (gender, age)
- Duration and clinical manifestations of CTS symptoms
- The CTR procedure that was executed, utilizing either scalpel (200 patients) or scissors (90 patients)
- Observed intraoperative findings and complications
- The occurrence and characteristics of vascular injuries (such as arterial or venous damage) and superficial palmar nerve injuries

**Outcome Metrics:** During CTR surgery, the incidence of vascular injury served as the principal outcome metric. Vascular injury was operationally defined as any damage that occurred during the procedure to the vascular structures, which necessitated surgical intervention or led to the formation of a hematoma, impaired blood flow or other notable clinical outcomes.

**Statistical Analysis:** For summarizing the data, descriptive statistics were applied. The percentage and frequency of vascular injuries were determined. Analyses of patient demographics and clinical characteristics were conducted utilizing frequencies and percentages for categorical variables and means and

standard deviations for continuous variables using statistical software SPSS version 25.0 at the statistical significance of  $p < 0.05$ . A comparison was made between ECTR and OCTR in terms of the incidence of vascular injury and any significant associations with patient demographics or clinical characteristics were investigated.

**Ethical Considerations:** The research was carried out in adherence to the guidelines outlined in the Declaration of Helsinki. We obtained ethical approval from the DHQ Hospital Ethics Committee. Nevertheless, in order to uphold patient confidentiality, the data were anonymized.

## RESULTS

The demographic and clinical characteristics of patients undergoing CTR surgery provided comprehensive overview of the demographic and clinical characteristics of 290 patients. It indicated that there were no substantial disparities in age or gender between patients with and without surgical injuries. The average age was 45.3 years, with injured patients slightly older at 46.8 years, although this difference was not statistically significant ( $p > 0.05$ ). The incidence of injury was not significantly influenced by gender distribution, as 41.4% of the study cohort consisted of males and 58.6% of the cohort consisted of females. Similarly, there was no significant difference between the groups in the average duration of CTS symptoms prior to surgery, which was 18.4 months ( $p > 0.05$ ). It is important to note that the type of surgical technique had a significant impact: patients who underwent surgery with scissors experienced a higher incidence of injury (25.6%) than those who used a scalpel (5.5%). This underscored the necessity of considering surgical instruments in procedural planning (Table 1).

The data delineated the management strategies and outcomes for 34 patients who sustained injuries during Carpal Tunnel Release surgery. Of these, 32.4% (11 patients) necessitated surgical intervention to resolve their complications, while the greater number, 67.6% (23 patients), were treated conservatively. The effectiveness of both surgical and conservative management approaches was underscored by the fact that a substantial majority of the patients, 85.3% (29 patients), achieved full recovery, despite the incidence of injuries. Nevertheless, complications were not uncommon, affecting 14.7% (5 patients) of the injured. This suggested that, despite the fact that the majority of patients recovered, a significant minority may experience substantial adverse outcomes following the injury. This data underscored the significance of meticulous surgical planning and patient management in order to optimize recovery outcomes and reduce complications during CTR surgery.

The statistical analysis of the incidences of vascular injury between the two surgical techniques, Scalpel and Scissors, demonstrated substantial difference. The Scissors technique showed significantly higher

incidence rate of 25.6% among 90 patients, while the Scalpel technique resulted in lower injury incidence of 5.5% among 200 patients. The Scissors technique was associated with a potentially higher risk, as evidenced by the statistical analysis ( $p < 0.05$ ). This marked difference was statistically significant (Table 2). The demographic analysis of 290 patients, which correlated vascular injuries with age and gender, did not reveal a statistically significant association. The chi-squared tests for both gender and age groups produced p-values that were substantially higher than the standard significance level (age and gender:  $p > 0.05$ ). This indicated that neither factor significantly influenced the likelihood of vascular injury during CTR surgery (Table 3).

Statistical experiments conducted across a range of symptom durations and severity levels did not reveal any significant relationships in the correlation between the clinical presentation of symptoms and the incidence of vascular injuries. These clinical characteristics do not predict the risk of vascular injury in this patient population, as evidenced by the high p-values for both the duration of symptoms and the severity levels ( $p > 0.05$ ) (Table 4). The absence of substantial differences within groups was underscored by the comprehensive analysis of vascular injury incidences by gender within each surgical technique. The Scalpel technique had similar low injury incidences for both males and females (male: 6.0%, female: 5.1%). Although the Scissors technique exhibited higher overall injury rates, there was no statistically significant difference between sexes (male: 32.4%, female: 20.8%;  $p > 0.05$ ) (Table 5).

The management and outcomes of vascular lesions in 34 patients indicated that 47.1% necessitated surgical intervention, while 44.1% were managed conservatively. The majority, 85.3%, accomplished the complete recovery, while 14.7% experienced complications. The chi-squared tests conducted on these outcomes did not reveal any statistically significant differences between the management strategies employed with Scalpels and Scissors, indicating that effective management was consistent regardless of the surgical technique employed (Table 6).



**Figure No. 1: Carpal Tunnel Syndrome Surgery**  
(Source: <https://www.orthoracle.com/library/carpal-tunnel-decompression/>)

**Table No. 1: Demographic and Clinical Characteristics of Patients Undergoing CTR Surgery**

Characteristic	Total (n = 290)	With Injury (n = 34)	Without Injury (n = 256)	$\chi^2$	p-Value
Age (years), mean $\pm$ SD	45.3 $\pm$ 10.2	46.8 $\pm$ 9.7	45.1 $\pm$ 10.3	0.53	0.47
Gender, n (%)				0.15	0.70
Male	120 (41.4)	15 (44.1)	105 (41.0)		
Female	170 (58.6)	19 (55.9)	151 (59.0)		
Duration of CTS Symptoms (months)	18.4 $\pm$ 6.7	19.1 $\pm$ 7.2	18.3 $\pm$ 6.6	0.27	0.61
Type of CTR Surgery, n (%)					
Scalpel	200 (69.0)	11 (32.4)	189 (73.8)		
Scissors	90 (31.0)	23 (67.6)	67 (26.2)		

**Table No. 2: Comparison of Vascular Injury Incidence between Surgical Techniques**

Surgical Technique	Number of Patients	Injuries (n)	Incidence (%)	$\chi^2$	p-Value
Scalpel	200	11	5.5	22.2	0.002*
Scissors	90	23	25.6		
<b>Total</b>	290	34	11.7		

**Table No. 5: Surgical Techniques and Vascular Injury Incidence**

Surgical Technique	Gender	No. of Patients	Injuries (n)	Incidence (%)	$\chi^2$	p-Value
Scalpel		200	11	5.5	0.0	1.0
	Male	83	5	6.0		
	Female	117	6	5.1		
Scissors		90	23	25.6	1.01	0.315
	Male	37	12	32.4		
	Female	53	11	20.8		
<b>Total</b>		290	34	11.7	22.22	0.002*

**Table No. 6: Management and Outcomes of Vascular Injuries**

Outcome/Management	No. of Patients (n = 34)	Percentage (%)	With Scalpel (n = 11)	With Scissors (n = 23)	$\chi^2$	p-Value
Surgical Intervention Required	16	47.1	7	9	0.0	1.0
Conservative Management	15	44.1	8	7	0.0	1.0
Full Recovery	29	85.3	19	10	0.0	1.0
Complications	5	14.7	3	2	0.0	1.0
Type of Vascular Injury						
Arterial Injury	9	26.5	4	5		
Venous Injury	8	23.5	3	5		
Hematoma Formation	5	14.7	2	3		
Compromised Blood Flow	2	5.9	1	1		

**Table No. 3: Demographic Characteristics and Vascular Injury Correlation**

Characteristic	Total (n = 290)	With Injury (n = 34)	Without Injury (n = 256)	$\chi^2$	p-Value
<b>Age Group, n (%)</b>					
<30 years	39	4	35	0.190	0.910
30-50 years	161	20	141		
>50 years	90	10	80		
<b>Total <math>\chi^2</math> (Age)</b>					
<b>Gender, n (%)</b>					
Male	120	15	105	0.026	0.873
Female	170	19	151		

**Table No. 4: Clinical Presentation and Vascular Injury Correlation**

Clinical Characteristic	Total (n = 290)	With Injury (n = 34)	Without Injury (n = 256)	$\chi^2$	p-Value
<b>Duration of Symptoms</b>					
<12 months	60	7	53	0.054	0.973
12-24 months	141	16	125		
>24 months	89	11	78		
<b>Symptom Severity</b>					
Mild	81	9	72	0.077	0.962
Moderate	129	15	114		
Severe	80	10	70		

## DISCUSSION

The results of our analysis included 290 patients who underwent CTR surgery, have offered critical insights into the vascular injury patterns that are associated with various surgical techniques. In comparison to the Scalpel technique, which had 5.5% incidence, Scissors technique had a significantly higher incidence of vascular injuries at 25.6%. The potential hazards associated with the Scissors technique are underscored by this substantial discrepancy, which is supported by a chi-squared value of 22.2 and p-value of 0.002. This may require additional scrutiny and potential modifications to surgical practice.

In contrast to our initial assumptions, the incidence of vascular injuries did not demonstrate a statistically significant correlation with demographic factors such as age and gender. The p-values for both age and gender were significantly higher than the significance threshold in the chi-squared tests, indicating that these factors do not independently predict the likelihood of vascular complications<sup>13-14</sup>. This discovery was consistent with prior research that has demonstrated the non-specificity of demographic variables in predicting surgical risks in CTR procedures.

Additionally, the duration and severity of CTS symptoms were examined to ascertain their potential impact on surgical outcomes. High p-values in our statistical tests suggested that there was no significant correlation between the incidence of vascular lesions and these clinical presentations, as our results demonstrated. This contradicted the prevalent belief that surgical risks are inherently elevated by the duration or severity of symptoms. Rather, it implied that the inherent risks may be more closely associated with the surgical technique and execution than the patient's preoperative condition<sup>15-17</sup>.

The surgical outcomes that were emphasized in this study were particularly informative. While 53.3% of patients with vascular injuries required surgical intervention, a substantial 46.7% were successfully managed with conservative treatments. The efficacy of current management strategies was emphasized by the overall high recovery rate of 85.3%. However, the 14.7% complication rate underscored the necessity of vigilant surgical monitoring and postoperative care to mitigate potential adverse outcomes<sup>18-22</sup>.

The comparative safety of Scalpel versus Scissors techniques, particularly when analyzed across gender-specific outcomes, did not demonstrated a statistically significant difference. This indicated that both techniques can be equally safe when executed efficiently. This revelation supports the personalized approach in surgical method selection, which should consider the patient's specific anatomical and clinical profile rather than a generalized preference for one technique over another<sup>23</sup>.

The implications of our research were not limited to the selection of procedures; they emphasized the critical importance of proactive management of potential

complications and precise surgical execution. The absence of significant predictors based on demographic and clinical characteristics further implied that surgical risks may be more effectively managed through technique refinement and skill enhancement rather than patient selection.

The findings of our study would be enhanced by validation through multicenter trials, as it is a single-center investigation. Further investigation into how anatomical variations influence the risk of vascular injuries could also enhance preoperative assessments and planning, potentially reducing the incidence of such injuries.

Our research corroborated some aspects of existing literature regarding the non-consequential nature of demographic and symptom severity factors on surgical risks, it crucially highlighted the differential risk associated with surgical techniques. The detailed examination of these techniques not only enriched our understanding but also paves the way for more targeted and safer surgical practices in the treatment of CTR.

## CONCLUSION

This study provides a comprehensive evaluation of vascular injuries occurring during carpal tunnel release (CTR) in a cohort of 290 patients, demonstrating an overall incidence of 11.7%. The findings indicate no statistically significant association between vascular injury and demographic or clinical variables, including age, gender, duration of symptoms, and disease severity. Notably, a marked difference in injury rates was observed between surgical techniques. The scissors technique was associated with a significantly higher incidence of vascular injury (25.6%) compared to the scalpel technique (5.5%). These results suggest that the choice of instrument for flexor retinaculum division plays a critical role in determining intraoperative safety. Therefore, careful selection of surgical technique—taking into account anatomical considerations and operative context—is essential to minimize the risk of vascular complications during CTR.

### Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Raza Man, Khalid Mehmood, Muhammad Saqib
Drafting or Revising Critically:	Yousaf Gul, Shahid Nawaz, Asim Zia
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# The Relationship Between the Duration of Type 2 Diabetes Mellitus and Obesity with Complications Neuropathy

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## ABSTRACT

**Objective:** To observe the type 2 diabetes mellitus in combination with obesity levels affects diabetic neuropathy occurrence.

**Study Design:** Cross-sectional study

**Place and Duration of Study:** This study was conducted at the Department of Biochemistry of Al-Rafai Education Hospital in Dhi Qar Iraq from 31<sup>st</sup> December 2024 to 31<sup>st</sup> March 2025.

**Methods:** A total of 90 participants assigned to three separate groups consisting of healthy control patients and participants with type 2 diabetes mellitus without neuropathy as well as type 2 diabetes mellitus patients with confirmed diabetic neuropathy. Researchers took blood samples which they used to measure fasting blood sugar and haemoglobin A1c and hemoglobin and ferritin along with the lipid profile.

**Results:** Diabetic patients especially those with neuropathy experienced a marked increase in fasting blood sugar and haemoglobin A1c and body mass index levels when tested. The diabetic neuropathy patient group demonstrated elevated ferritin compared to other participant groups. A high relationship emerged between haemoglobin A1c measurements and both fasting blood sugar levels and body mass index values as well as ferritin levels.

**Conclusion:** Both type 2 diabetes mellitus duration extending in years and obesity condition increase the susceptibility to neuropathic complications.

**Key Words:** Type 2 diabetes mellitus, Diabetic neuropathy, Obesity, Ferritin

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## INTRODUCTION

People with type 2 diabetes mellitus (T2DM) show persistent elevated blood glucose levels due to insulin resistance combined with inadequate insulin action. An impaired  $\beta$ -cell status prevents patients from increasing their insulin secretion levels successfully. Multiple factors including genetics and inflammation together with visceral obesity contribute to the exact pathogenesis of insulin resistance in T2DM.<sup>1</sup> The defective insulin-mediated receptor phosphorylation occurs at the receptor kinase domain tyrosine position.

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Research showed that Glut4 molecules on adipocytes play a significant role in downregulating PPAR $\gamma$  coactivator 1 $\alpha$  (PGC1 $\alpha$ ) gene activity in patients with T2DM which contributes to insulin resistance. Endogenous free fatty acids (NEFA) affect glucotoxicity in pancreatic islet cells by creating higher reactive oxygen species (ROS) concentrations which generate increased oxidative stress. The reduction in number of  $\beta$ -cells becomes the final cause of developing T2DM.<sup>2</sup> Throughout its development T2DM results in declining insulin secretion from pancreatic cells because apoptosis is prevented in  $\beta$ -cells throughout the body. The numbers of people with T2DM have grown remarkably since the previous years. The population numbers of both adult and adolescent T2DM patients have demonstrated rising figures. By 2025 the level of T2DM diagnosis is expected to increase substantially for adults between 20 to 79 years of age.<sup>3</sup>

Death statistics show that diabetes complications now appear with greater frequency. The occurrence of diabetic peripheral neuropathy in diabetes exists from 30-50% of patients. Late detection of diabetes often results in amputation of feet requiring removal because of ulcers along with gangrene development. T2DM develops when genetic and immune and metabolic

dysfunctions together cause persistent high blood glucose levels. Tissues and functions of nerves undergo biochemical transformations that modify structure because of persistent high blood glucose levels. The length of time someone has diabetes stands alone as a critical risk factor in developing diabetic neuropathy.<sup>4,5</sup>

The initial pathological changes from glycosylating end products formation in diabetes affect synapses far from the cell body first. The long axons experience structural support alterations which produce Wallerian degeneration together with distal segment dying back while proximal neuron nerve function declines.<sup>6</sup> The conditions which define T2DM include different types of metabolic problems with fat compounds and proteins alongside carbohydrates. Over the period diabetes type 2 patients who are overweight have grown in numbers. The condition develops due to reduced insulin sensitivity in end organs and reflects weight problems and inactive lifestyles. Type 2 diabetes connected to obesity has produced increased adult diabetes cases which ultimately result in delayed complications. Patient diagnoses need to consider diabetic neuropathy because it ranks as the second manifestation that makes life worse for diabetic patients. Research currently fails to explain all of the factors which trigger neuropathy progression in diabetic patients.<sup>7</sup>

Millions of people worldwide experience substantial health problems because of diabetes which has become the speediest spreading long-term disease today. Global healthcare providers diagnosed 382 million people with diabetes during the count of 2013. The research indicates that diabetes will affect 591.9 million people worldwide during 2035. Researchers indicate that diabetes patients amount to 50% of the global population who live in five key countries. The five leading countries with diabetes cases are the United States of America alongside India, Brazil, China and Indonesia. The two countries with the most frequent occurrences of diabetes are China followed by India among the five nations examined. These countries face a realistic possibility of becoming known as "diabetes capitals" if they fail to implement effective diabetes control measures because of their worsening situation.<sup>8</sup> The rate at which people suffer from diabetes differs between separate nations around the world. Diabetes prevalence throughout South and Southeast Asia ranked in the third position in 2013 with 72 million cases yet the highest case counts were detected in Western Pacific and North American regions. The population of Malaysian adults with diagnosed diabetes passed 2.4 million in 2013. Research data indicates that diabetes cases will increase by 123% to reach this number by 2035. The leading complication of neuropathy affects all body regions starting from autonomic and continuing to cranial and spinal nerves. High levels of glucose within the bloodstream of diabetes patients lead to nerve destruction and degeneration. The lengthy

nerves belong to the group most susceptible to damage thus rendering them the first targets of this process. Researchers consider peripheral neuropathy to be among the most prevalent diabetes-related conditions.<sup>9,10</sup>

## METHODS

This cross-sectional research was conducted at the Department of Biochemistry of Al-Rafai Education Hospital in Dhi Qar Iraq from 31<sup>st</sup> December 2024 to 31<sup>st</sup> March 2025 vide letter No. 43egr/QM/Approval/rgEEUI3 dated September 24, 2024. A total of 90 participants were enrolled. The number of samples included 30 apparently healthy adults (C, aged 30-50 years of healthy volunteers), 30 patients with type 2 diabetes (DM, aged 38-65 years) and 30 patients with type 2 diabetes with neuropathy (DF, aged 40-80 years). All study participants underwent their annual medical examination at Al-Rifai Teaching Hospital. The subjects were those diagnosed with type 2 diabetes and its complications as defined by the World Health Organization diagnostic criteria. This study excluded patients who were found to have nephropathy, retinopathy, chronic kidney disease, chronic liver disease, tuberculosis, arthritis, systemic lupus erythematosus, mononucleosis, Bechet's disease, or cancer. Screening interviews were used where specific questions were asked to collect basic facts.

Venous blood samples of 5 ml each were collected in fasting state from the subjects using sterile method. 3 ml of blood sample was taken in gel tube for measurement of random blood sugar (RBS) in mg/dl and lipid profile including Triglyceride, Total Cholesterol HDL, LDL, VLDL in mg/dl unit and serum HB, firetine levels. We collected 2 ml of blood in EDTA tube for determination of hemoglobin A1c levels using automated biochemical analyzer. For measurement of hemoglobin A1c, a semi-automated biochemical analyzer known as HumaLyzer-3500 was used. However, the remaining parameters were analyzed using a spectrophotometer manufactured by Biotech Engineering. BMI is given as weight expressed in kilograms divided by square height measured in meters. The data was analyzed using SPSS-26. The Kolmogorov-Smirnov test was used to divide the variables across the research groups. The one-way ANOVA test was used to compute and compare the means and standard deviations of the homogeneously distributed variables. P values less than  $P < 0.05$  were used by Medcalc to calculate the ROC and statistical significance.

## RESULTS

The DF patients who had diabetic neuropathy displayed considerably elevated FBS, HbA1c, and ferritin levels than participants in both control and T2DM groups. The

DF group presented with the greatest FBS mean values at 256.10±106.36 mg/dl whereas both results differed significantly from control patients and those with T2DM (p < 0.001). The mean HbA1c levels in DF

patients stood at 8.88±1.72% above those measured in control and T2DM groups at p<0.001 significance. The DF group showed elevated ferritin levels which supported the notion of disease severity relationships.

**Table No. 1: Comparison of HbA1c, HB, Firretin and BMI, among different groups (n=90)**

Parameter	Controls	Diabetes mellitus	Diabetic nephropathy	P value	
				Control_DM	0.001**
Age (mg/dl)	41.40±11.23	49.23±7.62	58.03±9.064	Control*DF	0.001**
				Diabetic*DF	0.001**
				Control*DM	0.041*
Gender	1.46± 0.507	1.36±0.49013	1.500±0.5085	Control*DF	0.031*
				Diabetic*DF	0.051
				Control*DM	0.9621
BMI (Kg/m2 )	27.36±3.60	34.61±5.76	30.82±5.186	Control*DF	0.065
				Diabetic*DF	0.031*
				Diabetic*DF	0.031*
Duration		34.61±5.76	42.82±5.186	Diabetic*DF	0.031*

\*\*p<0.01 is extremely significant. \*p<0.05 is significant

**Table No. 2: Comparison of FBS, HbA1c, HB, Firetin and BMI, among different groups (n=90)**

Parameter	Controls	Diabetes mellitus	Diabetic nephropathy	P value	
				Control*DM	0.001**
FBS (mg/dl)	136.40±17.64	195.43±40.45	256.10±106.36	Control*DN	0.001**
				Diabetic*DN	0.001**
				Control*DM	0.001**
HbA1c %	4.87±0.51	7.79±1.43	8.88±1.72	Control*DN	0.001**
				Diabetic*DN	0.001**
				Control*DM	0.9621
HB	13.40±1.762	13.36 ± 1.53	11.75±2.31	Control*DN	0.065
				Diabetic*DN	0.031*
				Control*DM	0.9621
Ferritin	96.29±83.274	131.61 ± 89.71	162.76±180.98	Control*DN	0.065
				Diabetic*DN	0.031*
				Control*DM	0.9621

\*\*p<0.01 is extremely significant. \*p<0.05 is significant

**Table No. 3: Comparison of lipid profile, Triglyceride, Cholesterol, HDL, LDL and VLDL among different groups (n=90)**

Parameter	Controls	Diabetes mellitus	Diabetic nephropathy	P value	
				Control*DM	0.001**
Triglyceride	185.00±54.14	200.50±65.26	240.90±136.37	Control*DF	0.001**
				Diabetic*DF	0.001**
				Control*DF	0.001**
Total cholesterol	190.500±30.33	209.13±34.37	206.06±44.84	Conytrol*DF	0.001**
				Diabetic*DF	0.001**
				Control*DM	0.001**
HDL	47.200±13.324	54.7000±13.61	42.1667±15.66	Control*DF	0.001**
				Diabetic*DF	0.001**
				Control*DM	0.030
LDL	108.93±27.48	111.63±29.109	106.73±39.82	Control*DF	0.004
				Diabetic*DF	0.744
				Control*DM	0.030
VLDL	35.03±9.21	43.40±10.47	49.96±29.76	Control*DF	0.004
				Diabetic*DF	0.744
				Control*DM	0.030

\*\*p<0.01 is extremely significant. \*p<0.05 is significant

**Table No. 4: The Pearson correlation of haemoglobin A1c activity**

Parameters	Hemoglobin A1c					
	Diabetes mellitus (n= 30)			Diabetic nephropathy (n=30)		
	r	p	Sig.	r	P	Sig.
FBS (mg/dl)	0.69538**	0.001	HS	0.7521**	0.001	HS
Hb	0.8601**	0.001	HS	0.876**	0.001	HS
Ferritin	0.4382**	0.058	S	0.4919**	0.0042	HS
BMI (Kg/m2)	0.46799**	.0071	HS	0.4528*	0.0450	S

Pearson coefficient (r), Significant (S) at  $p \leq 0.05$ , \*\*Highly significant (HS) at  $p \leq 0.01$

**Table No. 5: The Pearson correlation of haemoglobin in males and females**

Parameters	Hemoglobin					
	DM group (n= 30)			DF group (n=30)		
	r	p	Sig.	r	P	Sig.
FBS (mg/dl)	0.6991**	0.001	HS	0.636**	0.001	HS
HbA1c (%)	0.8601**	0.001	HS	0.876**	0.001	HS
BMI (Kg/m2)	0.6592**	0.001	HS	0.491**	0.004	S
Ferritin	0.3342	.0623	NS	0.3972*	0.0253	S

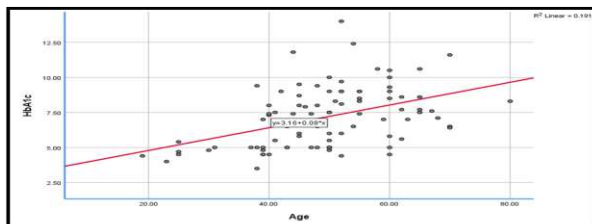
Pearson coefficient (r), Not significant (NS)  $p > 0.05$ \*, Significant (S) at  $p \leq 0.05$ , \*\*Highly significant (HS) at  $p \leq 0.01$

**Table No. 6: The Pearson correlation of ferritin**

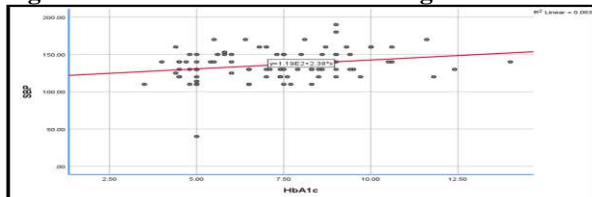
Parameters	Ferritin					
	DM group (n= 30)			DF group (n=30)		
	r	p	Sig.	r	P	Sig.
FBS (mg/dl)	0.5863**	0.031	HS	0.5839**	0.001	HS
HbA1c (%)	0.4382**	0.058	S	0.4919**	0.0042	H
BMI (Kg/m2)	0.5863**	0.001	HS	0.3836*	0.0300	S
HB	0.4909**	0.0430	S	0.280	0.1182	No S

Pearson coefficient (r), Not significant (NS)  $p > 0.05$ \*, Significant (S) at  $p \leq 0.05$ , \*\*Highly significant (HS) at  $p \leq 0.01$

The DF group displayed temporary mild decreases in hemoglobin levels yet this change was not considered statistically important. Both HbA1c and FBS levels showed statistically significant correlations with BMI measurement in the DF group because obesity acts as an important risk factor. Diabetic patients with neuropathy show increasing metabolic problems (Tables 1-6, Figs. 1-2).



**Figure No. 1: Correlation between Age and HbA1C**



**Figure No. 2: Correlation between SBP and HbA1C**

## DISCUSSION

The widespread chronic disease known as Type 2 diabetes mellitus causes several complications which diabetic neuropathy (DN) represents one of the major issues among patients.<sup>11</sup> Multiple research studies show poor diabetes management together with being overweight and having abnormal lipids play critical roles in developing diabetic neuropathy. The research evaluated fasting blood sugar, hemoglobin A1c, body mass index and ferritin levels together with hemoglobin and lipid profile results to establish their connection with diabetic neuropathy.<sup>12</sup>

Fasting Blood Sugar and HbA1c concentrations increased substantially in diabetic patients especially those with neuropathy compared to people in the control group according to this research. Extensive research has confirmed that prolonged high blood sugar levels play a central role in causing nerve damage for diabetic patients.<sup>13</sup> A Diabetes Care study established that a 1% rise in HbA1c increases diabetic neuropathy risk by 10%. Chronic high blood sugar levels generate oxidative stress with inflammation and damage the endothelium and these processes jointly harm nerve cells. The reduction of neuropathic complications in

diabetic patients is achievable when maintaining HbA1c levels at or below 7%.<sup>14</sup> The study data shows obesity measured through BMI creates substantial connections between HbA1c and FBS levels mainly among patients who have neuropathy. The dual process of elevated insulin resistance and increased systemic inflammation intensifies diabetic complications because of obesity.<sup>15</sup> The study documenting how weight gain beyond safe limits leads to elevated inflammation and damage to nerve cells stands confirmed through research findings. The analysis uncovered evidence that weight reductions between 5 to 10 percent enhance insulin sensitivity and minimize the risk of diabetic neuropathy. The data demonstrates how effective weight control remains vital to stop diabetic neuropathy from appearing in patients with diabetes.<sup>16</sup> The medical community recognizes dyslipidemia as a well-established diabetic complication risk factor that includes neuropathy as part of its effects. The research on diabetic neuropathy patients found that their blood TG and LDL cholesterol concentrations were higher and their HDL cholesterol levels were lower.<sup>17</sup> The DN group displayed significantly higher triglyceride level values which indicate that triglycerides could contribute to the damage of nerves. Scientific research shows that triglyceride (TG) levels exceeding 204 mg/dL lead to increased susceptibility for diabetic polyneuropathy.<sup>18</sup> The available evidence suggests high LDL levels may cause microvascular complications yet other researchers found no direct connection while healthcare providers should keep LDL levels under 100 mg/dL to reduce vascular and nerve complications. Reductions in HDL levels stand as a risk factor to develop DN.<sup>19</sup> Research based in Denmark proved that male participants with HDL below 39 mg/dL and female participants with HDL below 50 mg/dL showed increased rates of diabetic polyneuropathy. The connection between lipid management and diabetic patients became essential according to these results.<sup>20</sup> The diabetic neuropathy participants had elevated ferritin levels when compared to other patient groups. When ferritin levels increase in the body they signal high iron stores yet these elevated levels frequently trigger oxidative stress together with chronic inflammation that damages nerves. Elevated ferritin levels in Egyptian diabetic patients with diabetic nephropathy support an expanded role of this factor in diabetes complications according to research findings.<sup>21</sup> Assessing ferritin levels in diabetic patients provides useful information to evaluate their neuropathy development risk.<sup>22</sup> Studies revealed no meaningful distinctions in hemoglobin levels neither among the groups nor did they show an actual change in neuropathy participants. Researchers identify similar findings because diabetes causes impairment to red blood cell production through

its impact on erythropoietin secretion.<sup>23</sup> Diabetic patients are more prone to developing mild anemia mainly because this condition makes neuropathy progression more likely. Veterinary practice involves recommending routine anemia testing for diabetic patients because it helps provide enough oxygen to peripheral nerves.<sup>24</sup>

## CONCLUSION

Long-term type 2 diabetes combined with obesity together with lipid disturbances leads directly to diabetic neuropathy development. The levels of HbA1c, FBS, ferritin and triglycerides show that metabolic disorder plays an important role in causing nerve damage. Biochemical markers monitoring along with proper lifestyle adjustments can decrease the amount of diabetic complications patient's experience. Academics should pursue research to develop new ways to prevent neuropathy in diabetic patients.

### Author's Contribution:

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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# Classification of Environmental and Clinical Acinetobacter species Based on Some Antibiotics and Heavy Metals Resistant Genes

Evaluate Cadmium Resistance and Genetic Diversity in Acinetobacter Isolates

Ali Khanjar Jaber, Saba Riad Kkudhaier and Ali Murtatha Hasan

## ABSTRACT

**Objective:** To evaluate cadmium resistance and genetic diversity in Acinetobacter isolates from clinical and environmental sources within hospitals.

**Study Design:** Experimental study

**Place and Duration of Study:** This study was conducted at the multiple hospitals in Baghdad City, Iraqi from 1<sup>st</sup> November 2024 to 31<sup>st</sup> March 2025.

**Methods:** Sixty Acinetobacter species isolates were collected; 44 *A. baumannii* isolates (41 clinical, 3 environmental) and 16 Acinetobacter complex isolates (13 clinical, 3 environmental). The isolates were identified using the VITEK-2 system, and the diagnosis was confirmed using polymerase chain reaction for the blaOXA-51 gene. 22 representative isolates were selected for further analysis. Cadmium resistance tests were performed using the minimum inhibitory concentration method, and the *czcB* gene was detected using polymerase chain reaction. Genetic diversity was analyzed using enterobacterial repetitive intergenic consensus-polymerase chain reaction and biotyping tools.

**Result:** All 22 isolates showed resistance to low cadmium concentrations (0.25mg/L, 0.125mg/L, and 0.0625 mg/L). The *czcB* gene was detected in 7 isolates (4 from *A. baumannii* and 3 from *A. complex*). Enterobacterial repetitive intergenic consensus-polymerase chain reaction demonstrated significant genetic diversity among the isolates. Cluster diagrams, heatmaps, and association matrices highlighted the phylogenetic relationships and unique characteristics of some isolates.

**Conclusion:** Acinetobacter isolates showed significant resistance to cadmium, partly attributable to the presence of the *czcB* gene. Enterobacterial repetitive intergenic consensus-polymerase chain reaction has proven effective in elucidating genetic diversity and tracking potential sources of infection.

**Key Words:** Acinetobacter baumannii, Acinetobacter complex, Cadmium resistance, Genetic diversity

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## INTRODUCTION

The genus Acinetobacter, particularly *A. baumannii*, has emerged as a major cause of healthcare-associated infections due to its remarkable ability to survive in hospital environment and acquire resistance to antibiotics and disinfectants.<sup>1</sup>

These bacteria have also gained attention for their potential to tolerate and resist toxic heavy metals, including cadmium (Cd), which poses a serious

environmental and health hazard due to its persistence and toxicity.<sup>2</sup>

In addition to phenotypic resistance, the genetic diversity of bacterial isolates is critical for understanding their adaptability and environmental persistence. The Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR technique is a widely used genotyping method to assess genomic variability among bacterial strains, including Acinetobacter spp.<sup>3</sup>

Cadmium is an SAM has no function in a plant, animal or human when its accumulation in college stays out, causing high blood pressure and kidney disease, and the difficulty to remove output Lead and Cadmium for direct damage neurons, because it prevents the formation of steel Colin and activates the enzyme choline esterase inhibitor) reduce the embarrassment of cadmium in the soil of 3-5 mg/kg this limit does not cause increased accumulation of a toxic concentration of lead occurring cadmium 5-10 mg/kg to reduce production and lay the seriousness of this element in it (SAM) in low concentrations.<sup>4</sup> Cadmium, often used in

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batteries and pigments, is a heavy metal. Stanford Advanced Materials (SAM) supplies various cadmium products, including cadmium acetylacetonate, cadmium sulfide powder, cadmium selenide powder, and cadmium telluride powder. SAM also offers cadmium metal, rods, evaporation materials, and sputtering targets.<sup>6</sup>

Generally, many studies had proven that when heavy metals present under traces were toxic to humans. Firstly, they combine with proteins and did not cause any poisoning. However, when concentration increase above the threshold level, they become a real health problem. These toxic metals could interact with important cellular components by forming covalent and ionic bonding, furthermore, when it became at high levels, both essential and non-essential metals causes cell membrane damage, enzyme specificity alteration, cellular function disruption and DNA structure damage.<sup>5-8</sup>

The purpose of this study is to evaluate the cadmium resistance of clinical and clinical environment *Acinetobacter* spp. isolates, detect the presence of the *czcB* gene, and assess their genetic diversity using ERIC-PCR.

## METHODS

This experimental study was conducted at multiple hospitals in Baghdad City, Iraqi from 1<sup>st</sup> November 2024 to 31<sup>st</sup> March 2025 vide letter No. BCSMU/1221/00048M dated 1<sup>st</sup> September 2024. A total of 204 clinical and clinician environment samples were collected, comprising 136 clinical and 68 clinical environmental samples. *Acinetobacter* spp. was isolated from 54 clinical samples and 6 clinical environment samples. The clinical specimens were obtained from various sources including: wound swabs, burn swabs, sputum, urine, and blood, while the clinical environment samples were collected from operating rooms and outpatient clinics.

**Diagnosis and identification of *Acinetobacter* species:** All the isolates were cultured on ordinary media (MacConkey agar and blood agar) and incubated for 24 hrs at 37°C. Depending on microscopically examination for Gram stain culture's characteristics, and biochemical tests (oxidase, catalase, kligler iron agar test) utilized for initial diagnosis. Identification was confirmed using the VITEK-2 system, and PCR amplification of *blaOXA-51* gene.

Cadmium susceptibility testing was performed using serial dilution. Detection of *czcB* gene was conducted by PCR. ERIC-PCR was used to evaluate genomic diversity. The specific primers for the study were confirmed by BLAST were detected by PCR Technique (Table 1). The chi-square statistical analysis was applied with significance at  $p \leq 0.05$  to measure the significance between variables.

## RESULTS

Collection and isolation of *Acinetobacter* species were categorized in Table 2. *Acinetobacter* spp. was isolated from 54 clinical samples and 6 clinical environment samples. The clinical specimens were obtained from various sources including: wound swabs, burn swabs, sputum, urine, and blood, while the clinical environment samples were collected from operating rooms and outpatient clinics (Table 3).

**Table No. 1: Primer sequence of *bla-oxa-51*, ERIC, and *czcB* genes<sup>6</sup>**

Gene	Sequences (5'-3')	Product size/bp
bla oxa 51	Forward: TAATGCTTTGATCGGCCTTG	353
	Reverse: TGGATTGCACTTCATCTTGG	
ERIC	Forward: ATGTAAGCTCCTGGGGATTCA	Variable
	Reverse: AGTAAGTGACTGGGGTGAGCG	
czcB	Forward: GCTGTGGCTGGAGATGAGAA	400
	Reverse: TTTTGCTCGGCATCCAAACG	

The clinical and clinician environmental strain isolates (n=60) of the isolated bacterial strains were in agreement with those reported for typical strains of 44 *A. baumannii* (41 clinical isolates and 3 clinician environmental isolates), and 16 *A. complex* (13 clinical isolates and 3 clinician environmental isolates), respectively. Therefore, after classifying 60 *Acinetobacter* spp. isolates (54 clinical isolates and 6 clinical environment isolates), based on phenotype (Biochemical tests, the Vitek-2 system, and confirmed through molecular identification by using the *blaOXA-51* gene), we started with sequencing the 16S rRNA gene to determine the genetic identity of the isolates. However, due to the significant similarity between members of the *Acinetobacter calcoaceticus baumannii* complex (ACB complex), this analysis was not sufficient for precise differentiation. Therefore, we performed an analysis to detect the *bla-oxa-51*-like gene, which is a characteristic gene that is naturally present in *A. baumannii* as previously mentioned, this gene is considered a distinctive diagnostic genetic marker for the *A. baumannii* bacteria (due to the *bla-oxa-51*-like genes carrying up on a bacterial chromosomal specially). Through this, the isolates demonstrated morphological and genetic concordance with isolates of both *A. baumannii* and *A. complex*. based on this phenotypic and genetic concordance of the isolates (and its importance in providing high

accuracy in bacterial identification, which helps guide appropriate treatment and combat the spread of infection), we worked on reducing the number of isolates required to diagnose some antibiotic-resistant genes and heavy metals specific to this study. Therefore, we established a dendrogram Phylogenetic

tree for the isolates of both *Acinetobacter* spp., a dendrogram typing for *A.baumannii* and a dendrogram typing for *A. complex*. Thus, the final isolates for the molecular study scientifically according to the theory of phenotypic and genotypic compatibility (Table 4, Fig. 1).

**Table No. 2: Distribution of *Acinetobacter* spp. in clinical and clinical environment samples**

Sample Type	Total samples	Negative culture (No growth)	Non-Positive for <i>Acinetobacter</i> spp.	Positive for <i>Acinetobacter</i> spp.
Clinical	136	9 (6.6%)	73 (53.7)	54 (39.7%)
Clinical Environment	68	46 (67.7%)	16 (23.5%)	6 (8.8%)
Total	204	55 (27%)	89 (43.6%)	60 (29.4%)

**Table No. 3: Incidence of *A. baumannii* and *A. complex* isolates in various clinical and clinician environmental samples**

Sources of Infection		<i>A. Baumani</i> (N, Rate of isolates %)	<i>A. Complex</i> (N, Rate of isolates%)
Clinical	Wound	12 (27%)	3 (19%)
	Burns	18 (41%)	9 (56%)
	Sputum	4 (9%)	-
	Urine	1 (2%)	-
	Blood	6 (14%)	1 (6%)
Clinician environment	Operating rooms	2 (5%)	2 (13%)
	Outpatient clinics	1 (2%)	1 (6%)
Total		44 (100%)	16 (100%)

**Table No.4: Final *Acinetobacter* spp. isolates according to Dendrograms typing of 60 primary *Acinetobacter* spp. isolates**

13 <i>A. baumannii</i> isolates	No.	9 <i>A. complex</i> isolates
A.b-Cli 1	1	A.c- Cli 43
A.b-Cli 2	2	A.c-Cli 56
A.b-Cli 17	3	A.c-Cli 42
A.b -Cli 25	4	A.c – En 46
A.b-Cli 27	5	A.c -Cli 53
A.b- En 29	6	A.c-Cli 49
A.b-Cli 30	9	A.c-Cli 57
A.b-Cli 32		A.c- En 48
A.b-Cli 36		A.c-Cli 55
A.b-Cli 40		
A.b-Cli 41		
A.b -En 47		
A.b-Cli 54		

**Table No. 5: Cadmium resistance profile of *A. baumannii* and *A. complex* isolate**

Tendency	S	D1	D2	D3	D4	D5	D6	D7	D8
<b><i>A. baumannii</i> (n= 13)</b>									
Resistance of Cd <sup>2+</sup> (Growth)	-	-	-	-	-	-	7 (53.8%)	13 (100%)	13 (100%)
Susceptible of Cd <sup>2+</sup> (No growth)	13 (100%)	13 (100%)	13 (100%)	13 (100%)	13 (100%)	13 (100%)	6 (46.2%)	-	-
<b><i>A. complex</i> (n= 9)</b>									
Resistance of Cd <sup>2+</sup> (Growth)	-	-	-	-	-	-	7 (77.8%)	9 (100%)	9 (100%)
Susceptible of Cd <sup>2+</sup> (No growth)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	9 (100%)	2 (22.2%)	-	-

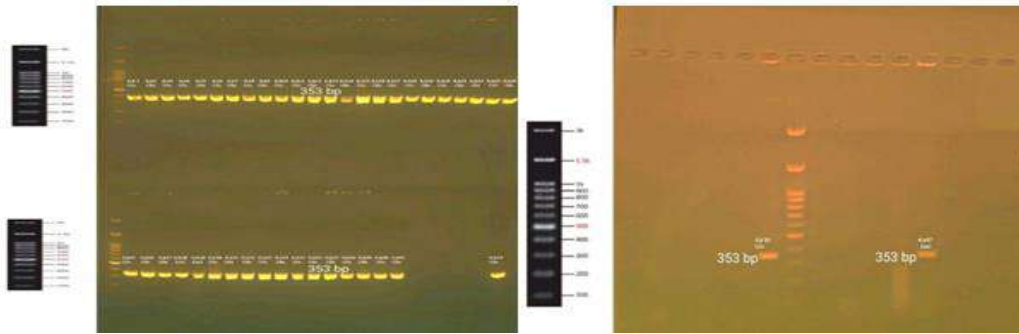


Figure No. 1: The amplification of the blaOXA-51 gene of A.baumannii samples was fractionated on 1.5% agarose gel (60 min at 7v/cm<sup>2</sup>), (353 bp amplicon) M: 100bp ladder marker



Figure No. 2: The amplification of the ERIC gene of Acinetobacter species samples was fractionated on a 1.5% agarose gel (60 min at 7v/ cm<sup>2</sup>), (variable amplicons) lane: 100bp ladder marker

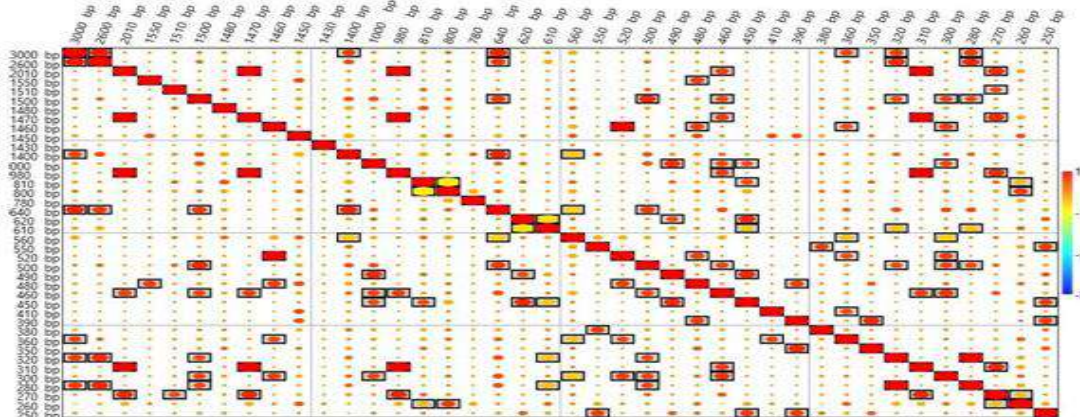


Figure No. 3: The Pearson matrix is a statistical method for determining the linear correlation coefficient between variables in ERIC gene

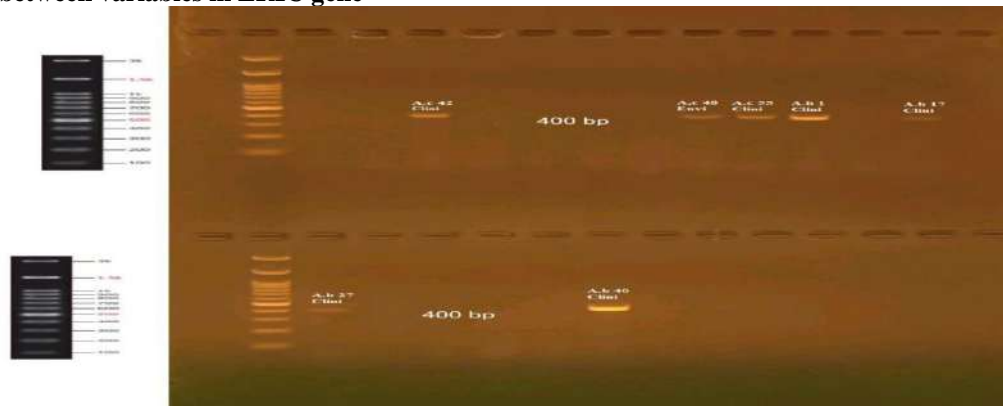


Figure No. 4: The amplification of CzcB gene of Acinetobacter species samples was fractionated on 1.5% agarose gel (60 min at 7v/ cm<sup>2</sup>), (400 bp amplicon) lane: 100bp ladder marker

Molecular Detection of Genetic Variation ERIC Gene: The Enterobacterial Repetitive Intergenic Consensus-PCR amplification of *A. baumannii* and *A. complex* produced unique polymorphic banding patterns, as seen in the agarose gel electrophoresis in Figure 2. Significant genetic variety among the strains was indicated by the numerous DNA bands of different sizes that were seen in the ERIC-PCR profiles of all 22 isolates. The band sizes varied between roughly 250 bp to 3 kb. Importantly, a variety of isolates showed strong bands at 590 bp, 610 bp, 800 bp, and 1400 bp, suggesting that these areas are frequently amplified between various strains. On the other hand, only a small number of isolates had particular bands, like those at 280 bp, 450 bp, and 1430 bp. Different isolates had different band numbers and extents, which could be due to strain-specific insertions or deletions or genomic rearrangements. To further analyse the dendrogram that displays the distribution of ERIC amplicons. This heat map representation illustrates the genetic fingerprinting of each isolate based on the observed bands. The X-axis represents different DNA fragment sizes, while the Y-axis lists the various isolates, which are represented by black squares indicating the presence of specific DNA fragments, while white spaces denote their absence. A strong correlation existed between the dendrograms and gel electrophoresis banding patterns.

Here we observe in the linear Pearson matrix, for example, the band for isolates of molecular size (3000bp) repeats or is associated with several bands, as the band for isolates of molecular size (3000bp) has repeated with, or is associated with, the band of molecular size (1400bp), and the band of molecular size (640bp), and the band of molecular size (360bp), and the band of molecular size (320bp), and the band of molecular size (280bp), but it is closely associated with the band of molecular size (640bp) (the size and position of the red circle inside the rectangle is very large, as it occupies most of the rectangle's size) [Fig. 3].

The presence of the heavy metal resistance *czcB* gene in 22 *Acinetobacter* spp. isolates as 13 *A. baumannii* isolates (11 clinical isolates and 2 clinical environment isolates), and 9 *A. complex* isolates (7 clinical isolates and 2 clinical environment isolates), was detected using PCR. The amplification of the *czcB* gene resulted in a distinct 400 bp band, confirming its presence in the positive isolates (Fig. 4).

The wells diffusion method, which involved serial dilutions (9-fold) of different cadmium concentration (Stock: 16 mg/l, D1: 8 mg/l, D2: 4 mg/l, D3: 2 mg/l, D4: 1 mg/l, D5: 0.5 mg/l, D6: 0.25 mg/l, D7: 0.125 mg/l, and D8: 0.0625 mg/l respectively), was used to evaluate the cadmium resistance. It was observed that all isolates resisted cadmium at the specified concentrations (D6, D7, and D8), including the isolates that appeared in the molecular (genetic) examination

with the presence of the gene *czcB*. The results were identical in both examinations (genetic and phenotypic). The other isolates also showed resistance to cadmium in the phenotypic examination, but did not show genetic resistance to cadmium associated with this gene. We believe that these other isolates possess another gene within the *czcABC* system, such as the gene *czcA* or the gene *czcC*, or *czcE*. All 22 isolates did not grow at high concentrations ( $\geq 0.5$  mg/l), but grew at the following concentrations 0.25 mg/l (D6): *A. baumannii* = 7/13 (53.8%), *A. complex* = 7/8 (77.8%). All isolates grew at 0.125 mg/l (D7) and 0.0625 mg/l (D8) = 100% growth. Statistical analysis indicates no significant difference in cadmium resistance between *A. baumannii* and *A. complex* at 0.25 mg/l ( $P > 0.05$ ), indicating similar levels of resistance at lower concentrations (Table 5).

## DISCUSSION

The strong correlation between phenotypic and genotypic characteristics resulted in reducing the number of isolates from 60 to 22 representative isolates, helping to reduce costs and analysis time. The absence of significant statistical difference suggests that cadmium resistance may not be solely dependent on species type. ERIC-PCR revealed considerable genomic diversity, consistent with previous reports. *czcB* gene is positive in 4 of 13 *A. baumannii* isolates. 3 of 9 *A. complex* isolates.<sup>9-12</sup>

There are other isolates possess another gene within the *czcABC* system, such as the gene *czcA* or the gene *czcC*, or *czcE*. Evaluating cadmium resistance in *Acinetobacter* species, specifically *A. baumannii* and *A. complex*, provides important insights into how these bacteria respond to heavy metal stress. The relationship between DNA sequence and form is frequently referred to as the genotype-phenotype map (GPM), a term that is used in quantitative genetics about the effects of specific genomic regions on traits of interest. Likewise, in medicine, the GPM may refer to the association between mutations at specific loci and a disease or other condition. Given the complexity of form, researchers have sometimes sought to understand principles of the GPM by looking at specific processes such as RNA folding.<sup>13-15</sup>

## CONCLUSION

*Acinetobacter* species showed resistance to cadmium in various and specific concentrations that represented possible adaptations in the environment by using some genetic factors that may affect metal tolerance. The Resistance to heavy metals in *Acinetobacter* species is mostly mediated by *CzcB* genes that encode to the *CzcCBA* efflux system. ERIC-PCR analysis provided insights into the genetic diversity among the isolates.

**Author's Contribution:**

Concept & Design or acquisition of analysis or interpretation of data:	Ali Khanjar Jaber, Saba Riad Kkudhaier
Drafting or Revising Critically:	Ali Khanjar Jaber, Ali Murtatha Hasan
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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# Role of Photodynamic Therapy in Dentistry

Photodynamic  
Therapy in  
Dentistry

Heba Mahmoud Ashi

## ABSTRACT

**Objective:** To provide a comprehensive overview of the applications, mechanisms, and clinical effectiveness of photodynamic therapy in dentistry across a wide range of oral diseases and conditions.

**Place and Duration of Study:** This study was conducted at the Department of Dental Public Health, Faculty of Dentistry, King Abdulaziz University, Jeddah, Saudi Arabia from January 2025 to October 2025.

**Methods:** A narrative review of the literature was conducted, synthesizing evidence from in vitro studies, randomized controlled trials, clinical investigations, and systematic reviews. The review focused on the mechanisms of PDT, types of light sources and photosensitizers, and its clinical applications in pain management, periodontal and peri-implant diseases, dental caries, endodontic infections, post-extraction complications and oral infections.

**Results:** The reviewed evidence demonstrates that PDT exerts its therapeutic effects through ROS-mediated destruction of microbial cells and modulation of inflammatory processes. Clinically, PDT has shown promising outcomes in reducing pain, improving healing, and decreasing microbial load across various dental conditions. In periodontal and peri-implant diseases, adjunctive PDT enhances clinical parameters such as probing depth and plaque index. In endodontics and caries management, it improves disinfection and reduces bacterial counts. Additionally, PDT has demonstrated effectiveness in managing oral infections and reduced risk of antimicrobial resistance. However, variability in treatment protocols, photosensitizers, and irradiation parameters limits direct comparison across studies.

**Conclusion:** Photodynamic therapy represents a promising, safe, and minimally invasive approach in modern dentistry, with broad applications as both a stand-alone and adjunctive treatment. Despite encouraging clinical outcomes, further well-designed, large-scale studies are required to standardize protocols and establish long-term efficacy.

**Key Words:** Photodynamic therapy; Photosensitizers; Reactive oxygen species (ROS); Periodontal diseases; Peri-implantitis; Dental caries; Endodontic infections.

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## INTRODUCTION

Photodynamic therapy (PDT) is a therapeutic modality that employs light to activate a photosensitizing agent (PS) in the presence of oxygen. The activated PS initiates cytotoxic and vasculotoxic reactions that result in targeted tissue damage<sup>1</sup>. Depending on the agent, photosensitizers can be delivered topically, orally, or intravenously<sup>1</sup>. The relative simplicity of PS activation has generated significant clinical interest<sup>1</sup>, and PDT has already gained regulatory approval in several countries, including Japan, Russia, Canada, the European Union, and the United States<sup>2</sup>.

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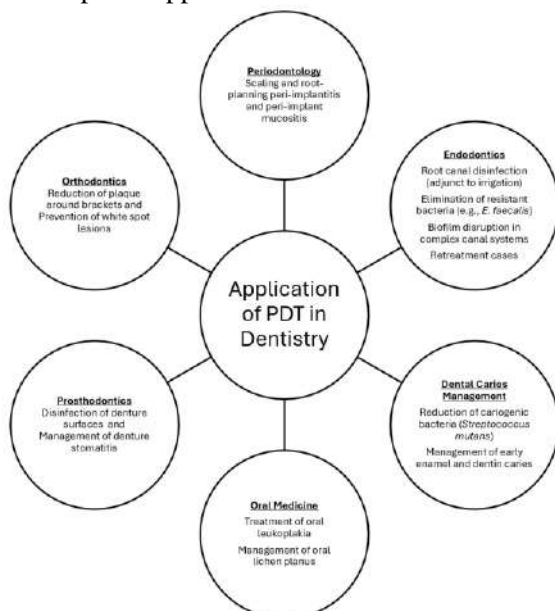
While PDT is most widely used in oncology, growing evidence demonstrates its antimicrobial efficacy<sup>3</sup>. This has given rise to photodynamic antimicrobial chemotherapy (PACT), which has emerged as a potential alternative against drug-resistant bacteria, fungi, and viruses.

PDT integrates photochemical and photophysical processes to achieve biological effects. The core components-light, PS, and oxygen-are individually non-toxic, yet their interaction produces cytotoxic species that induce cell death through multiple molecular pathways<sup>4</sup>. When activated by light of an appropriate wavelength, the PS undergoes excitation, and in the presence of oxygen, reactive oxygen species (ROS) are generated. These species disrupt cellular structures and functions, leading to microbial or tumor cell destruction. Two reaction pathways are described: Type I, in which electron or hydrogen transfer generates free radicals, and Type II, in which energy transfer produces singlet oxygen<sup>5</sup>.

**Light sources:** Various light systems have been explored for PDT activation, including copper vapor, gold, Nd:YAG, and argon lasers, though their high cost and complexity limit routine use<sup>5</sup>. Diode lasers, by contrast, are more portable, economical, and widely

adopted in clinical settings. Alternative sources such as halogen lamps and light-emitting diodes (LEDs) have also shown favorable results<sup>6</sup>. Additionally, the use of intracanal optical fibers has been proposed to improve precision and enhance therapeutic efficiency in dental applications<sup>7</sup>.

**Photosensitizers:** An ideal PS should possess favorable photophysical, chemical, and biological properties, including selective uptake by target cells, strong absorption within the optical transmission window of biological tissues, high stability, reproducibility, low intrinsic toxicity, and minimal post-treatment photosensitivity<sup>5</sup>. In dentistry, phenothiazine derivatives, particularly methylene blue (MB) and toluidine blue O (TBO)-have been extensively studied and shown effectiveness against antibiotic-resistant microbial strains as well as tumor cells<sup>8</sup>. Other candidates, such as pheophorbide a-polylysine, chlorin e6, and riboflavin, activated by blue light sources (380–520 nm), have been proposed for oral PDT<sup>9</sup>. However, limitations in suitable PS availability and incomplete clinical validation remain barriers to widespread application.



**Figure No. 1: Applications of PDT in Dentistry**

## PDT IN PAIN MANAGEMENT

PDT has emerged as a versatile modality for alleviating various types of dental and orofacial pain, including aphthous ulcers, actinic cheilitis, oral lichen planus (OLP), necrotizing ulcerative gingivitis (NUG), pain associated with dental injections, post-operative discomfort in soft tissue surgeries, orthodontic pain, temporomandibular disorders (TMDs), trigeminal neuralgia, and post-extraction pain<sup>10-12</sup>.

In patients with aphthous ulcers, PDT has been shown to significantly reduce lesion size and pain severity while accelerating healing. A randomized controlled trial (RCT)<sup>10</sup> demonstrated that a single session of PDT was sufficient to achieve meaningful clinical improvements, highlighting its practicality as a treatment option, although the study was limited by a small sample size and lack of microbiological analysis<sup>10</sup>. Similarly, in actinic cheilitis, both conventional PDT and indoor daylight PDT (idl-PDT) produced comparable reductions in lesion size and severity; however, idl-PDT was better tolerated, with lower pain scores and milder inflammatory responses<sup>11</sup>. For OLP, PDT has demonstrated symptomatic relief and improved functionality, although current evidence is limited by small patient populations and heterogeneous study designs. Systematic reviews indicate significant reductions in lesion size and pain, with some studies suggesting PDT may outperform corticosteroid therapy, particularly in corticosteroid-resistant cases. Nonetheless, further large-scale, RCTs are needed to establish standardized protocols and validate comparative efficacy<sup>12</sup>.

PDT has also shown clinical benefits in NUG management. When combined with mechanical debridement (MD), it produced superior pain reduction and decreased bacterial load, including *Fusobacterium nucleatum* and *Prevotella intermedia*, as assessed by PCR over a 12-week follow-up period<sup>13</sup>. In the context of dental injection pain, photobiomodulation therapy (PBM) has been shown to alleviate needle-related discomfort, with several RCTs reporting significant reductions in pain scores<sup>14</sup>.

## PDT IN PERI-IMPLANT DISEASES

Peri-implant diseases, including peri-implant mucositis and peri-implantitis, represent inflammatory conditions driven by bacterial biofilms on implant surfaces. Conventional treatment typically involves MD, sometimes supported by systemic or local antimicrobials. Given concerns about antibiotic resistance, antimicrobial photodynamic therapy (aPDT) has been investigated as an adjunctive or alternative therapy<sup>15</sup>.

Systematic reviews and meta-analyses suggest that aPDT may enhance clinical outcomes, particularly in reducing probing depth (PD) and plaque scores. Zhao et al<sup>16</sup> reported greater improvements in smokers treated with adjunctive aPDT compared to MD alone, though heterogeneity and limited sample sizes warrant cautious interpretation. Similarly, Shahmohammadi and colleagues<sup>17</sup> observed significant short-term reductions in plaque index (PI) and PD with adjunctive aPDT but emphasized the lack of consistent long-term evidence.

Microbiological analyses further support the antimicrobial potential of aPDT in peri-implantitis. Lopez et al<sup>18</sup> demonstrated significant reductions in bacterial loads on implant surfaces following aPDT, while a meta-analysis by Fraga et al<sup>19</sup> confirmed significant decreases in *Prevotella intermedia*,

*Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*. Collectively, these findings indicate that aPDT is effective in reducing peri-implant pathogenic bacteria and may serve as a viable alternative to antibiotics. However, inconsistencies in light parameters, PS types, and follow-up durations underscore the need for rigorously designed, long-term RCTs before definitive recommendations can be made.

### PDT IN PERIODONTAL DISEASES

Conventional management of periodontal disease relies primarily on MD to remove plaque, calculus, and biofilm, often supplemented by systemic or local antibiotics. While antibiotics enhance treatment outcomes, their frequent use raises concerns about resistance development, prompting the exploration of alternative antimicrobial strategies. Over the past two decades, aPDT has emerged as a promising adjunctive modality<sup>20</sup>.

aPDT exerts bactericidal effects through the light-activated generation of ROS, leading to disruption of microbial cell structures. In vitro investigations have demonstrated that common periodontopathogens, including *A. actinomycetemcomitans* and *P. gingivalis*, are highly susceptible to aPDT. Similarly, photodynamic inactivation using erythrosine or TBO has achieved more than a 10-fold reduction in *Streptococcus sanguinis*, *S. sobrinus*, and *S. mutans* biofilms<sup>21</sup>.

Clinical studies consistently report improvements in PD, bleeding on probing (BOP), and PI when aPDT is used alongside scaling and root planing (SRP)<sup>22</sup>. However, head-to-head comparisons of antibiotics versus aPDT have yielded variable microbiological and clinical outcomes, with some trials favoring antibiotics while others show comparable efficacy<sup>23</sup>. A recent study concluded that aPDT, when combined with SRP, produces clinical improvements similar to systemic antibiotic regimens such as amoxicillin with metronidazole<sup>24</sup>. Nevertheless, substantial heterogeneity in study design, PS type, irradiation parameters, and pre-irradiation times limits definitive conclusions. Collectively, current evidence positions aPDT as a potentially valuable alternative or adjunctive therapy in periodontics, though greater standardization is required to establish its optimal clinical application<sup>25</sup>.

### PDT IN DENTAL CARIES

Dental caries is a biofilm-mediated, multifactorial disease characterized by cycles of demineralization and remineralization of tooth hard tissues. Effective management relies on disrupting cariogenic biofilms, and aPDT, also referred to as PACT, has gained attention as a potential adjunctive strategy<sup>26</sup>. This approach employs PS activated by specific light sources, resulting in the production of reactive oxygen species that destroy cariogenic bacteria<sup>27</sup>.

Early laboratory and clinical studies by Bevilacqua and Wilson confirmed the antimicrobial potential of TBO-mediated PDT with LED or laser activation,

demonstrating significant bacterial reduction in both in vitro and in vivo models. Interest in this modality declined during the antibiotic era but has resurged with the emergence of multidrug-resistant microbes and recognition of the role of dental plaque in caries progression<sup>28</sup>. A key advantage of PDT over antibiotics is its low risk of resistance development, as bacteria are unlikely to adapt to the cytotoxicity of singlet oxygen and ROS<sup>29</sup>. Furthermore, polysaccharides within dental plaque biofilms are also susceptible to photodynamic damage, providing an added benefit compared with antimicrobial drugs<sup>30</sup>.

Systematic reviews and meta-analyses further support PDT's utility<sup>31</sup>. Ornellas et al<sup>31</sup> concluded that PDT significantly reduces microbial counts in deep carious lesions. De Oliveira et al<sup>32</sup> emphasized methodological heterogeneity but acknowledged PDT's therapeutic potential, while Cieplik et al<sup>33</sup> reported consistent reductions in cariogenic bacteria following mechanical caries removal combined with adjunctive aPDT. Despite some limitations, including variable protocols and lack of standardized controls, the cumulative evidence suggests that PDT is a promising adjunctive approach for reducing bacterial burden in deep caries before restorative procedures.

### PDT IN PERIAPICAL INFECTIONS

Periapical periodontitis, typically arising from inadequate root canal disinfection, is another condition where PDT has shown a promising result. Garcez et al<sup>34</sup> evaluated PDT as an adjunct to two-visit root canal therapy in patients with radiographically evident periapical lesions. Adjunctive PDT significantly enhanced bacterial log reduction compared to conventional treatment alone<sup>34</sup>. Other clinical investigations comparing calcium hydroxide dressings with diode laser therapy found similar levels of periapical healing, though diode laser application reduced lesion size without reaching statistical significance<sup>35</sup>. Collectively, these studies suggest that PDT, when integrated with standard endodontic protocols, may improve antimicrobial efficacy and enhance periapical healing outcomes.

### PDT IN POST-EXTRACTION COMPLICATIONS

PDT has also been evaluated for the prevention and management of post-extraction complications such as dry socket and pain. In a controlled trial, Neugebauer et al<sup>36</sup> compared HELBO Blue-mediated PDT with conventional care in patients undergoing bilateral extractions. The PDT-treated sites showed a markedly reduced incidence of dry socket (2% vs. 26% in controls) and significantly lower pain scores both immediately post-extraction and at one-week follow-up. These findings suggest that PDT may provide a reliable adjunctive strategy to reduce morbidity following dental extractions.

## PDT IN ORAL INFECTIOUS DISEASES

Beyond oncology, aPDT is gaining traction for managing oral infections. Recurrent oral candidiasis, often resistant to topical antifungals, has shown favorable responses to PDT<sup>37</sup>. Laboratory studies demonstrated that Photofrin®-mediated PDT effectively inactivated multiple *Candida* species<sup>38</sup>. Likewise, methylene blue-mediated PDT has been successfully applied in herpes labialis, reducing recurrence rates, accelerating lesion healing, and improving patient comfort without significant adverse effects<sup>39</sup>.

## CONCLUSION

Photodynamic therapy (PDT) has emerged as a versatile and promising modality in dentistry, offering both antimicrobial and therapeutic benefits through the generation of reactive oxygen species. The evidence reviewed highlights its effectiveness across a wide spectrum of applications, including pain management, periodontal and peri-implant diseases, dental caries, endodontic and periapical infections, post-extraction complications, and oral infectious conditions. PDT demonstrates significant advantages such as broad-spectrum antimicrobial activity without inducing resistance, minimal invasiveness, targeted tissue action, and favorable patient outcomes including pain reduction and enhanced healing. Additionally, its potential as an adjunct to conventional therapies often results in improved clinical and microbiological outcomes. Despite these encouraging findings, the heterogeneity in study designs, photosensitizers, light sources, and treatment protocols limits the ability to establish standardized clinical guidelines. Therefore, further well-designed, large-scale randomized controlled trials are required to optimize treatment parameters and confirm the long-term efficacy and clinical applicability of PDT in routine dental practice.

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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# Psychological Intervention for Caregiver Distress During Prolonged Pediatric Burn Hospitalization: A Consultation Liaison Psychiatry Case Report

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## ABSTRACT

Severe pediatric burns often require prolonged hospitalization and are accompanied by significant psychological burden affecting both the child and caregivers. Despite this, structured psychological interventions for caregivers within inpatient settings remain underreported. We describe a case involving a 6-year-old boy with extensive burn injury affecting 57 percent of total body surface area and a complicated seven-month hospital course. His mother, the primary caregiver, developed marked parental stress and caregiver burden characterized by persistent guilt, intrusive recollections, anxiety symptoms, and anticipatory concerns about her child's recovery and future functioning. A brief structured cognitive behavioral intervention was delivered by the consultation liaison psychiatry team at the bedside, focusing on maladaptive guilt related cognitions, problem solving, and emotional regulation strategies. At follow up, the caregiver demonstrated clinically meaningful improvement across standardized measures of stress, anxiety, and caregiver burden, alongside reduced rumination and improved functional coping. This case highlights the potential value of integrating targeted psychological support for caregivers within pediatric burn care. Early intervention may reduce maladaptive guilt, improve caregiver resilience, and support sustained engagement in the child's recovery.

**Key Words:** Burns, pediatric, caregiver distress; liaison psychiatry; Psychological

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## INTRODUCTION

Severe pediatric burns often require prolonged hospitalization and repeated procedures, creating sustained psychological strain not only for the child but also for caregivers.

Parents, particularly primary caregivers, frequently experience persistent anxiety, guilt, and emotional exhaustion during the treatment course, especially when

recovery is complicated by recurrent medical setbacks.<sup>1,2</sup> Parental stress and caregiver burden, while related, reflect different dimensions of this experience. Parental stress arises from the perceived imbalance between caregiving demands and coping resources, whereas caregiver burden encompasses the broader emotional, functional, and socioeconomic impact of long-term care.<sup>3-5</sup> Both are associated with impaired psychological well-being and reduced capacity to engage effectively in treatment. Despite growing recognition of caregiver distress in pediatric settings, structured psychological interventions during hospitalization remain limited.<sup>3-5</sup> This case highlights the role of consultation liaison psychiatry in addressing caregiver distress through a brief, targeted cognitive behavioral approach integrated within inpatient care.

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## CASE REPORT

A 6-year-old boy was admitted following scald injury from boiling broth, resulting in deep partial and full thickness burns involving approximately 57 percent of total body surface area. He underwent repeated surgical debridement and grafting procedures and experienced a prolonged and complicated hospital course marked by recurrent infections, graft failure, and intermittent readmission. At the time of psychiatric evaluation in February 2026, he remained hospitalized. Behaviorally,

the child showed a clear deviation from premorbid functioning. Previously described as cheerful and cooperative, he developed irritability, tantrums, aggression toward his mother, and sleep disturbance. These symptoms were closely associated with pain and febrile episodes. Psychiatric assessment supported a diagnosis of adjustment related emotional disturbance. The child's 42-year-old mother served as the sole caregiver throughout hospitalization. She reported persistent guilt related to the injury, intrusive recollections of the event, somatic anxiety symptoms, and ongoing concern regarding her child's future. Despite these difficulties, she remained actively engaged in caregiving and adhered to medical recommendations. There was no prior psychiatric history.

Psychometric evaluation revealed mild depressive symptoms, moderate anxiety, and moderate stress on DASS 21. Caregiver burden was elevated, with a ZBI score indicating moderate to severe burden, and parental stress was markedly high based on PSS assessment. Mental status examination demonstrated intact orientation, coherent thought processes, and preserved insight, though affect was dominated by anxiety and guilt. There was no evidence of suicidality. The clinical formulation suggested a stress related affective condition driven by prolonged caregiving demands, recurrent medical setbacks, and maladaptive guilt related cognitions.

A brief cognitive behavioral intervention was delivered across four sessions at the bedside. The intervention targeted maladaptive guilt, rumination, and anticipatory anxiety through cognitive restructuring, problem solving strategies, and behavioral techniques for emotional regulation and sleep. Psychoeducation was provided regarding burn recovery and normal caregiver responses to stress. Practical strategies were introduced to improve coping during medical setbacks and to support planning for the child's reintegration into daily activities.

At follow up, the caregiver reported reduced frequency and intensity of self-blaming thoughts, improved sleep, and greater ability to manage distress during clinical fluctuations. Objective measures demonstrated improvement across all domains, including reductions in anxiety, stress, and caregiver burden. Functional improvements included better concentration and more structured problem solving.

## DISCUSSION

This case reflects a common but often under-recognized clinical scenario in pediatric burn care, where caregiver distress evolves alongside the child's prolonged medical course. The intensity of caregiving demands, combined with uncertainty and repeated clinical setbacks, creates conditions that reinforce maladaptive cognitive patterns such as guilt and catastrophizing.

Existing literature consistently highlights the association between caregiver burden and reduced psychological well-being, particularly in contexts of limited social support. In pediatric burn populations, the treatment phase itself often represents the period of greatest psychological strain, as caregivers assume active roles in care while managing emotional responses to the child's suffering.<sup>1,2</sup> What stands out in this case is how central maladaptive cognition was in maintaining distress. The caregiver's belief that she was responsible for the injury amplified emotional burden and interfered with adaptive coping. Addressing these beliefs directly through cognitive behavioral strategies appeared to be a key mechanism of improvement. Although caregiver support is widely recommended, structured psychological interventions remain inconsistently implemented in inpatient settings. This case suggests that even brief, targeted interventions delivered within a consultation liaison framework can lead to measurable improvements in caregiver functioning. From a clinical perspective, supporting caregivers is not only beneficial for their own mental health but may also influence treatment adherence, communication with the medical team, and ultimately the child's recovery trajectory.

## CONCLUSION

Prolonged pediatric burn hospitalization can lead to significant caregiver distress, even in individuals without prior psychiatric history. In this case, maladaptive guilt and sustained stress were key drivers of psychological burden. A brief cognitive behavioral intervention delivered within a consultation liaison psychiatry setting was associated with meaningful improvement in both emotional symptoms and caregiving capacity. These findings support the integration of early, structured psychological support for caregivers as part of routine pediatric burn care.

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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**Authors Contributions:** W.S. contributed to patient assessment, data collection, and drafting of the manuscript. A.K. conceptualized the study, supervised the clinical intervention, and critically revised the manuscript for important intellectual content. I.H. contributed to clinical management of the patient and provided surgical input relevant to the case. All authors reviewed and approved the final version of the manuscript.

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# Attachment Style and Pain Perception in Burn Injury: A Case Report

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Anxious Attachment and Burn Pain Perception

## ABSTRACT

Burn pain is strongly influenced not only by tissue injury but also by psychological factors. We report the case of a 55-year-old man admitted with partial-thickness electrical burns involving approximately three percent total body surface area. Although the physical injury was limited, he reported severe and persistent pain accompanied by marked anxiety about disability and burdening his family. Psychiatric consultation was requested. His developmental history and current coping pattern were consistent with an anxious attachment style, which appeared to amplify pain perception and catastrophic thinking. He was diagnosed with adjustment disorder with anxiety related to burn injury and received supportive psychotherapy, cognitive-behavioral interventions, and FRAMES-based counseling alongside standard burn care. As anxiety decreased, his reported pain intensity also improved. This case underlines the importance of considering attachment style and psychological distress in burn pain management and supports the role of early psychiatric involvement in multidisciplinary burn care.

**Key Words:** Burns; Electric Injuries; Pain Perception; Attachment Behavior; Anxiety Disorders; Adaptation, Psychological

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## INTRODUCTION

Burn pain is a complex clinical problem shaped not only by tissue injury but also by emotional and cognitive responses. Psychological factors, including attachment style, influence how individuals interpret and cope with stress, illness, and pain.<sup>1</sup> Attachment style reflects relational patterns formed early in life and carried into adulthood, and has been linked to differences in distress tolerance and threat perception. Individuals with insecure attachment, particularly anxious attachment, may experience greater worry and amplified pain perception when faced with medical trauma.<sup>2</sup> Recognizing this relationship may help clinicians provide more holistic care for burn patients.

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This case report describes a burn patient with anxious attachment style whose pain perception and anxiety improved following targeted psychological interventions.

## CASE REPORT

A 55-year-old married man who worked intermittently as a construction laborer and farmer was admitted to the burn unit after accidental electrocution while repairing the ceiling of his home. He sustained partial-thickness second-degree burns involving about three percent of the total body surface area, affecting the left hand and both feet. Although initially shocked by the incident, he expressed a strong desire to recover quickly so he could return to work, as he was the main financial provider for his family and continued to support a school-aged child. On admission he reported severe pain rated 8 out of 10, which worsened during dressing changes and debridement and improved only temporarily with intravenous analgesia. Over time he became increasingly worried that the pain would be permanent, that he would no longer be able to work, and that he would become a burden to his wife. These concerns contributed to sleep disturbance and significant anxiety. He denied previous psychiatric illness and described himself as disciplined and emotionally reserved. He recalled a childhood marked by inconsistent caregiving, with a strict father and a permissive mother who nevertheless reported his misbehavior to his father, which led him to suppress emotions to avoid conflict. On examination he was alert and hemodynamically stable, with normal neurological and laboratory findings. The burns were confirmed as partial-thickness electrical injuries involving approximately three percent total body surface area. Because the intensity of reported pain appeared disproportionate to the physical

severity of the burns, the burn team requested a psychiatric consultation.

Psychiatric assessment revealed marked anxiety focused on pain, disability, and financial responsibility, accompanied by a tendency toward catastrophizing and concerns about dependency on others. His presentation and developmental history were consistent with an anxious attachment style that appeared to heighten pain perception and emotional distress. He was diagnosed with adjustment disorder with anxiety related to burn injury. The patient continued to receive standard burn care and underwent five debridement procedures under general anesthesia, with medical pain management and rehabilitation therapy to prevent contractures. Psychiatric treatment included supportive psychotherapy, FRAMES-based counseling to encourage adaptive coping, and cognitive behavioral techniques to address catastrophic beliefs and attachment-related fears. Clobazam 5 mg nightly was prescribed to support anxiety relief and sleep. With coordinated multidisciplinary care, his anxiety gradually improved, and reported pain intensity decreased from 8 out of 10 to about 3 out of 10. By the time of discharge, he expressed greater confidence in recovery and increased willingness to communicate his emotional needs to both his family and the treatment team.

## DISCUSSION

Pain in burn injury reflects not only tissue damage but also psychological and emotional processing.<sup>1</sup> In some patients, reported pain intensity may exceed what would be expected from the physical injury alone. Anxiety, catastrophic thinking, and relational insecurity can heighten attention to pain and lower tolerance.<sup>2,3</sup> In this case, the patient experienced severe and persistent pain despite relatively limited burn involvement. His worries about disability, loss of income, and burdening his family appeared to amplify the subjective pain experience. Attachment theory provides a useful lens for understanding this pattern. Individuals with anxious attachment often struggle with insecurity, high threat sensitivity, and a strong need for reassurance in stressful situations. Early caregiving inconsistency can impair emotional regulation, making acute illness particularly distressing.<sup>4</sup> For this patient, longstanding tendencies to internalize emotion and avoid conflict contributed to heightened anxiety when hospitalized, which in turn intensified pain perception.

Psychological intervention played an important role alongside medical care.<sup>5</sup> Supportive psychotherapy and cognitive behavioral techniques helped the patient explore and reframe catastrophic fears, while the FRAMES approach encouraged active coping. As emotional distress improved, his reported pain intensity declined. This reinforces the value of integrating psychological care into burn management, particularly for patients with insecure attachment or significant worry. Although this is a single case, it highlights how attachment style may influence the pain experience in burn injury. Early psychiatric consultation can support

pain control, improve coping, and prevent prolonged distress. Future research may clarify how screening and targeted intervention could strengthen multidisciplinary burn care.

## CONCLUSION

This case illustrates how psychological factors, particularly attachment style, can influence the perception of pain in burn injury. In this patient, an anxious attachment pattern contributed to heightened worry, catastrophic thinking, and amplification of pain despite relatively limited tissue damage. When psychological distress was identified and addressed through supportive and cognitive-behavioral approaches, both anxiety and reported pain intensity improved. These findings stress the value of early psychiatric consultation and multidisciplinary collaboration in burn care. Awareness of attachment related vulnerability may help clinicians provide more holistic treatment, strengthen coping, and reduce the risk of prolonged distress in patients recovering from burn trauma.

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