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Editorial

Requirement of Food and Fluid for Body Fitness Before, During and After Exercise

Prof. Dr. Azhar Masud Bhatti
Editor-in-Chief

Recently, the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine published a joint position statement regarding nutrition and athletic performance that included recommendations about food and fluid consumption before, during, and after exercise[1].

Appropriate fueling before, during, and after exercise can assist in supporting all of the following: energy balance, weight management, health, and fitness.

Before Exercise:

In general, before-exercise meals should be consumed 3 to 4 hours before exercise. Meals should contain between 1 to 4 g CHO/kg or 0.5 to 2 g CHO/lb of body mass[1] (e.g., whole grains, cereals, pasta, rice, potatoes, vegetables, fruit), moderate protein (e.g., chicken, tofu, fish, low fat dairy, eggs), and some fat (e.g., olive oil, nuts). To meet weight loss goals, half of the plate should be from vegetables and fruit, one fourth of the plate from lean protein, and the rest from whole grains or legumes (e.g., beans, lentils). This approach offers a balanced, nutrient-dense meal that is relatively low in calories but satisfies the appetite[2].

Thus, more processed, easily digestible CHO, including starchy vegetables, are appropriate for prolonged exercise, high-intensity or intermittent-type physical demands because these foods can be absorbed quicker than less processed starches, and if consumed in higher amounts and sufficiently before the onset of exercise, they contribute to stored energy (e.g., liver and muscle glycogen) for the exercise session.[3]

Four hours before a workout, individuals are advised to drink 5 to 7 mL of water or sport drink/kg body mass (~1.5 to 2 cups)[4].

During Exercise:

CHO intake during exercise has been shown to maintain energy levels and improve exercise capacity and performance of endurance and intermittent type sports.[5,6,7]

Fluid replacement during exercise should occur according to sweat rate, which can vary with environmental factors such as heat and humidity, exercise intensity, sport, age, and sex.[4]

After Exercise:

The aim of recovery nutrition is to replace what is lost during exercise (e.g., fluid, glycogen) and to support an optimal hormonal and metabolic environment to promote muscle building and repair, ultimately resulting in training adaptations. Therefore, the initial strategy for recovery nutrition should include fluid, electrolytes (e.g., sodium), CHO, and protein.

To achieve optimal rehydration after exercise, 1.5 times more fluid should be consumed than what was lost. Consuming rehydration beverages with electrolytes or consuming water with a snack and continuing to rehydrate with subsequent meals/ snacks will optimize fluid and electrolyte replacement.

The window for optimal recovery of muscle energy (glycogen) stores ranges from 30 minutes to 4 hours after exercise. To fully replenish glycogen stores (e.g., after a marathon, soccer match, or heavy 2-hour lifting protocol), 24 hours are needed generally[8].

Generally don't need to refuel with carbohydrates after a modest activity like a brisk walk. If your exercise was more intense, it probably depleted your glycogen reserves, which is the body's preferred energy source for intense exercise. Muscle tissue starts to break down when the muscles' glycogen stores are low. Eating carbs is essential for supporting the body's repair and regrowth. "Carbohydrates help the body release insulin after exercise, which replenishes the glycogen stores that were just depleted during your workout. Pairing carbs with protein improves how well they accomplish this. The pace at which the body stores glycogen is accelerated by the combination of proteins and carbohydrates.

Carbohydrates help prevent post-workout fatigue. Reintroducing carbohydrates to your diet can make you recover more quickly and with less fatigue. Additionally, studies show that eating carbohydrates after exercise increases one's capacity for endurance during subsequent workouts.

Carbohydrates help blood sugar control and blood sugar levels drop when you exercise in most cases. Never hold off on grabbing a food that is high in carbohydrates to bring your glucose back in the positive range. By doing this, you can avoid unpleasant hypoglycemia symptoms including trembling, headache and a quick heartbeat.

Carbohydrates aid in Muscle Recovery. Although you may believe that protein is the only macronutrient that helps you gain muscle, carbohydrates also play a supportive role in this process. Amino acids are the building blocks of protein, and carbohydrates help them reach muscles more quickly, accelerating muscular growth and recovery.

Carbohydrates lower cortisol levels. In response to low blood sugar, cortisol – often referred to as "the stress hormone" – increases. It's possible that replenishing your carbohydrate reserves to maintain a stable blood sugar level will also lower cortisol levels.

After exercise complex carbohydrate foods include whole grains, vegetables, beans, and potatoes, which are also high in fibre. Eating high-fibre foods soon after engaging in vigorous exercise may occasionally cause digestive issues. Simple carbohydrates may be tolerated better post-workout.

The Academy of Nutrition and Dietetics advises consuming carbohydrates an hour after a strenuous activity.

Recent research suggests that consuming approximately 15 to 25 g of protein, typically found in milk (8 g of protein per cup), a Greek yogurt (15 to 20 g of protein per cup), or commercially available recovery products (e.g., CHO protein mix, CHO-protein bar), is the maximum needed to stimulate muscle repair and growth after exercise. Protein should be ingested as part of a recovery snack or beverage as soon as is possible after exercise, especially after resistance exercise.[9]

You can add after heavy exercise the following proteins;

Hard-Boiled Egg

Make a batch of hard-boiled eggs and keep them in the refrigerator for a quick, gluten-free, grab-and-go snack. Each large egg provides 6 grams of protein, 5 grams of fat, and less than a gram of carbohydrate. Egg whites offer 4–5 grams of protein and almost no fat. The yolk provides about 2.7 grams of protein and different types of fat, including healthy mono- and polyunsaturated fats.

Cottage Cheese with Blueberries

A 3.5 ounce serving of 2% low-fat cottage cheese provides 11 grams of protein, 84 calories, 2.3 grams of fat, and 4.3 grams of carbohydrate.

Chia Pudding

Chia seeds are full of fibre, protein, and healthy omega-3 fatty acids. One ounce of chia seeds provides 4.7 grams of protein, 138 calories, 8.7 grams of fat, and 12 grams of carbohydrate.

Tuna Salad

Fresh, canned, or water-packed tuna is a great source of protein and there are so many ways to prepare and eat it. A whole can (165g) of tuna provides 42 grams of protein, 191 calories, 1.4 grams of fat, and 0 grams of carbohydrate.

Mixed Nuts

Plain or roasted nuts are delicious, crunchy, and easy to eat,15 making them a perfect high-protein snack. Have a generous handful (about ¼ cup) to get 6 grams of protein. Almonds may be one of the most popular nuts, but you also can choose pecans, macadamia nuts, or even Brazil nuts. All have plenty of protein.

Chilled Shrimp

Cooked, chilled shrimp is a great high-protein snack to grab when you need something light but meaty. A single 3-ounce (85 gram) serving of shrimp provides about 20.4 grams of protein, 84 calories, 0.2 grams of fat, and 0.2 grams of protein. The number of shrimp in a single serving will vary depending on the size and the type of shrimp you buy. Shrimp is also a great source of energising vitamin B12, and brain-boosting choline along with zinc, iron, and selenium.

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Original Article

Frequency of Thyroid Dysfunction in Patients with Gestational Diabetes

Thyroid Dysfunction in Gestational Diabetes

Nazia Hakeem, Wajiha Karim, Rabia Jamil, Afshan Shahid, Pooja Seetlani and Kanwal Jan Memon

ABSTRACT

Objective: To determine the frequency of thyroid dysfunction in patients with gestational diabetes.
Study Design: Cross sectional study
Place and Duration of Study: This study was conducted at the Department of Obstetrics and Gynaecology, Dow International Medical College from June 2023-December 2023.
Methods: Informed consent was taken. All pregnant women who visited to OPD during 24-28 weeks gestation with gestational diabetes confirmed from 75gm oral glucose tolerance test(OGTT), fasting ≥ 5.1 mmol/l (92 mg/dl); 1-h ≥ 10.0 mmol/l (180 mg/dl); or 2-h ≥ 8.5 mmol/l (153 mg/dl) were subjected for the assessment of serum TSH level for thyroid dysfunction. If patient with serum TSH levels < 0.3 was considered as hyperthyroidism. Patients with serum TSH levels > 4.0 was considered as hypothyroidism. In both cases patient were categorized for thyroid dysfunction. Patients demographic information, such as age, BMI and socioeconomic status were recorded in a self-designed proforma. Women with preexisting diabetes and thyroid dysfunction were excluded.
Results: This study was conducted on 213 pregnant patients presenting with gestational diabetes. The mean age of the patients recorded was 29.60±5.93 years. The mean height of the patients was 1.65±0.03 meter. The mean weight of the patients was 72.60±441 kg and the mean BMI recorded was 26.70±20.01 kg/m^2. In our study there were 113 (53.1%) patients in the age group of 20 to 30 years and there were 100 (46.9%) patients in the age group of 31 to 40 years. The frequency of thyroid dysfunction in patients with gestational diabetes in our study was 30 (14.1%)
Conclusion: The prevalence of thyroid dysfunction in gestational diabetes was 14.08%, therefore screening should be offered to high risk pregnant women.
Key Words: Gestational diabetes, Pregnancy, Thyroid dysfunction, Third trimester, maternal outcome

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INTRODUCTION

"Gestational diabetes (GDM) is defined as any degree of glucose intolerance that begins or is first detected during pregnancy". Previously, screening for gestational diabetes was done via history and examination. Personal or family history of type 2 diabetes, obstetrics history such as recurrent pregnancy losses, overweight baby and anomalous baby. Sedentary lifestyle and presence of obesity all were included in the screening of gestational diabetes. This screening strategy was not effective despite it's proper use, about half of the pregnant women with GDM were missed by this screening strategy. [1, 2]

The thyroid is an endocrine gland that sits underneath the inferior half of the larynx at the superior segment of the trachea in the anterior midline of the neck. It is made up of two oval lobes joined by an isthmus .[3]The thyroid gland is made up of spherical follicles that are surrounded by follicular cells and contain a material known as colloid, which is thyroglobulin. This thyroglobulin, along with the circulating iodine forms monoiodotyrosine and diiodotyrosine, are precursors of the thyroid hormones tetraiodothyronine (T4) and triiodothyronine (T3), respectively.[4]

In general population, thyroid dysfunction is fairly common, predominantly among women; additionally, it has been proposed that the total of undetected cases of thyroid dysfunction may be twice as high as the sum of detected cases. Thyroid dysfunction is typically categorized as hyperthyroidism or hypothyroidism. Hypothyroidism is a condition characterized by a lessening in thyroid gland function and thyroid hormone secretion while Hyperthyroidism is a condition characterized by surplus of thyroid hormone production. [5, 6]

Testing for thyroid dysfunction in diabetic pregnant patients is of great value in detecting thyroid disorders, and the screening for thyroid dysfunction in pregnancy remains a contentious issue.[7] Hypothyroidism in pregnancy increases the risks of miscarriage, preeclampsia, placental abruption, preterm delivery, intrauterine fetal death, fetal neurological disorders,

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mental illness and lower than average IQ later in life.[7] It is therefore suggested to increase the screening range for thyroid disorders so that various maternal and fetal morbidities could be timely prevented.[7] A study reported in India showed the prevalence of hypothyroidism in patients with GDM was 87.2%, which is considerably very higher. Another study conducted in Iran showed the prevalence of thyroid dysfunction 16.6% in women with GDM.[8]

Thyroid dysfunction in pregnancy could lead to serious complications and pose risks to mother and neonate lives. Therefore, in time diagnosis of thyroid dysfunction would help in preventing unfavorable situations related to feto-maternal outcome. The primary aim of the study is to determine the frequency of thyroid dysfunction in patients with gestational diabetes in our local setup. The outcome of this study will be shared with health care professionals in understanding the risks associated with this condition and adopting effective approach to counter unfavorable outcomes.

METHODS

This cross sectional study was carried out in the department of Obstetrics and Gynaecology, Dow International Medical College from June 2023-December 2023 after receiving approval from the hospital's ethics board and the research unit of the College of Physicians and Surgeons of Pakistan. Informed consent taken. All pregnant women who visited to OPD during 24-28 weeks gestation with gestational diabetes confirmed from 75gm oral glucose tolerance test(OGTT), fasting ≥ 5.1 mmol/l (92 mg/dl); 1-h ≥ 10.0 mmol/l (180 mg/dl); or 2-h ≥ 8.5 mmol/l (153 mg/dl) were subjected for the assessment of serum TSH level for thyroid dysfunction. If patient with serum TSH levels < 0.3 was considered as hyperthyroidism. Patients with serum TSH levels > 4.0 was considered as hypothyroidism. In both cases patient were categorized for thyroid dysfunction. Patients demographic information, such as age, BMI and socioeconomic status were recorded in a self designed proforma. Women with preexisting diabetes and thyroid dysfunction were excluded.

RESULTS

This study was conducted on 213 pregnant patients presenting with gestational diabetes. The mean age of the patients recorded was 29.60±5.93 years. The mean height of the patients was 1.65±0.03 meter. The mean weight of the patients was 72.60±441 kg and the mean BMI recorded was 26.70±20.01 kg/m^2. In our study there were 113 (53.1%) patients in the age group of 20 to 30 years and there were 100 (46.9%) patients in the age group of 31 to 40 years. The frequency of thyroid dysfunction in patients with gestational diabetes in our study was 30 (14.1%). Regarding the socioeconomic status, there were 44 (20.7%) patients having income between 10000 to 20000 Rs/month. There were 137 (64.3%) patients having income between 20000 to 50000 Rs/month and there were 32 (15%) patients having income > 50000 Rs/month as shown in table 1.

Table No.1: Sociodemographic characteristics and presence or absence of Thyroid dysfunction

Age distribution	Frequency (n)	Percent (%)
20 to 30 years	113	53.1
31 to 40 years	100	46.9
Socioeconomic status 10000 to 20000 Rs/Month	44	20.7
20000 to 50000 Rs/Month	137	64.3
> 50000 Rs/Month)	32	15.0
Thyroid dysfunction Yes	30	14.1
No	183	85.9

Table No.2: Stratification of thyroid dysfunction with Age, BMI and Socioeconomic status.

Age	Yes	No	Total	P value
20-30 years	12(5.6%)	101(47.5%)	113(53.1%)	0.12
31-40years	18(8.4%)	82(38.5%)	100(46.9%)	
BMI				
22 to 24.9 kg/m^2	5(2.3%)	42(19.7%)	47(22.1%)	
25 to 30 kg/m^2	21(9.8%)	130(61.0%)	151(71.0%)	0.29
> 30 kg/m^2	4(1.8%)	11(5.16.%)	15(7.0%)	
Socioeconomic status				
10000-20000	10(4.69%)	34(15.96%)	44(20.65%)	
20000-50000	16(7.51%)	121(56.8%)	137(64.31%)	0.17
>50000	4(1.8%)	28(13.14%)	32(14.94%)	

DISCUSSION

Thyroid dysfunction and gestational diabetes during pregnancy are two most common illnesses that affect pregnancy outcomes. Diabetes, which has a frequency of 3.5–5% during pregnancy, is one of the most prevalent metabolic illnesses and is characterised by high blood glucose levels and metabolic changes in lipids, carbohydrates, and proteins. Preeclampsia, premature labour, miscarriage, congenital malformations, shoulder dystocia, and stillbirth for the foetus are among its known prenatal problems. As a result, it poses a significant risk for unfavourable pregnancy outcomes. About 40% of women with GDM will develop overt diabetes during the next 20 years since the consequences of diabetes persist even after childbirth. It has been established that thyroid malfunction during pregnancy has negative consequences on the development of the foetus.

The manufacture and release of foetal thyroid hormones do not start until the 20th week of pregnancy, so foetal growth in the first trimester is entirely dependent on the thyroxine supplied by the mother. The foetus needs thyroxine for appropriate growth, particularly for brain development. Maternal thyroid function and pregnancy outcomes are closely correlated, and it has been noted that early pregnancy foetal death increases when thyroid autoantibodies are present in the mother's blood. Negative pregnancy outcomes are associated with both hypothyroidism and untreated thyrotoxicosis. Subclinical hypothyroidism has been linked to an increased risk of pregnancy problems, including placental abruption (which increased three times), preterm labour (which increased twice), and low birth weight babies (increased twice)[9]. Additionally, a number of studies showed a correlation between elevated thyroid peroxidase antibodies (TPO) in euthyroid pregnant women and an increase in pregnancy problems, such as prelabor ruprure of membranes (PROM), miscarriages and preterm labour. It has been estimated that 6–19% of pregnant women who are asymptomatic have thyroid peroxidase antibodies and that 10% of pregnant women in the 16th week of pregnancy have TPO-Ab, which may be associated to hyperthyroidism. Pregnant women with type 1 diabetes have a prevalence of thyroid dysfunction that is almost three times higher than that of the general population. Even in other studies, 40% of pregnant women also had thyroid disease and type 1 diabetes concurrently.

Subclinical hypothyroidism is more common among thyroid disorders than other disorders. Like GDM, clinical and subclinical hyperthyroidism of the thyroid is an insulin resistance condition that may point to a connection between the two illnesses. According to several studies, maternal diabetes during pregnancy may have an impact on the fetus's active T4 to T3 conversion or T3 secretion. This supports the connection between thyroid problems and diabetes. According to reports, Iran's GDM prevalence ranges from 4.7 to 7.4%. Ten to fifteen percent of expectant mothers' experience thyroid dysfunction throughout the first part of their pregnancies. According to certain research, women with GDM had significant rates of anti-TPO and hypothyroxinaemia. Despite of several studies the true link between thyroid dysfunction and diabetes has not yet been established. Because the research of this link includes a wide range of additional explanations such as racial differences, genetics, ethnicity, environmental factors, underlying disorders, diabetes diagnostic criteria and thyroid dysfunction.

In our study the mean age of the participants was 29.60±5.93 years which is similar to the study conducted by M Alan et al, mean age was 30.9±5.0 years[10]. Another study conducted by Rizwana Arif et al showed mean age was 33.1±4.5 years[11]. Mean BMI of our study was 26.70±20.01 kg/m^2 whereas another research showed mean BMI was 26.96±4.55 kg/m^2 which is similar to our study.

In our study the prevalence of thyroid dysfunction was 14.08%, while the study conducted by Mahmood et al observed subclinical hypothyroidism 17% and clinical hypothyroidism 10.48% in gestational diabetes[12]. Our results are in agreement with a study[8] which reported a prevalence of 16.6% of thyroid dysfunction in women presenting with gestational diabetes. However, the study conducted by researcher where prevalence of thyroid dysfunction in type 1 diabetes patients were 26%. Another study conducted by Gallas PR[13] showed thyroid dysfunction in pregnant women with type 1 diabetes was 40% and 40.9% respectively; which is higher than our study. The reason for this difference is that all of our patients had gestational diabetes, whereas the patients in Gallas PR had type 1 diabetes. Another study showed hyperthyroidism was in 13.9% and hypothyroidism was observed in 6.59%.

Another study conducted by Fatima Sana et al[14] demonstrated prevalence of subclinical hypothyroidism in gestational diabetes versus healthy control was 61.5% vs 6%. A study conducted in India showed that prevalence of thyroid dysfunction was 87.2% which is much higher than other studies[15]. Different sample size, ethnicity, race, nutritional deficiency specifically iodine deficiency and regional differences are main factors which leads to such differences in studies.

Haddow and colleagues observed various maternal, fetal and neonatal adverse outcomes and recommend thyroid screening in high risk patients.[16] Several studies observed association of thyroid antibodies in gestational diabetes. Antithyroid antibodies can cross the placenta and affects neurodevelopment of fetus. The levels of anti-TPO, TSH, T3 and T4 were assessed in 61 patients with GDM and 35 healthy women in a study conducted by Fouyang Ouyang et al observed that thyroid

dysfunction was comparable between the GDM group and the control group.[17]

South Korean study conducted by Bian observed that thyroid dysfuction cause metabolic abnormalities and unfavourable pregnancy outcomes, therefore; women with gestational diabetes should be checked for thyroid dysfunction.[18]

CONCLUSION

The prevalence of thyroid dysfunction in gestational diabetes was 14.08%, therefore screening should be offered to high risk pregnant women.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Nazia Hakeem, Wajiha Karim, Rabia Jamil
Drafting or Revising Critically:	Afshan Shahid, Pooja Seetlani, Kanwal Jan Memon
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Isolation and Diagnosis of Bacteria Contaminating the Hospital Environment in Diyala Governorate

Salih Saood Yagoub and Ali Shakir Al-Ezee

ABSTRACT

Objective: To find out which antibiotic has the greatest effectiveness against bacteria.
Study Design: Cross sectional study
Place and Duration of Study: This study was conducted at the Department of Biology, College of Education for Pure Sciences, University of Diyala, 32001, Baqubah, Iraq from 10th December to 31st December 2024.
Methods: Thirty swabs were taken from beds and waste at Baqubah Teaching Hospital, Iraq and cultured on two types of media (blood agar and MacConkey agar). Five types of antibiotics (ciprofloxacin, amikacin, ampicillin, cefoxitin, and co-trimoxazole) were then applied to the growing bacteria.
Results: Ciprofloxacin, Amikacin, Ampicillin, Cefoxitin and Co-Trimoxazol on Escherichia bacteria grown on molar Hinton agar where taken from hospital beds to show any antibiotics have the greatest effect on them. The Ciprofloxacin antibiotic was the best for killing Escherichia, with Inhibition zone diameter 1.25 cm, while Amikacin and Ampicillin killing Escherichia bacteria, with Inhibition zone diameter 1 cm, and the antibiotics Cefoxitin and Co-Trimoxazol, killing bacteria, with Inhibition zone diameter of 30 mm
Conclusion: Ciprofloxacin antibiotic was the best for killing Escherichia bacteria, with inhibition zone diameter 1.25 cm, while Amikacin and Ampicillin kill Escherichia bacteria, with inhibition zone diameter 1 cm, and the antibiotics Cefoxitin and Co-Trimoxazol, killing bacteria, with inhibition zone diameter of 30 mm.

Key Words: Hospital beds, Antibiotic, Escherichia coli

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INTRODUCTION

Environmental factors like the availability of materials and equipment, administrative apathy, or personal factors like education and experience can all have an impact on adherence to safety precautions (SPs).[1] According to health-care organizations, health-care workers should closely follow established protocols to prevent illnesses linked to healthcare.[2] By reducing the frequency of infections linked to healthcare, adherence to fundamental safety measures is a practical and efficient method to improve the quality of healthcare. Protecting patients, communities, nurses, and other healthcare professionals is also essential. Particularly in resource-poor communities, there is a high frequency of serious, contagious diseases like HIV, Hepatitis B, and C, and very few prophylactic measures are in place.[1,3]

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Theodor Escherich, a German pediatrician who lived from 1857 to 1911, identified the germs Escherichia coli from infants' feces in 1885. Gram-negative, non-sporulating, rod-shaped, facultatively anaerobic, and coliform, Escherichia coli is a common bacteria found in food, the environment, and the lower stomach of warm-blooded animals. It is the most extensively researched prokaryotic model organism in the fields of microbiology and biotechnology. It is commonly employed as an indicator organism for water contamination and may survive for extended periods of time in soil, water, and feces. Under aerobic conditions, the bacterium grows quickly in new feces for two to three days, but then its numbers progressively decline. Gram-negative, straight, rod-shaped, non-sporing, non-acid fast, and bacilli that can exist alone or in pairs, E. coli is a type of bacteria. Usually formed like a rod, cells have dimensions of 1-3 μm × 0.4-0.7 μm (micrometer), which is approximately 1 μm long, 0.35 μm broad, and 0.6-0.7 μm in volume. Very few strains are non-motile because of the peritrichous flagellar configuration, which makes it motile. Although 37°C (98°F) is the ideal temperature for E. coli development, many lab strains can thrive at as high as 49°C (120.2°F). Under ideal circumstances, reproduction can occur in as little as 20 minutes. There are two types of fibriated strains: motile and non-motile. Certain strains of E. coli that were isolated from extraintestinal illnesses have been found to contain a polysaccharide

capsule. Using negative staining techniques, which create a bright halo over a dark background, the E. coli capsules are easily visible. They only have one or two peptidoglycan layers in their thin cell wall.

Antibiotic resistance is becoming a major worldwide issue that poses a threat to human health. Worldwide, Enterobacteriaceae have developed resistance in recent years[4]. Multidrug-resistant (MDR) bacteria are known to be most prevalent in South Asia, and in Pakistan, the number of multidrug-resistant Enterobacteriacea which are resistant to ampicillin, chloramphenicol, and co-trimoxazole is rising alarmingly.[5] Because these resistant enteric strains prolong illness and increase the likelihood of complications, they are considerably more difficult to manage and avoid in developing nations.[6] It is anticipated that resistant enteric bacteria, such as S. typhi and E. coli, will keep expanding in Karachi, Pakistan. This is because there hasn't been any notable success in reducing infectious diarrhea over the last ten years.[7] Furthermore, India and Pakistan have the highest incidence rates of acute gastroenteritis and enteric fever among Asian nations, with 214.2 and 451.7 cases per 100,000, respectively.[8] Managing MDR Salmonella (S) Typhi outbreaks can be challenging, especially in developing nations with limited resources.[9] In addition to first-line medicines, it has been observed that S. typhi is becoming more resistant to fluoroquinolones and cephalosporins worldwide, and Karachi is also seeing these resistant strains.[10] Additionally, it has been discovered that the E. coli strains in Karachi are resistant to the antibiotic Ciprofloxacin.[11] Since third-generation cephalosporins have been shown to be safe when used against E. coli, they can be utilized to treat resistant strains.[12] But third-generation cephalosporin resistance is also developing.[13] According to a single-center prospective research carried out in Karachi, strains of E. coli were resistant to ceftriaxone and ciprofloxacin but sensitive to imipenem and amikacin.[14]

Due to overuse and abuse of antibiotics, the problem of antibiotic resistance has spread, especially in poor nations like Pakistan.[15] According to a cross-sectional study carried out in Karachi, the primary cause of the city's rising antibiotic resistance is the local population's ignorance of antibiotic usage and doctors' illogical prescribing of antibiotics.[16] The management of frequent, prevalent enteric illnesses in our community is a critical future concern, especially in light of the threat posed by inadequate sanitation, contaminated water supplies, irrational antibiotic usage, and rising antibiotic resistance. Testing for resistance and sensitivity patterns at regular intervals is necessary to prevent the emergence of resistance and to guide local doctors.[17] Thus, this study's primary goal is to evaluate and contrast the effectiveness of four distinct antibiotics - Amikacin, Ceftriaxone, Ciprofloxacin, and Imipenem - against Salmonella typhi and Escherichia coli.

METHODS

Thirty swabs were taken from beds and waste at Baqubah Teaching Hospital and were cultured on two types of media (blood agar and MaCkonkey agar).The swabs taken from hospital beds are grown on culture media and placed in the incubator for 24 hours at 37°C after identifying the bacteria growing on the culture media for all samples (Escherichia coli). The antibiotics are applied to determine the extent of response to each of them; ciprofloxacin, amikacin, ampicilin, cefoxitin and co-trimoxazol.

RESULTS

Ciprofloxacin, Amikacin, Ampicillin, Cefoxitin and Co-Trimoxazol on Escherichia bacteria grown on molar Hinton agar where taken from hospital beds to show any antibiotics have the greatest effect on them. The Ciprofloxacin antibiotic was the best for killing Escherichia, with Inhibition zone diameter 1.25 cm, while Amikacin and Ampicillin killing Escherichia bacteria, with Inhibition zone diameter 1 cm, and the antibiotics Cefoxitin and Co-Trimoxazol, killing bacteria, with Inhibition zone diameter of 30 mm (Table 1, Fig.1).

Table No.1: Activities not used in the study

Antibiotic	Sensitivity	Resistance
Ciprofloxacin	100%	-
Amikacin	80%	20%
Cefoxitin	100%	-
Co-Trimoxazol	100%	-
Ampicillin	80%	20%

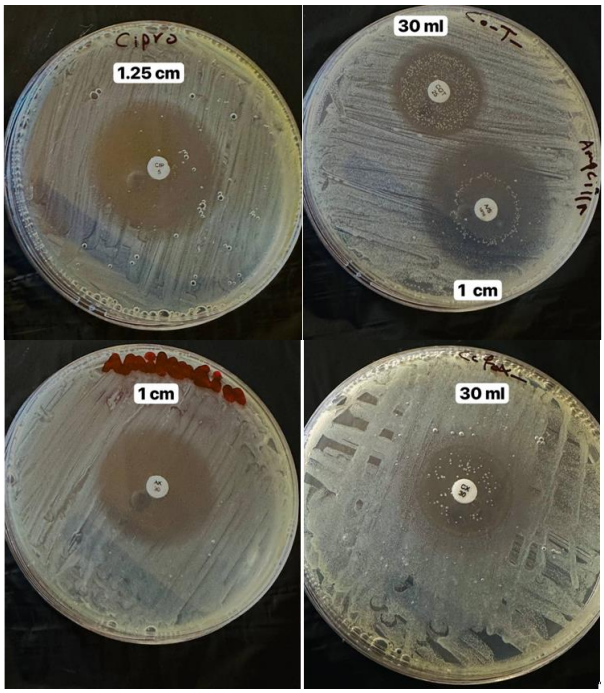

Figure No. 1: Escherichia coli bacteria with different types of antibiotics.

DISCUSSION

Ciprofloxacin was the most effective antibiotic against Escherichia bacteria. Ciprofloxacin is a fluoroquinolone antibiotic known for its strong activity against Gram-negative bacteria, including Escherichia coli. According to CLSI guidelines, inhibition zones for Ciprofloxacin against E. coli typically range between 1.5-3.0 cm depending on resistance profiles. The observed inhibition zone of 1.25 cm suggests reduced susceptibility, which may be due to increasing fluoroquinolone resistance globally.

Amikacin is an aminoglycoside antibiotic often used for Gram-negative bacterial infections. Global studies indicate variable effectiveness due to the emergence of aminoglycoside-modifying enzymes, leading to resistance. Ampicillin is a β-lactam antibiotic, but resistance is widespread due to E. coli producing β-lactamases (e.g. TEM, SHV, and CTX-M) results (1 cm inhibition zone) align with global trends showing that many E. coli strains exhibit resistance to Ampicillin, making it less effective in hospital-acquired infections.[18]

Cefoxitin, a second-generation cephalosporin, is commonly used as a surrogate marker for detecting Methicillin-resistant Staphylococcus aureus (MRSA), but it also has activity against E. Coli. If your inhibition zone is 30 mm (3 cm), it suggests significant susceptibility. However, many global reports indicate rising cephalosporin resistance in E. coli, particularly in extended-spectrum beta-lactamase (ESBL)-producing strains.[18] Co-Trimoxazole (Trimethoprim-Sulfamethoxazole) is frequently used for E. coli infections, but resistance rates vary widely (from 20-60% in hospital settings) observed 30 mm inhibition zone suggests that the tested strain is highly susceptible, which is less common in many hospital-acquired E. coli infections.[19-21]

Recommendations

1. Nurses and doctors must be careful and wear medical gloves and special clothing to prevent the transmission of bacteria and viruses from one patient to another and to workers.
2. Continuous use of sterilizers when dealing with patients for fear of transmission of infection
3. Take breaks from work to focus and avoid mixing tools specific to each disease.

CONCLUSION

Escherichia coli bacteria are the most common type found in hospital beds. The antibiotic ciprofloxacin is best for killing these bacteria with an inhibition zone of 1.25 cm but Amikacin and Ampicillin killing Escherichia bacteria, with Inhibition zone diameter 1 cm, and the antibiotics Cefoxitin and Co-Trimoxazol, killing bacteria, with Inhibition zone diameter of 30 mm.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Salih Saood Yagoub, Ali Shakir Al-Ezee
Drafting or Revising Critically:	Salih Saood Yagoub, Ali Shakir Al-Ezee
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Development of a Sustainable High-Performance Liquid Chromatography (HPLC) Method for Quantifying Metformin, Sitagliptin, and Empagliflozin in Type 2 Diabetes Treatment

Mahmood Shakir Al Samarrai and Eman Thiab Al Samarrai

ABSTRACT

Objective: To develop and validate a green, environmentally sustainable high-performance liquid chromatography (HPLC) method for the simultaneous determination of three commonly used antidiabetic agents: Metformin Hydrochloride (MET), Sitagliptin Phosphate (STG), and Empagliflozin (EMP).

Study Design: Experimental analytical study.

Place and Duration of Study: This study was conducted at the State Company for Drugs Industry and Medical Appliances Samarra (SDI) Iraq from April 2024 to May 2024.

Methods: The chromatographic separation was achieved using an isocratic elution on a C10 column (4.6 × 250 mm, 5 µm) with a mobile phase consisting of phosphate buffer (20 mM), methanol, and acetonitrile in the ratio of 65:30:5 (v/v/v), adjusted to pH 2.9. The flow rate was maintained at 1.0 mL/min, with a column temperature of 30°C and a total run time of 15 minutes. Detection was performed using UV spectroscopy at 208 nm. Linearity was established for MET (0.08–0.13 mg/mL), STG (0.035–0.065 mg/mL), and EMP (0.014–0.026 mg/mL), with correlation coefficients exceeding 0.998 and RSD% not exceeding 1.06%. The method's greenness was evaluated using AGREE and GAPI tools. Additionally, the Blue Applicability Grade Index (BAGI) was employed to assess operational and environmental suitability.

Results: The method exhibited high accuracy, reproducibility, and compliance with green analytical chemistry standards. The AGREE and GAPI assessments confirmed the method's minimal environmental impact. BAGI scoring yielded a value of 82.5, indicating strong sustainability and applicability. The method was successfully applied to the analysis of 13 commercial antidiabetic products, including branded and generic formulations available in the Iraqi market.

Conclusion: The developed HPLC method provides a reliable, accurate, and environmentally sustainable approach for the simultaneous analysis of MET, STG, and EMP. It demonstrates strong analytical performance and practical utility, contributing to green pharmaceutical quality control and sustainable laboratory practices.

Key Words: Green Chemistry; Diabetic mellites; Environmental sustainability; Metformin; Sitagliptin; Empagliflozin; AGREE; GAPI; BAGI

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INTRODUCTION

Diabetes mellitus is a chronic disorder of glucose metabolism caused by insulin deficiency or resistance. Its global prevalence is rising rapidly, with projections estimating 629 million affected adults by 2045 – nearly one in ten people. In response, the WHO designated November 14 as World Diabetes Day.[1-2]

Metformin, or 1,1-dimethylbiguanide hydrochloride, is a well-established antihyperglycemic agent for type 2 diabetes. It works by inhibiting hepatic gluconeogenesis, enhancing peripheral glucose uptake, and reducing intestinal glucose absorption. Recent studies suggest it may also act via the gut microbiome.[3-5] Beyond diabetes, Metformin shows promise in treating certain cancers, infections (including COVID-19 and malaria), aging-related conditions, and polycystic ovary syndrome (PCOS), where it improves insulin sensitivity, menstrual regularity, and fertility.[3,6]

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Sitagliptin, a DPP-4 inhibitor, also acts as an alpha-glucosidase inhibitor. It enhances incretin activity (GLP-1 and GIP) by inhibiting DPP-4, leading to increased insulin secretion and reduced glucagon in a glucose-dependent manner.[7-9] Beyond type 2 diabetes, recent studies highlight its anti-inflammatory and antioxidant effects, including potential benefits in non-diabetic COVID-19 patients through modulation of immune responses and reduction of pro-inflammatory cytokines.[10-12]

Empagliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor used in type 2 diabetes management. By blocking SGLT2, it reduces renal glucose reabsorption, lowering the threshold for glucose excretion and increasing urinary glucose output.[13,14] Besides its glucose-lowering effect, Empagliflozin shows diuretic and natriuretic properties by enhancing sodium excretion. Clinical trials report reduced cardiovascular mortality and heart failure hospitalization in diabetic patients with systolic heart failure.[15-17]

Green Analytical Chemistry (GAC) promotes safe, eco-friendly practices based on the 12 principles by Anastas and Warner.[18-20] Tools now assess method greenness, with advances like green HPLC methods for Metformin, Sitagliptin, and Empagliflozin using eco-solvents, miniaturization, and automation to reduce environmental impact.[18,19]

To evaluate the practicality of the proposed analytical method, the Blue Applicability Grade Index (BAGI) was employed. BAGI is a recent color-based metric (dark blue to light blue) that complements green metrics by assessing 10 critical attributes, including analysis type, number of analytes, instrumentation, sample preparation efficiency, throughput, reagents, need for preconcentration, and automation level.[21]

BAGI uses a scoring system from 25 to 100, with higher scores being better. It has user friendly open-source tools to make it easy to use and help researchers identify strengths and weaknesses in methods and get them accepted in the chemical community.[21] Applying these HPLC methods supports the quality of 13 antidiabetic products in Iraq and aligns with global efforts for sustainable pharmaceutical practices.

METHODS

The APIs used in this study were Metformin Hydrochloride (Sohan Healthcare, India, gifted by SDI), Sitagliptin Phosphate (Indexim International, India, gifted by Pioneer), and Empagliflozin (Nanjing Chico Pharmaceutical, China), all with purity ≥99.9%. Placebo excipients included pharmaceutical-grade Maize starch, Sodium starch glycolate, Polyvinylpyrrolidone, Avicel PH 302, Talc, Aerosil, and Magnesium stearate, sourced from Indian suppliers. HPLC-grade Acetonitrile and Methanol (Merck, Germany, 99.95% purity) were used in the mobile phase, and Monopotassium phosphate (A-Z Chem, 95–100.5%) was used for buffer preparation. All pharmaceutical products were obtained from a local pharmacy.

The analytical method was developed and validated using a Shimadzu HPLC system (Japan) comprising an LC-20AD pump, SPD-20A UV-Vis detector, DGU-20A5 degasser, and CTO-20A column oven. Separation was achieved on a reversed-phase L10 (CN) column (4.6 × 250 mm, 5 µm) from MACHEREY-NAGEL (Germany). Supporting equipment included a magnetic stirrer and pH meter (Jenway, Belgium), analytical balance (Sartorius, Germany), ultrasonic bath (ISOLAB, Germany), and UV-Vis spectrophotometer (UV1900, Shimadzu, Japan). Sample preparation involved extraction, dilution, and filtration. The method was validated per regulatory guidelines, with data acquisition and analysis performed using LC Solution software (Shimadzu, Kyoto, Japan) and appropriate statistical methods to support study conclusions. The mobile phase was prepared using 20 mM phosphate buffer (pH 2.35, adjusted with phosphoric acid), mixed with methanol (30% v/v) and acetonitrile (5% v/v), and adjusted to pH 2.9 if needed. The solution was filtered and degassed, contributing to the method's ruggedness.

Standard solutions of Metformin HCl (0.1 mg/mL), Sitagliptin Phosphate (0.05 mg/mL), and Empagliflozin (0.02 mg/mL) were prepared using 70% acetonitrile and ultrasonication, followed by dilution with mobile phase. For test solutions, ten tablets were crushed, average tablet weight calculated, equivalent API amounts transferred to 100 mL flasks, dissolved in 70% acetonitrile (70 mL), sonicated for 5 minutes, diluted to volume with mobile phase, and filtered. Placebo solutions were prepared by dissolving common excipients in the mobile phase, sonicated for 5 minutes, shaken, and filtered.

The simultaneous quantification of Metformin Hydrochloride, Sitagliptin Phosphate, and Empagliflozin was carried out using a Shimadzu HPLC system (Japan) featuring an LC-20AD binary pump, DGU-20A5 degasser, manual injector with 100 µL loop (USA), and a CN reversed-phase column (250 mm). Detection was performed using an SPD-20A UV/VIS detector at 208 nm. The column temperature was maintained at 30°C, with isocratic elution at a flow rate of 1 mL/min. Data acquisition and processing were conducted using LC Solution software and a CBM-20A communication module (Shimadzu, Japan).

To select the optimal wavelength for simultaneous analysis, UV-Vis spectra (190–300 nm) of individual drug solutions revealed a common strong absorbance at 208 nm, suitable for quantifying Metformin HCl, Sitagliptin Phosphate, and Empagliflozin.

RESULTS

According to USP 40, system suitability tests ensure the reliability of the HPLC system. Key parameters include tailing factor (Tf) <2.0 for peak symmetry, and resolution (Rs) ≥1.5 between peaks. The method showed excellent resolution: Rs=10 between Metformin

HCl (Rt=9.01) and Sitagliptin Phosphate, and clear separation from Empagliflozin (Rt=14.75), with all Tf values < 2. Theoretical plates and HETP were calculated per USP guidelines. The average retention times for six injections had %RSD <2%, confirming method consistency (Table 1).

The method showed excellent linearity with correlation coefficients (r) > 0.99. LOD and LOQ were calculated using the standard deviation of response and slope of the calibration curves (Table 2)

The accuracy of the method was validated by spiking known API concentrations at 80%, 100%, and 120% of target levels. Recovery results confirmed high accuracy, with recoveries of 97.7–102.6% for Metformin, 98.8-101.0% for Sitagliptin, and 98.6-101.1% for Empagliflozin. These findings confirm the method's capability for accurate API quantification (Table 3).

The method showed excellent precision, with repeatability assessed over six replicates (n=6) producing RSD% values below 2%. Intermediate precision across different days also yielded RSD% under 2%, confirming consistent performance. The method accurately quantified API content in test samples, with results aligning closely with expected values verifying its accuracy, precision, and reliability for pharmaceutical analysis (Tables 4-6).

Thirteen antidiabetic formulations sourced from Iraqi pharmacies were quantitatively assessed for Metformin HCl (500–1000 mg), Sitagliptin Phosphate (100 mg), and Empagliflozin (25 mg) using a validated RP-HPLC method. Sample solutions (0.1, 0.05, and 0.02 mg/mL, respectively) complied with USP acceptance criteria (90-110%), affirming dosage accuracy and highlighting the necessity of continuous quality assurance (Table 7).

Table No.1: The test of the system suitability method

APIs	Retention time (Rt)(min)	Tailing factors (Tf) NMT 2	Resolutions (Rs) ≥1.5 (USP)	Theoretical plates (N)	HETP (USP) (mm)
Metformin HCl	3.0	1.27	-	1726	87.0
Sitagliptin Phosphate	6.0	1.38	9.01	4334	34.6
Empagliflozin	13.2	1.05	14.75	7300	20.5

Table No.2: Linearity curve of parameter data results

APIs	Regression equation	Linearity range (mg/ml)	Slope	Intercept	Mean (%)	Standard deviation	Relative standard deviation RSD (%)	R-squared value	LOD (mg/ml)	LOQ (mg/ml)
Metformin HCl	y= 269,304,684.7x +12,258,479.156	(0.08-0.13)	269,304, 684.7	12258479.1	99.01	2.7132	$6*10^{-6}$	0.998	0.0266	0.0807
Sitagliptin Phosphate	y= 188,171,535.7x + 207,345.0714	(0.035-0.065)	188,171, 535.7	207345.1	99.9	0.4412	0.441	0.9995	0.0048	0.0147
Empagli-flozin	y=490,796,827.3x 100,663.8810 -	(0.014-0.026)	490,796, 827.3	-100663.8	99.9	0.7006	$7*10^{-6}$	0.9991	0.0023	0.0070

Table No.3: Accuracy data test of Metformin HCl, Sitagliptin Phosphate, and Empagliflozin

APIs	Levels (%)	Theoretical conc. (mg/mL)	Average AUP (mV) N*=3	Recovery (%)	Relative standard deviation RSD (%) +2%
Metformin HCl	80%	0.080	33351885	98.0	0.40
	100%	0.100	39784313	102.2	0.30
	120%	0.120	43952513	98.07	0.20
Sitagliptin Phosphate	80%	0.040	77040450	99.6	0.04
	100%	0.050	96856120	100.7	0.19
	120%	0.060	11366286	98.8	0.02
Empagliflozin	80%	0.016	76520520	98.7	0.08
	100%	0.020	98196600	101.1	0.07
	120%	0.024	11556651	99.0	0.10

Table No.4: Metformin HCl Precision Intra-day, Inter-day

APIs	Taken Conc. (mg/mL)	Intra-day		
		Initial test AUP (mV)	After 4-hour AUP (mV)	Day-1 AUP (mV)
Metformin HCl	0.1	39875805	39704636	39704636
		39760814	39559362	39559362
		39866014	38923332	38923332
		39701319	39819463	39819463

		39729076	39997477	39997477
		39874572	39760309	39760309
Mean		39801267	39627429	39894804
Founded (mg/ml)		0.10227	0.10163	0.10262
Recovery (%)		102.3	101.6	102.6
SD		79949.11	373582	79932.1
RSD (%)		0.200871	0.94274	0.20036

Table No.5: Sitagliptin Phosphate Precision Intra-day, Inter-day

APIs	Taken Conc. (mg/mL)	Intra-day		
		Initial test AUP (mV)	After 4-hour AUP (mV)	Day-1 AUP (mV)
Sitagliptin Phosphate	0.05	9918527	9726208	9929224
		9906403	9747459	9839959
		9831801	9662725	9905762
		9932309	9920181	9934757
		9898239	9911092	9872268
		9953124	9830785	9959943
Mean		9906734	9906734	9906986
Founded (mg/ml)		0.05155	0.05098	0.05098
Recovery (%)		103.1	102.0	103.1
SD		41550.97	104673	44203.3
RSD (%)		0.419422	1.06812	0.44618

Table No.6: Empagliflozin Precision Intra-day, Inter-day

APIs	Taken Conc. (mg/mL)	Intra-day		
		Initial test AUP (mV)	After 4-hour AUP (mV)	Day-1 AUP (mV)
Empagliflozin	0.02	9946005	9878348	9910687
		9957256	9766222	9966082
		9972385	9789566	9741066
		9883858	9723323	10021428
		9898239	9913315	9779291
		9963535	9738927	10040576
Mean		9936880	9801617	9909855
Founded (mg/ml)		0.02045	0.02018	0.0204
Recovery (%)		102.3	100.9	102.0
SD		36807.61	77229.7	125088
RSD (%)		0.370414	0.78793	0.78793

Table No.7: Assay of sample tablets (method application)

Samples	Origin	APIs	Samples Conc. (mg/ml)	AUP (mV)	Found Conc. (mg/ml)	Recovery (%)
Glucophage®500mg	Merch,France	Metformin HCl	0.10	38996561	0.099	99.3
Glifor® 1000mg	bilim, Turkey	Metformin HCl	0.10	38772264	0.098	98.5
METFORAL® 500mg	Menarini, Germany	Metformin HCl	0.10	40515318	0.105	104.9
Januvia® 100mg	MSD, USA	Sitagliptin Phosphate	0.05	12643634	0.066	94.5
Sitagla® 100mg	Maddox, Germany	Sitagliptin HCl	0.05	12766454	0.067	95.4
SITAVIA® 100mg	PIONEER, Iraq	Sitagliptin Phosphate	0.05	13324290	0.069	99.7
Jardiance® 25mg	Boehringer Ingelheim, USA	Empagliflozin	0.02	9771733	0.020	100.5
EMPOLI® 25mg	SAMI, Pakistan	Empagliflozin	0.02	10722886	0.022	110.2
EMPO® 25mg	Motakadema, Jordin	Empagliflozin	0.02	9661833	0.019	99.5
Emglif® 25mg	GENIX,Pakistan	Empagliflozin	0.02	10400108	0.021	106.9
Jard® 25mg	Future, Canada	Empagliflozin	0.02	10032391	0.020	103.2

EMPAGIT® 25mg	Getz, Pakistan	Empagliflozin	0.02	10265236	0.021	105.6
Empadil L® 25mg	Ajanta, India	Empagliflozin	0.02	10193317	0.020	104.8

DISCUSSION

The developed HPLC method achieved baseline separation of metformin, sitagliptin, and empagliflozin in 15 minutes using a CN column and isocratic elution (phosphate buffer: methanol: acetonitrile 65:30:5, pH 2.9). Validation studies demonstrated excellent linearity ($r^2 > 0.998$) across concentration ranges of 0.08-0.13 mg/mL (metformin), 0.035-0.065 mg/mL (sitagliptin), and 0.014-0.026 mg/mL (empagliflozin), with LODs of 0.0023-0.0266 mg/mL. Accuracy (98-102% recovery) and precision (<2% RSD) met pharmacopeial requirements. It is suitable for routine quality control and meets USP and ICH standards.[22]

The method showed good environmental performance (AGREE score 0.65, BAGI 82.5) with low solvent consumption (15 mL/analysis).[23] Successful application to 13 commercial formulations confirmed robustness against minor operational variations (±0.2 pH units, ±5% flow rate, ±2°C temperature). This validated approach combines rapid analysis with green chemistry principles for reliable quality control of antidiabetic medications. Future work could explore biological sample applications or alternative detection methods.[24]

CONCLUSION

The developed RP-HPLC method effectively quantified metformin HCl, sitagliptin phosphate and empagliflozin in pharmaceutical forms, showing excellent linearity ($r^2 \geq 0.998$), precision (RSD% <2%), and ruggedness (RSD% ≤1%). Greenness assessment gave an AGREE score of 0.65 and a GAPI profile with 13 green, 9 yellow, and 3 red zones. The method is suitable for routine quality control, with potential for further environmental optimization

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Mahmood Shakir Al Samarrai, Eman Thiab Al Samarrai
Drafting or Revising Critically:	Mahmood Shakir Al Samarrai, Eman Thiab Al Samarrai
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Overview of Immunization Coverage and Determinants for Children

Hajer S. Essa, Duha Majid Abdulrahem, Hazim N. Waheeb

ABSTRACT

Objective: To determine the vaccination coverage rate for Basrah's infants and toddlers aged 0–2 in 2024.
Study Design: Descriptive / cross-sectional study
Place and Duration of Study: This study was conducted at the Community Health Nursing, College of Nursing, University of Basrah, Iraq from January 2024 to November 2024.
Methods: The descriptive (cross-sectional) investigation into the factors influencing vaccination coverage in Basrah City's primary care facilities. One hundred and fifty women were interviewed directly between January 2024 and November 2024. A sample was used to select from four primary healthcare centers in the Basrah Center.
Results: The immunization coverage, the coverage of immunization for children was 95 (63%) completed immunization, while 55 (36%) of the sample had partial immunization.
Conclusion: The coverage rate is good, though the defaulters' rate is relatively high due to fear of vaccines and the mothers being busy.

Key Words: Immunization, Coverage, Determinants, Children

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INTRODUCTION

Many people consider vaccination to be one of the greatest advancements in public health throughout the 20th century.[1] Depending on the vaccine-preventable disease, 90 to 95% of the population must be immunized for it to be effective.[2] The number of children who receive late vaccination doses throughout their first year of life is used to estimate coverage.[3,4] To guarantee that all children, in all nations, received life-saving immunizations, the 27th World Health Assembly decided in May 1974 to build on the achievements of the smallpox eradication program and created the Expanded Program on Immunization (EPI).[5] As understanding of the disease's immunological components grew, new vaccines were created and added to the EPI's list of suggested vaccinations.[5] EPI was well-established in Iraq in 1985, providing vaccination services to certain populations.[6] EPI is thought to have prevented 2 million lives worldwide in 2003.[7]

Evaluating vaccination coverage aids in determining how well program goals are being met and how well services are being provided.

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Furthermore, the assessment of vaccination coverage shows whether significant strides are being made in reaching vaccination goals.[8] A population's vaccination coverage is the percentage of its members who have received vaccinations during a specific time frame. For both single and multi-dose vaccines, it is approximated[9], for every dose (e.g., a vaccine containing diphtheria, tetanus, and pertussis; DTP1, DTP2). Usually, a percentage of the targeted children is used to display it. The dropout rate indicator between the first and last doses of the vaccine during the children's first year of age is used to measure the health system's ability to finish the child's vaccination course.[10] 10% is the highest allowable dropout rate. Higher rates worldwide point to ineffective health care, service interruptions at fixed posts, mothers not being informed about returning for follow-up dosages, and outreach or mobility teams not making follow-up visits.[11]

Other factors that contributed to dropout rates included sex, marital status, and having children. Two approaches are used to monitor immunization programs: surveys conducted in the community and an administrative institution-based approach.[12] The purpose of this study was to determine the vaccine coverage rate for Basrah children aged 0–2 in 2024. It also looks at the reasons behind partial immunization and the vaccine dropout rate.

Immunization is a procedure that is artificially initiated in which a person's immune system is protected from disease by the development of protective factors for the exclusion of a particular antigen following the introduction of the immunogenic.[13] The immunological response is triggered when the immune system reacts outside of the microorganism's molecules. The immune

system then gains the capacity to react swiftly to a subsequent encounter with the new agent because of immunological memory.[14]

METHODS

The descriptive (cross-sectional) study about the determinants of immunization coverage in primary care centers of Basrah city were conducted from January 2024 to November 2024. Data was collected via direct interviews with 150 mothers using an Arabic questionnaire. The sample was selected from four primary care centers in the Basrah Center. The researcher developed the following tools for the study in two parts; part one is demographic characteristics including (gender, child birth order, mother education, mother occupation, mother age group, family type, and place of birth). The causes of incomplete vaccination are covered in Part 2. The data was entered and analyzed through SPSS-25.

RESULTS

Maximum proportion of mothers 58.6% were in the age range of 21-30 years, followed by 31-40 years were 26.6% and those under 20 years were 14.6%. There were 80 (53.3%) males and 70 (46.6%) females. The childbirth orders was 4th i.e. 55 (36.6%) and 3rd i.e. 20 (13%), the place of delivery the high percentage in the hospital 145 (96.6%) the lower percentage 5 3.3% (Tables 1-2). The immunization coverage, the coverage of immunization for children 95 (63%) completed immunization while 55 (36%) of the sample had partial immunization. The type of vaccine (BCG) has a coverage rate of 150 (100%) while the lower coverage is 120 (80%) measles (Table 3). The high percentage of 27 (49%) reasons for the other causes, 7 (12.7%) children were ill 6 (10.9%) mothers were too busy 5 (9.09%) family problems (Table 4).

A high percentage of mothers were educated at secondary school (92.5%) and had complete immunization, while a high percentage of partial immunization for children (86.1%) was illiterate. Where the mothers were unemployed (66.4%) completed immunization for mothers' children, while (33.5%) had partial immunization. Mothers employed (62.5%) had partial immunization, while (37.5%) had complete immunization. The family type of the sample has a high percentage of single-generation families (84.9%), where complete immunization and a lower percentage (15%) of partial immunization. The multi-generation had a high percentage (51.5%) of complete immunization, and the lower percentage (48.4%) was partial immunization. In the childbirth order of the study sample, the high percentage (90.9%) was 2nd while the lower percentage (22.2%) 4th was complete immunization, but the high percentage (77.7%) partial immunization was the childbirth order 4th while the lower percentage (9%) were the childbirth order 2nd (Table 5).

Table No.1: Sociodemographic data of the mothers (n=150)

Variable	No.	%
Age (years)		
< 20	22	14.6
21-30	88	58.6
31 – 40	40	26.6
Gender		
Level of Education		
Illiterate	36	24.0
Primary	39	26.0
Secondary	54	36.0
Graduate and higher	21	14.0
Occupation		
Housewife	134	89.4
Worker	16	10.6
Family type		
Single generation	53	36.0
Multi generation	97	64.0

Table No.2: Sociodemographic data of the children (n=150)

Variable	No.	%
Gender		
Male	80	53.4
Female	70	46.6
Child birth order		
1st	30	20.0
2nd	55	36.6
3rd	20	13.0
4th	45	30.0
Place of delivery		
Hospital	145	96.6
Home	5	3.4

Table No.3: Immunization coverage (n=150)

Immunization coverage	No.	%
Overall immunization status		
Complete	95	63.0
Partial	55	37.0
Unimmunized	-	-
Type of vaccine		
BCG, OPV0, HepB1	150	100.0
DTP1, OPV1, HepB2	141	94.0
DTP2, OPV2	136	90.0
DTP3, OPV3, HepB3	129	86.0
Measles	120	80.0

Table No.4: Causes of partial immunization (n=55)

Cause	No.	%
Mother too busy	6	10.9
Place of immunization not known	2	3.6
Child too young	1	1.8
Child ill	7	12.7
Family problem	5	9.1
Vaccine not available	1	1.8
Unaware of the need for immunization	1	1.8
Fear of side effects	4	7.2
Not faith in immunization	1	1.8
Other causes	27	49.1

Table No.5: Association of sociodemographic data with state of immunization

Variables	Complete Immunization	Partial Immunization
Mother's education		
Illiterate(n =36)	5 (13.8%)	31 (86.1%)
Primary(n=39)	30 (76.9%)	9 (23.07%)
Secondary (n =54)	50 (92.5%)	4 (7.4%)
Graduate (n =21)	10 (47.6%)	11 (52.3%)
Mother's occupation		
Unemployed (n=134)	89 (66.4%)	45 (33.5%)
Employed (n=16)	6 (37.5%)	10 (62.5%)
Family type		
Single generation (n=53)	45 (84.9%)	8 (15.09%)
Multi-generation (n=97)	50 (51.5%)	47 (48.45%)
Childbirth order		
1st (n=30)	25(83.3%)	5 (16.6%)
2nd (n=55)	50 (90.9%)	5 (9.1%)
3rd (n=20)	5 (25%)	15 (75%)
4th (n=45)	10 (22.2%)	35 (77.7%)

DISCUSSION

Sociodemographic characteristics of mothers in the present study showed 58.6% of the mothers' between 21-30 years, 26.6% between 31-40 years, and 14.6% were age group <20. This results are consistent with previous studies[15,16] which reveals that most of the ages were between 31-40 years.

This study showed that a high percentage of children were males, 80 (53.3%), and the lower were females, 70 (46.6%). The results are inconsistent with others researchers[17,18] which reveal that most of the participants were females.

The childbirth orders showed a high percentage was 4th stage 55 (36.6%), and the lower percentage was 3rd 20 (13%). The results of this study are consistent with previous research[19] which reveals that most of the participants were from the 4th stage.

The highest percentage of children was delivered in the hospital 96.6% in the present study. This results are consistent with other studies[20,21] which reveal that most of the participants were delivered at the hospital.

This study shows the immunization coverage, coverage of immunization for children 95 (63%) completed immunization while 55 (36%) had partial immunization. The type of vaccine (BCG) had a coverage rate of 150 (100%), while the coverage of measles was lower at 120 (80%). The results of this study are consistent with Tiryag et al[22] which reveal that most of the participants complete immunization.

In this study a high percentage of 27 (49%) reasons for the fear of the vaccines, 7 (12.7%) children were ill 6 (10.9%) mothers were too busy and 5 (9.09%) family problems. This study's results are consistent with previous research[23], which reveals that most of the participants fear immunization.

In the current study, a high percentage of mothers were educated at secondary school 92.5% and had complete immunization, while a high percentage of partial immunization for children 86.1% were illiterate. The findings of this study agree with Mohammad et al[24], which reveal that most of the participants had secondary school.

Where the mothers were unemployed 66.4% completed immunization for mothers' children, while 33.5% had partial immunization while mothers were employed 62.5% had partial immunization, while 37.5% had complete immunization. This study's results are consistent with Mohammad et al[25], which reveal that most of the participants were unemployed.

This study showed that family type has single-generation families was 84.9%, where complete immunization was 15% of partial immunization. In the multi-generation, 51.5% had complete immunization and 48.4% had partial immunization. The results of this study are consistent with Mohammad et al[26] which reveals that most of the participants were single-generation.

According to childbirth order of this study, 90.9% was 2nd while 22.2% was 4th was complete immunization, but 77.7% has partial immunization was the childbirth order 4th while 9% were the childbirth order 2nd. The results of this study are consistent with Asuman et al[27] which reveal that most of the participants had 2nd childbirth order.

In 2015, Maki et al[6] reported that vaccination coverage rate was 80.7%, while the dropout rate was 19.3%, which is quite high. The primary reasons for the dropout were mothers' inability to attend Primary Health Care Centers (50%) and their lack of knowledge

(31%). In another study, Odusanya et al[28] indicated the 339 children and 339 mothers participated in the study; each mother had one eligible child. Except for breathing difficulties (a sign of diphtheria), the majority of mothers (99.1%) had highly positive views toward vaccination, and more than half (>55%) were typically aware of the signs of diseases that can be prevented by vaccination.

The rate of full immunization was substantially linked with mothers' vaccination at a privately funded health institution ($p<0.001$) and their awareness of immunization ($p = 0.006$), according to multiple logistic regression. In another study in Kenya by Gupta et al[29], the mother's age, literacy level, place of delivery, and birth order all significantly influence how often Kenyans use vaccination services, at a 5% significance level. The use of immunization services was positively correlated with the mother's age, literacy, and place of delivery, whereas the immunization of children was adversely correlated with birth order.

CONCLUSION

The coverage rate is good, although the default rate is relatively high. The reasons for that were fear of the vaccine, also the other reason the mothers were busy.

Author's Contribution:

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Drafting or Revising Critically:	Hajer S. Essa, Hazim N. Waheeb
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Agreement to accountable for all aspects of work:	All the above authors

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Original Article

The Role of Job-Related Stress and Burnout in Increasing Heart Attack Risk Among Young Professionals

Shirjeel Hussain[1], Urooj Shuja[2], Sidra Rasheed[3], Saboohi Irfan[2] and Nazeer Ahmed Memon Humaira Zakir[2]

ABSTRACT

Objective: This study explored the relationship between job-related stress, burnout, and heart attack risk among young professionals aged 25 to 40 across various industries, including corporate, healthcare, education, and technology.

Study Design: A cross-sectional study.

Place and Duration of Study: This study was conducted at the Al-Tibri Medical College and Hospital, Karachi during June 2023 to June 2024.

Methods: A cross-sectional design was employed, with a sample size of 240 participants. Data were collected using structured questionnaires that assessed job-related stress through the Perceived Stress Scale (PSS), burnout using the Maslach Burnout Inventory (MBI), and medical history regarding heart attacks.

Results: The results indicated that 50% of participants reported high levels of job-related stress, while a similar percentage experienced high emotional exhaustion, a core dimension of burnout. Correlation analysis revealed significant positive relationships between job-related stress ($r = 0.35$, $p < 0.01$) and burnout ($r = 0.40$, $p < 0.01$) with heart attack risk.

Conclusion: The findings highlight the urgent need for interventions aimed at mitigating stress and burnout among young professionals to reduce the associated cardiovascular risks.

Key Words: Stress and Burnout, Heart Attack, Young Professionals

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INTRODUCTION

Work-related burnout and stress have evidently accounted the major concerns affecting human health and general wellness in the contemporary work setting especially in professionals aged between 25 to 40 years[1]. This generation is many a times aggressive to prosper, has high expectations from employers, and also struggles to manage work and family life. When people attempt to advance through such grueling career paths, the stresses and enervations that their jobs inflict upon their mental and emotional faculties become increasingly alarming and in some cases frightening[2,3]. Young people are often required to work for long hours accompanied with a heavy load of responsibilities, work under tension with hardly any time deadlines, and work on tight deadlines while maintaining top notch work which induces stress. This kind of stress is prolonged in duration and has detrimental physical health effects, raising the propositions for coronary heart diseases such as heart attacks.[4,5] studies that have been carried out on the subject have support the view that extending the exposure to work related stress by any straight or indirect means is harmful to the health of a person in the long run. Developed stress cannot be equated with aggravated primary stress as in acute stress, prolonged primary stress signifies a state of multiple tensions which can be brought on by locus problems, high levels of work demand, lack of support from peers and line managers, poor balance between work issues and family issues[6].

As stressors accumulate over time, they can give rise to a phenomenon called burnout, which is a psychological condition typified by overwhelming fatigue, depersonalization, and reduced individual accomplishment. Young professionals, who are still early in their careers, often experience their demanding workplace as an exciting challenge but could find themselves at odds with being able to deliver,[7,8] feeling more stressed and anxious. The impact of burnout is multi-directional, leading to poor performance at work,

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greater absenteeism, and most importantly, adverse health outcomes[9].

Several studies have established associations between numerous professions and cardiac disease. Long term stress activates the stress response system of the body, and this in turn leads to the secretion of hormones such as cortisol and adrenaline. In an attempt to extinguish the stress, the body prepares itself for a 'fight or flight' technique which causes an increase in the heart rate, high pressure, and inflammatory hyperactivation[10]. All these attributes may all cumulatively enhance the chances of atherosclerosis (the accumulation of plaques within the arteries) hypertension other cardiovascular disorders. For young professionals, who possibly already have some poor lifestyle practices such as poor diet, little exercise, and inadequate sleep thanks to work commitments, the risks are even greater. The combination of stress-induced physiological changes and lifestyle factors creates a veritable breeding ground for the potential development or manifestation of cardiovascular diseases which include but are not limited to heart attacks or other serious conditions[11].

METHODS

A cross-sectional design was conducted at Al-Tibri Medical College and Hospital, Karachi during June 2023 to June 2024, employed, with a sample size of 240 participants

Sample Size and Participants: The study composed of 240 participants from 25 to 40 years of age was conducted. The participants had a variety of work experiences representing their employment in different fields including corporate, healthcare, education, and information technology. A stratified sampling technique was deployed to ensure that each industry was sufficiently represented in the sample making it possible to appreciate the reasons behind differences in health management across industries. The subjects were recruited through their willingness to take part in the study and translation of the information into a consent form was provided before participation.

Data Collection: Data was mainly gathered using structured questionnaires that were handed out in person as well as electronically. The questionnaires were designed to obtain information concerning three main aspects: workplace stress, the levels of burnout and past heart attack incidences.

1. Stress Evaluation in the Workplace: The Perceived Stress Scale (PSS), known as a common tool evaluating one's stress perception, was also applied. The PSS contained some items that asked about the stressful' thoughts and feelings during the past month.

2. Private Lives of Participants Following Employment: In order to examine the extent of burn out amongst burn out, the Maslach Burnout Inventory (MBI) was given. This inventory made use of three dimensions of burnout: emotional exhaustion, depersonalization, and personal accomplishment.

3. Health Information: Participants were also inquired about their medical examination regarding heart attack, whether they had suffered from any heart attacks or suffered from any symptoms that were diagnostic of heart attacks

Data Analysis: The data was then entered into the SPSS software for statistical analysis after collection of data. To begin with, some descriptive results were calculated after studying the demographic aspects of the participants. Job related stress and job burnout were defined according to some cut offs established for PSS and MBI.

Two-tailed tests were conducted to test the significance of Pearson correlation coefficients computed to describe the relationship stress, burnout and risk of heart attack. This statistical method was opted for in this case because it seeks to establish the degree to which the two variables are correlated and the nature of the correlation. A significance level of $p < 0.05$ was used in all the analyses to determine the statistical significance in the effect of the analysed factors.

RESULTS

The findings of the study, focusing on the relationship between job-related stress, burnout, and heart attack risk among young professionals aged 25 to 40. A total of 240 participants completed the study, and the results are organized into sections addressing demographic characteristics, stress and burnout levels, and the correlation between these factors and heart attack risk.

1. Demographic Characteristics

Table 1 summarizes the demographic characteristics of the participants, including age, gender, and industry of employment.

Table No.1: Demographic Characteristics of Participants (n=240)

Demographic Variable	Frequency (n)	Percentage (%)
Age (years)		
25-30	80	33.3
31-35	90	37.5
36-40	70	29.2
Gender		
Male	124	51.7
Female	116	48.3
Industry		
Corporate	72	30.0
Healthcare	60	25.0
Education	48	20.0
Technology	60	25.0

2. Job-Related Stress and Burnout Levels

The prevalence of job-related stress and burnout was assessed using the Perceived Stress Scale (PSS) and the Maslach Burnout Inventory (MBI).

Table No.2: Levels of Job-Related Stress Among Participants

Stress Level	Frequency (n)	Percentage (%)
Low	36	15.0
Moderate	84	35.0
High	120	50.0

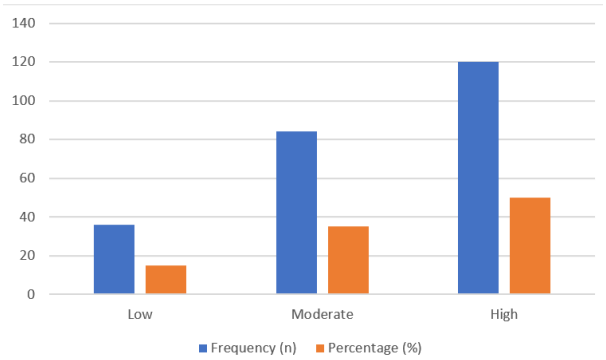

Figure No.1: Percentage heart attack symptoms

The results indicated that 50% of participants reported high levels of job-related stress, while 35% indicated moderate stress levels. Only 15% of participants experienced low stress levels.

Table No.3: Levels of Burnout Among Participants

Burnout Dimension	Frequency (n)	Percentage (%)
Low Emotional Exhaustion	48	20.0
Moderate Emotional Exhaustion	72	30.0
High Emotional Exhaustion	120	50.0
Low Depersonalization	96	40.0
Moderate Depersonalization	84	35.0
High Depersonalization	60	25.0
Low Personal Accomplishment	72	30.0
Moderate Personal Accomplishment	96	40.0
High Personal Accomplishment	72	30.0

3. Heart Attack Risk

Participants were asked about their medical history regarding heart attacks. The findings are summarized in figure 1.

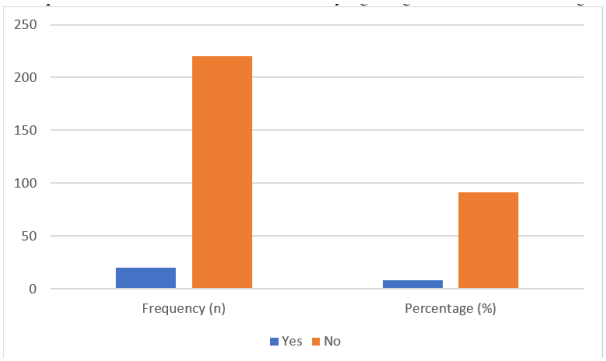

Figure No.2: Frequency percentage of patients

Figure 1: Only 8.3% of participants reported experiencing heart attack symptoms, while the vast majority (91.7%) had no such history.

4. Correlation Between Job-Related Stress, Burnout, and Heart Attack Risk

To examine the relationships between job-related stress, burnout, and heart attack risk, Pearson correlation coefficients were calculated. The results are presented in Table 4. The correlation analysis revealed a significant positive correlation between job-related stress and burnout ($r = 0.63$, $p < 0.01$), indicating that higher levels of stress were associated with increased burnout. Furthermore, a moderate positive correlation was observed between both job-related stress ($r = 0.35$, $p < 0.01$) and burnout ($r = 0.40$, $p < 0.01$) with heart attack risk. This suggests that as stress and burnout levels increased, so did the risk of experiencing heart attack symptoms.

Table No.4: Correlation Between Job-Related Stress, Burnout, and Heart Attack Risk

Variable	Job-Related Stress	Burnout (Emotional Exhaustion)	Heart Attack Risk
Job-Related Stress	1	0.63**	0.35**
Burnout (Emotional Exhaustion)	0.63**	1	0.40**
Heart Attack Risk	0.35**	0.40**	1

Note: **p < 0.01.s

DISCUSSION

The findings of this study emphasize that job-related stress and burnout among professionals aged 25-40 years are disturbingly common and suggest a potentially dangerous factor for cardiovascular health. Results have indicated that half of the respondents reported high job-related stress, while an equal number reported high emotional exhaustion characteristic of burnout yet another seasoned dimension. The scores for

stress-burnout-heart attack risk relationships were significant, therefore the need to factor in all of them in our contemporary world of work cannot be overemphasized[12].

Burnout and discriminatory job-related stress levels reported in this study must meet appropriate standards in previous studies. The correlational analysis showed that job-related stress and burnout both had a positive relationship with heart attack risk, which was within the moderate range. This has been previously documented among other studies. Researcher also provide evidence that high levels of stress increase the chances of cardiovascular disease events including heart attacks. Therefore, it brings in the perspective that chronic stress has health repercussions in young adults due to increase in heart rate, blood pressure among other factors. The findings of this study are of great importance to workplace health policies. The high rates of stress and burnout experienced by many young professionals must be addressed by organizations, and certain measures need to be adopted to prevent these issues. Studies have asserted that workplace interventions and programs, including stress management programs and work-life balance programs, have been effective in alleviating stress and enhancing the well-being of the employee[13]. As this study also suggests, organizations should contain the implementation of such initiatives as limiting stress to employees, instead, they should stress management within the organization comprehensively[14].

Certain similarities and dissimilarities can be outlined when comparing the outcome of this study with that of any previous research. Towards this end, 25% of the participants in the present study experienced high depersonalization, consistent with findings of a researcher and Leiter 2016, which shows the phenomenon of burnout exists in different dimensions. However, the findings also show that this study and a third of participants feel a lack of personal accomplishment suggesting the experience of young professionals will require more in-depth understanding than what has been captured in past literature[15].

While this study yields important understanding of the association between stress, burnout, and risks of heart attack, it has several limitations. The cross-sectional design does not allow making any causal conclusions, and self-reporting can be biased. More focus should be centered on the investigations on stress and burnout across professionals longitudinally considering these effects on cardiovascular health of the young professional population. It would also be beneficial to increase the size of the sample, especially the participants from various sectors to make the study more generalized.

CONCLUSION

To conclude, this study has highlighted the alarming prevalence of job-related stress and burnout has shifted towards young professionals and the consequences that arise from that alarming trend – an increase in the risk towards heart attacks. Based on the findings, stress, and emotional exhaustion were experienced by greater than a half of the participants, which supports other studies which have noted that this group experiences negative effects as a result of occupational demands. The association between stress and burnout with risk for heart attacks calls for more sensitivity from organizations in managing the mental wellbeing of employees as well as stress management. With the changes taking place in the workforce, especially due to the onslaught of modern professions, the problem of addressing job-related stress and burnout is not simply an issue of employee welfare rather it is a matter of workplace vitality that must be addressed. Future directions for research should examine more closely, the chronic consequences of stress and burnout, as Carolyn's on CVD employing longitudinal studies sampling more populations. Building more active strategies for safeguarding and enhancing mental health should encourage organizations to better ensure their healthier personnel are able to respond to pressures of the modern workplace.

Author's Contribution:

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Original Article

Efficacy and Safety of Combining Micro-Needling with Platelet-Rich Plasma (PRP) for the Treatment of Post-Acne Scars: A Clinical Outcome Analysis

Asim Ejaz[1], Muhammad Usman Amiruddin[2], Samia Aslam[3], Saleem Khan[4], Tayyaba Zahid[5] and Sara Gull[6]

ABSTRACT

Objective: To evaluate the efficacy and safety of microneedling combined with PRP compared to microneedling with saline placebo for the treatment of post-acne scars.

Study Design: A prospective study

Place and Duration of Study: This study was conducted at the Superior University Lahore during July 2024 to December 2024.

Methods: A prospective study was with 65 participants aged 18–45 years. Group A (n=33) received microneedling with PRP, and Group B (n=32) received microneedling with saline. Scar severity, patient satisfaction, and skin texture were assessed using Goodman and Baron's grading system, satisfaction scores, and imaging. Adverse effects were recorded. Statistical analysis included paired t-tests and chi-square tests, with $p<0.05$ considered significant.

Results: Group A demonstrated a significant improvement in scar severity (43%) compared to Group B (19%), with a p-value of <0.01. Patient satisfaction was higher in Group A (80% "very satisfied") versus Group B (53%, $p<0.01$). Both groups experienced mild, transient adverse effects such as erythema and edema, with no severe complications reported.

Conclusion: Microneedling combined with PRP is a safe and effective treatment for post-acne scars, offering superior improvements in scar severity and patient satisfaction compared to microneedling alone. Its favorable safety profile and clinical efficacy make it a promising option for scar management.

Key Words: Microneedling, platelet-rich plasma, PRP, post-acne scars, scar treatment, skin regeneration, minimally invasive therapy.

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INTRODUCTION

Post-acne scars are a prevalent and distressing dermatological condition affecting millions of individuals worldwide. These scars result from the inflammatory process of acne vulgaris, often leading to the destruction of collagen and elastic fibers in the dermis[1]. The physical manifestation of post-acne scars can range from atrophic scars, including ice pick, rolling, and boxcar scars, to hypertrophic scars and keloids.

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While acne itself is a temporary condition, its sequelae in the form of scars can persist for a lifetime, significantly impacting self-esteem, psychological well-being, and quality of life[2]. The treatment of post-acne scars remains a challenging endeavor due to the complex pathophysiology and variability in scar types. Various treatment modalities have been developed to address post-acne scars, including chemical peels, subcision, dermal fillers, laser therapies, and microneedling[3]. Among these, microneedling has emerged as a minimally invasive and cost-effective option for improving scar appearance. This technique involves the use of fine needles to create controlled micro-injuries in the skin, which stimulate the natural wound-healing cascade, leading to the production of new collagen and elastin[4]. Microneedling is particularly effective for atrophic scars, as it enhances the skin's structural integrity and reduces scar visibility. However, microneedling alone may require multiple sessions to achieve satisfactory results[5].

To enhance the outcomes of microneedling, platelet-rich plasma (PRP) has been introduced as an adjunctive therapy. PRP is an autologous concentrate of platelets

derived from the patient's blood, rich in growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), and epidermal growth factor (EGF)[6]. These bioactive molecules play a pivotal role in tissue repair, angiogenesis, and collagen synthesis. The application of PRP in combination with microneedling amplifies the regenerative effects by accelerating wound healing, enhancing neocollagenesis, and promoting the remodeling of extracellular matrix components. The combination of microneedling and PRP has gained significant attention in recent years due to its synergistic effects and its minimally invasive nature[7]. It is particularly appealing for patients seeking a safe and effective treatment with minimal downtime. While microneedling creates microchannels in the skin, PRP, when topically applied or injected, penetrates these channels, allowing for deeper delivery of growth factors[8]. This enhances cellular proliferation, improves skin texture, and reduces the depth and severity of scars. Despite its growing popularity, the combined efficacy and safety of microneedling with PRP for post-acne scars remain under continuous investigation. Clinical studies have reported promising results, indicating superior outcomes compared to microneedling or PRP alone[9-11]. However, variability in study designs, patient demographics, and treatment protocols necessitates further research to establish standardized guidelines and optimize therapeutic results. Additionally, while adverse effects are generally minimal, potential risks such as transient erythema, edema, and hyperpigmentation need to be evaluated to ensure patient safety[12].

METHODS

This prospective study was conducted at Superior University, Lahore, from July 2024 to December 2024. A total of 65 patients were enrolled in the study.

Inclusion Criteria

1. Patients aged 18–45 years.
2. Individuals with moderate to severe post-acne atrophic scars (Grade 3–4 on Goodman and Baron's grading system).
3. Patients willing to provide informed consent and adhere to follow-up visits.

Exclusion Criteria

1. Active acne or other inflammatory skin conditions.
2. Patients on isotretinoin within the past six months.
3. Pregnant or lactating women.
4. History of bleeding disorders, keloid formation, or hypersensitivity to treatments.
5. Use of anticoagulant medications.

Data collection: The 65 participants were divided into two nearly equal groups. Group A consisted of 33 patients who underwent microneedling combined with PRP, while Group B included 32 patients who received microneedling with a saline placebo. This grouping allowed for a direct comparison between the efficacy of PRP-enhanced microneedling and microneedling alone. The treatment began with thorough facial cleansing and the application of a topical anesthetic (lidocaine 2.5%) to minimize discomfort. For patients in Group A, 10 ml of blood was drawn and centrifuged to isolate PRP, which was rich in growth factors. A microneedling device with sterile, disposable needles (depth 1.5–2 mm) was used to create controlled micro-injuries on the scarred areas of the face. After microneedling, PRP was immediately applied to the skin in Group A to penetrate the microchannels, delivering growth factors to the dermis. Group B received a saline solution applied in the same manner. Patients were provided post-procedure care instructions, including avoiding direct sunlight, using sunscreen, and applying moisturizers. Follow-up visits were scheduled every two weeks for monitoring and progress evaluation. The primary outcome of the study was the improvement in scar severity, assessed using Goodman and Baron's grading system at baseline and the end of the study.

Statistical Analysis: Data were analyzed using statistical software SPS v26 to assess the significance of treatment outcomes. Paired t-tests were employed to compare pre- and post-treatment scores within groups, while chi-square tests compared outcomes between groups. A p-value of less than 0.05 was considered statistically significant, ensuring the reliability of the study's findings.

RESULTS

The study included 65 participants with a mean age of 28.6 years (range: 18–45). The majority of the participants were female (60%). At baseline, the Goodman and Baron's grading system showed no significant difference in scar severity between Group A (microneedling with PRP) and Group B (microneedling with saline), with mean scores of 3.7 ± 0.4 and 3.6 ± 0.5, respectively ($p = 0.42$). Both groups exhibited similar demographic and clinical profiles, ensuring comparability.

Table No.1: Baseline characteristics of the participants.

Characteristic	Group A (Micro-needling + PRP)	Group B (Micro-needling + Saline)	p-value
Mean Age (years)	28.6 ± 6.4	28.4 ± 6.2	-
Gender (Female)	60%	60%	-
Baseline Scar Severity (Goodman & B aron)	3.7 ± 0.4	3.6 ± 0.5	0.42

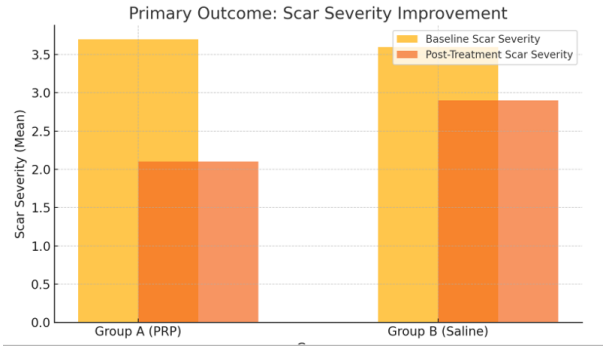

Figure No.1: Primary Outcome - Improvement in Scar Severity

Table No.2: Improvements in scar severity after treatment.

Group	Baseline Scar Severity (Mean)	Post-Treatment Scar Severity (Mean)	Improve-ment (%)
Group A (Microneedling + PRP)	3.7	2.1	43
Group B (Microneedling + Saline)	3.6	2.9	19

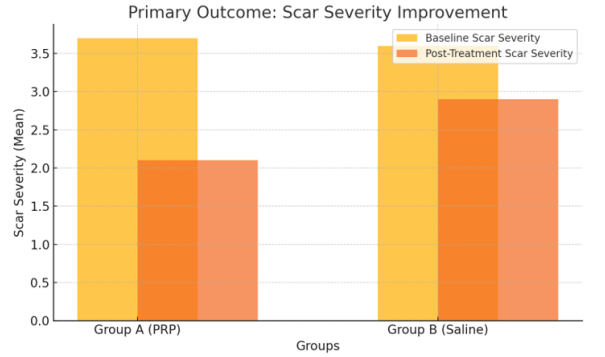

Figure No.2: Secondary Outcomes - Patient Satisfaction and Skin Texture Improvement

Table No.3: Patient satisfaction and skin texture improvement.

Outcome	Group A (Microneedling + PRP)	Group B (Microneedling + Saline)	p-value
Patient Satisfaction (Score ≥4)	80%	53%	<0.01
Skin Texture Improvement (%)	85%	60%	<0.01
Transient Erythema (%)	30%	25%	0.68

Table No.4: Adverse effects reported during the study.

Adverse Effect	Group A (Micro-needling + PRP)	Group B (Micro-needling + Saline)	p-value
Erythema	30%	25%	0.68
Edema	20%	15%	0.72
Hyper-pigmentation	0%	0%	-

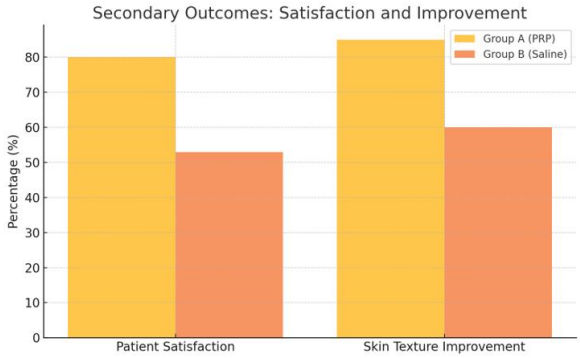

Figure No.3: Adverse Effects Observed

DISCUSSION

The results of this study demonstrate the efficacy and safety of combining microneedling with platelet-rich plasma (PRP) for the treatment of post-acne scars. The findings indicate that this combination therapy provides superior outcomes compared to microneedling alone, as evidenced by significant improvements in scar severity, higher patient satisfaction, and enhanced skin texture[13]. Microneedling, as a standalone procedure, has been widely recognized for its ability to stimulate collagen production through controlled dermal injuries. The addition of PRP enhances this process by delivering concentrated growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF)[14]. These growth factors accelerate tissue repair and collagen remodeling, which are critical for improving atrophic scars. The study results highlight that Group A (microneedling with PRP) achieved a 43% improvement in scar severity, significantly outperforming the 19% improvement observed in Group B (microneedling with saline).

The synergistic effect of microneedling and PRP is further supported by secondary outcomes. Patients in Group A reported higher satisfaction rates, with 80% rating their results as "very satisfied," compared to 53% in the control group[15]. This aligns with the noticeable improvements in skin texture observed through photographic and dermoscopic imaging, suggesting that the combination therapy provides both functional and aesthetic benefits. The safety of microneedling with PRP was evident in this study, as both treatment groups exhibited minimal and transient side effects[16]. The most

common adverse effects, such as erythema and edema, resolved within 48–72 hours and were comparable between groups. Importantly, no severe complications, including hyperpigmentation or infection, were reported, indicating that the combination therapy is well-tolerated when performed under proper clinical protocols[17].

The findings of this study align with previous research on microneedling and PRP for scar management. Studies have consistently demonstrated that PRP amplifies the regenerative effects of microneedling by enhancing collagen synthesis and angiogenesis[18]. However, variability in study designs, PRP preparation protocols, and patient demographics in the existing literature underscores the need for standardized methodologies to optimize treatment outcomes[19]. This study contributes to the growing evidence base by employing a robust, controlled design with clearly defined inclusion and exclusion criteria[20]. While the study provides valuable insights, certain limitations should be acknowledged. First, the sample size of 65 participants, though adequate, may limit the generalizability of the findings to a broader population. Second, the study focused exclusively on atrophic post-acne scars, and the efficacy of this combination therapy for hypertrophic scars or keloids remains unclear. Lastly, long-term follow-up was not conducted, which would have provided insights into the durability of the results.

CONCLUSION

It is concluded that the combination of microneedling with platelet-rich plasma (PRP) is a safe and effective treatment for post-acne scars, significantly improving scar severity, patient satisfaction, and skin texture compared to microneedling alone. Its minimally invasive nature and favorable safety profile make it a promising option for scar management. Further studies are encouraged to optimize protocols and evaluate long-term benefits.

Author's Contribution:

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Drafting or Revising Critically:	Saleem Khan, Tayyaba Zahid, Sara Gull
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Evaluation of Position of Occlusal Plane Relative to Parotid Papilla as an Aid in Complete Denture Fabrication

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ABSTRACT

Objective: To evaluate the reliability of the parotid papilla as a guide for orientation of occlusal plane in the Pakistani population.

Study Design: Cross-sectional, hospital-based study

Place and Duration of Study: This study was conducted at the Department of Prosthodontics of Islamic International Dental Hospital, Riphah International University, over a period of six months start from 01-12-2022 to 31-05-2023.

Methods: A total of 100 patients were examined. The subjects were examined and the vertical distance between the inferior border of parotid papilla and the mesiobuccal cusp tip of maxillary second molar on the right and left sides was measured using a periodontal probe. The measurements were recorded in a written performa, and mean values were calculated. Descriptive statistical analysis were used for qualitative and quantitative variables.

Results: Mean age of the participants was 33.23±7.20 years. The gender distribution of the patients reveals a higher proportion of males, with 57 (57.0%) participants, compared to females, who constituted 43 (43.0%). The mean position of the parotid papilla in relation to the maxillary occlusal plane was calculated as 3.66±0.50 mm.

Conclusion: The findings of this study conclude that the parotid papilla is present above the maxillary occlusal plane. This anatomical relationship was quantified by measuring the mean vertical distance, which was determined to be 3.69 mm.

Key Words: Occlusal plane, Parotid papilla, Complete denture

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INTRODUCTION

Upon replacing the lost teeth with artificial prosthesis, dentists not only aim to restore the esthetics and smile characteristics, but also regain the lost masticatory and functional stimuli. When it comes to restoration, it is imperative to enhance the esthetic features of a smile by making sure that the occlusal plane is correctly oriented.[1]

The GPT (Glossary of Prosthodontic Terms) defines "occlusal plane" as "the average plane established by the incisal and occlusal surfaces of the teeth".[2] A "consonant" smile should be achieved in the denture to establish proper esthetics.[3]

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The correct orientation of occlusal plane is associated with masticatory efficiency and neuromuscular coordination.[1] Moreover, it revealed a neutralised relation of the denture flanges with the soft tissues of the oral cavity.[3] This augments the resultant stability and retentive characteristics of the final denture which, in turn, provides a psychological uplift to the patient when the he is able to talk and bite on food without any hindrance from soft tissues and muscles on the denture.[3]

The parotid gland secretes saliva through the Stensen's duct, which drains in the oral cavity through the parotid papilla. This papilla is located opposite the upper second molar. An accessory parotid gland may also be present, in 21-69% of individuals.[4] It is a separate nodule of salivary tissue present anterior to parotid gland,[4] usually over the masseter muscle.

Both intraoral and extraoral anatomic structures are used to construct the occlusal plane during fabrication of complete dentures.[5] The Stensen's duct (parotid papilla) is possibly the only intraoral landmark that can be used for posterior maxillary denture plane orientation.[6] Extraorally, the Camper's line (line between ala of nose and tragus of ear) is used to establish the maxillary plane of occlusion in posterior region.[5]

ln the past, several researches have been conducted on dentate patients to estimate the distance of parotid papilla with maxillary occlusal plane. ln 1970, Researcher determined a sustained relationship of the parotid papilla and occlusal plane with no significant differences among the genders and race of the subjects included. A rather recent research was conducted in 2019, where Tantray and Zargar[6] evaluated the distance in the Kashmiri population and determined it to be 3.7mm. They divided the measurement of right and left parotid papillae to be 3.2mm and 3.6mm respectively.[6]

The most recent research was carried out in Nepal when Mehta and Chhetri concluded that the parotid papilla was located superior to the maxillary occlusal plane[7] and the mean measurement of distance of parotid papilla and maxillary occlusal plane was 3.69±1.19mm.[7]

There has been only one study[7] conducted in the recent past to evaluate the average distance of parotid papillae with the maxillary occlusal plane, thus providing us with the aim to conduct this study. Therefore, the objective of this study is to determine the location of parotid papilla in relation to the maxillary plane of occlusion in dentulous individuals and to predict its reliability to reposition the occlusal level in edentulous patients of the Pakistani population. The study will help in serving as an anthropometric guide for positioning of the maxillary posteriors in complete denture fabrication.

METHODS

A cross-sectional study was conducted over a period of six months from 01-12-2022 to 31-05-2023. After obtaining clearance from the Ethical Review Committee of Islamic International Dental Hospital (Ref No. IIDC/IRC/2022/10/018), the subjects were selected after obtaining written consent from patients and dental students at the Department of Prosthodontics of Islamic International Dental Hospital, Riphah International University, Islamabad. The data was kept anonymous throughout the procedure and evaluation forms were discarded after data entry.

Sample size was calculated by using the WHO sample size calculator, which was 100 with confidence level 95%, Population mean is 3.69mm.[7] Population standard deviation (±SD) is ±1.19mm. Absolute precision is 0.25mm.

Patients of both genders, with age range of 20 to 50 years with an Angles Class I molar relationship and unrestored, intact maxillary molars. Samples that were excluded from our study included those with missing maxillary molars or patients demonstrating wear facets and those with restorations in maxillary arch. Furthermore, patients with a history of orthodontic treatment and those with supraeruption, rotation or any malalignment of maxillary molars were also excluded.

The vertical distance of parotid papilla from the maxillary occlusal plane in dentate patients was measured. (Figure-1) Subjects reporting to the Department of Prosthodontics in IIDH filling the inclusion criteria were selected for the study. Before selection, each patient was directed and informed about the study and an informed written consent was taken for participation in the study. The subjects were examined. The distance between inferior border of parotid papilla and the mesiobuccal cusp tip of maxillary second molar on the right and left sides was determined. It was verified clinically that the teeth are in maximum intercuspation so that the parotid papilla remains in its unchanged form. Instrument that was used to measure the distance is William's Probe (Hu Friedy, Chicago). The probe is 10mm in length, with color coded markings at each millimeter and readings at 1-2-3-5-7-8-9-10mm. The right and left readings were compared and evaluated for any significant variation. The readings were recorded for both sides in a written performa.

Data analysis was done using Statistical Package for Social Studies version 23 (SPSS v23). Descriptive statistics were calculated for the variables. The quantitative variables like age, distance between parotid papilla and maxillary second molar cusp tip, mean±SD (standard deviation) was calculated. For qualitative variables like gender, percentages and frequencies were calculated. Effect modifiers like age and gender were controlled by stratification. Post-stratification independent sample t-test was applied. P value ≤ 0.05 was considered significant.

RESULTS

The distribution of patients by age shows that the majority of participants 61 (61.0%) were in the age group of 20–35 years. The mean age of the participants was 33.23±7.20 years, reflecting a relatively young patient population in this study (Table-1).

The gender distribution of the patients reveals a higher proportion of males, with 57 (57.0%) participants. This indicates a male predominance in the study population. Table-2 provides an overview of the mean position of the parotid papilla in relation to the maxillary occlusal plane, along with the distances measured on the right and left sides separately. The mean position of the parotid papilla in relation with the maxillary occlusal plane was calculated as 3.66 mm with a standard deviation (SD) of 0.50 mm. This indicates a consistent positioning of the papilla with relatively low variability among the participants. When examining the sides independently, the mean distance on the right side was slightly greater at 3.73 mm (SD = 0.63 mm) compared to the left side, which had a mean distance of 3.56 mm (SD = 0.58 mm). This slight variation suggests a minor asymmetry in the positioning of the parotid papilla between the right and left sides. The relatively small

standard deviations across all measurements indicate a high degree of consistency in the positioning of the parotid papilla among the studied individuals.

The stratification of the mean location of the parotid papilla in relation with the maxillary occlusal plane by age showed minimal variation between the two groups. The mean position in individuals aged 20–35 years (N=61) was 3.68 mm with a standard deviation of 0.50 mm, while in those aged 36–50 years (N=39), it was slightly lower at 3.61 mm with the same standard deviation of 0.50 mm. The difference between these age groups was not statistically significant, as indicated by a p-value of 0.480. This suggests that age does not have a notable impact on the vertical position of the parotid papilla, supporting its reliability as an anatomical reference across different age ranges (Table-3).

Table No.1: Patient distribution by age

Age of patients	Number of patients	Percentage
20-35 years	61	61.0
36-50 years	39	39.0
Total	100	100.00
Mean±SD	33.23±7.20	

Table No.2: Average distance between the parotid papilla and maxillary plane of occlusion

	Mean	S.D
Distance between parotid papilla and maxillary molar cusp	3.66	0.50
Right side distance	3.73	0.63
Left side distance	3.56	0.58

Table No.3: Stratification for age with regard to mean of parotid papilla location with the occlusal plane

Age (Year)	N	Mean	S.D
20-35	61	3.68	0.50
36-50	39	3.61	0.50
P value	P=0.480		

Table No.4: Stratification for gender with regard to mean of parotid papilla with the occlusal plane

Gender	n	Mean	S.D
Male	57	3.68	0.52
Female	43	3.62	0.47
P value	0.581		

The stratification by gender revealed negligible differences between males and females. Among males (n=57), the mean position was 3.68 mm with a standard deviation of 0.52 mm, while in females (n=43), the mean was slightly lower at 3.62 mm with a standard deviation of 0.47 mm. p-value was 0.581 indicating that this difference is not statistically significant. These findings suggest that gender does not significantly influence the vertical position of the parotid papilla in relation with the upper occlusal plane, confirming its consistency as an anatomical reference across both genders (Table-4)

DISCUSSION

Absence of natural teeth and supporting anatomical landmarks can make it difficult to determine the precise position of the occlusal plane.[8,9] The stomatognathic system functions most efficiently and harmoniously when the occlusal plane remains in the neutral zone.[10,11] Any deviation from this position may disrupt normal oral functions, potentially leading to issues such as impaired mastication, phonation difficulties, and discomfort.[12]

Additionally, the occlusal plane plays a pivotal role in enhancing the overall aesthetic appearance by maintaining facial symmetry and proportions, which are particularly important in edentulous patients[9] who may already experience facial sagging due to tooth loss. Reconstructing the occlusal plane involves careful consideration of various mechanical, aesthetic, and phonetic factors. By achieving an appropriate balance among these elements, clinicians can provide edentulous patients with prostheses that serve to have long-term patient satisfaction.[13]

The occlusal plane is represented as the average planar curvature formed by the incisal edges of anterior teeth and the occlusal surfaces of posterior teeth. However, when a patient experiences complete tooth loss, this occlusal plane is also lost,[9] posing a significant challenge for the restorative dentist. Several intraoral anatomical landmarks are utilized to aid in the reorientation of the lost occlusal plane. These landmarks include the incisive papilla[14,15], the retromolar pad[16], the ala-tragus line[17] and parotid papilla.[7] According to Sicher and DuBrul (20), the parotid papilla is an important anatomical feature that typically aligns near the maxillary second molar, though its exact position may vary. Researcher noted that it is located superior to the buccal cusp tips of the maxillary molars. These observations highlight the potential of the parotid papilla as a reference point in reconstructing the occlusal plane, although its variability emphasizes the need for individualized assessment and careful consideration in clinical practice.[6]

The present study holds significant clinical relevance as it provides insights into the location of parotid papilla in relation to the plane of occlusion in dentulous individuals. The findings of the study revealed that the distance of the parotid papilla from the occlusal plane was 3.73 ± 0.63 mm on the right side and 3.56 ± 0.58 mm on the left side. This measured distance of approximately 3.6 mm serves as a practical guideline for clinicians in establishing the occlusal plane during the fabrication of complete dentures. These findings align closely with a study conducted by Mehta and

Chhetri (9), who also reported that the parotid papilla is positioned superior to the maxillary occlusal plane. In this research, the average distance between the parotid papilla and the maxillary occlusal plane was concluded to be 3.69 ± 1.19 mm. This consistency across studies reinforces the reliability of the parotid papilla as a reference point, providing valuable guidance in prosthetic dentistry.

Another study conducted by Tantray and Zargar[6] evaluated the location of the plane of occlusion and parotid papilla in the Kashmiri population. Their findings revealed that the parotid papilla was located approximately 3.7 mm above the occlusal plane on average. When analyzed separately, the distance of the right papilla from the occlusal plane was 3.2 mm, while the left papilla was positioned at a slightly greater distance of 3.6 mm.[6] These results further support the variability in the position of the parotid papilla and its potential utility to be a reference landmark for re-establishing the plane of occlusion, particularly in region-specific populations.

The study carried out by a researcher measured the distance of parotid papilla from the cusp tip of maxillary molars. In comparison to this study, where the distance was 4 mm, the present study demonstrates a notable variation. This highlights the variability in anatomical measurements and underscores the importance of understanding findings within specific study populations and methodologies.

In our study, there can be certain biases including a selection bias. Although the sample size is 100 but if it's not randomly selected, this could affect the reliability of the results. Furthermore, human error, inconsistent techniques, or instrument calibration issues might result in inaccurate or unreliable data. These biases were accounted for by implementing random sampling techniques and it was ensured that measurements are taken by the same trained individual with standardized protocols and consistent methods. A clear exclusion and inclusion criteria were decided, so that the study population was as homogenous as possible.

Limitations: One of the limitations of is our reliance on a cross-sectional design, which only provides a snapshot of the relationship between the parotid papilla and the maxillary occlusal plane at a single point in time. As a result, causal inferences cannot be made, and the study does not account for any potential changes in the anatomical relationship over time. Furthermore, the gender distribution in the sample shows a higher proportion of males, which may introduce gender bias and affect the outcomes.

Recommendations: Based on the results of this study, it is advised that the parotid papilla be further explored as a reliable anatomical guide for occlusal plane determination. Additionally, future studies should consider expanding the sample size and including participants from diverse ethnic backgrounds to validate the generalizability of these. It would also be beneficial to conduct longitudinal studies to assess the stability of this anatomical relationship over time, providing more robust evidence for its use in prosthodontics. Furthermore, future research could explore the influence of other factors, such as age, gender, or systemic conditions, on the positioning of the parotid papilla.

CONCLUSION

In conclusion, the findings of this study highlight that the parotid papilla is positioned superior to the maxillary occlusal plane. This anatomical relationship was quantified by measuring the mean vertical distance, which was determined to be 3.69 mm. This result provides valuable insight into the spatial orientation of the parotid papilla relative to dental structures in the oral cavity.

Author's Contribution:

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Original Article

Estimation of Dectin-1 with Some Biochemical Parameters in Serum of Patients with Type 1 Diabetes Mellitus

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ABSTRACT

Objective: (1) To examine the correlation between serum Dectin-1 levels and various parameters of type 1 diabetes mellitus, including fasting blood glucose, HbA1C, urea, creatinine, uric acid, total cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein, through a comparative analysis between patients with type 1 diabetes mellitus and healthy individuals. (2) To ascertain if alterations in serum Dectin-1 could assist in the diagnosis of Type 1 diabetes mellitus.

Study Design: Comparative study.

Place and Duration of Study: This study was conducted at the Baqubah Teaching Hospitals in Diyala, Iraq from 10th October 2024 to 31st December 2024.

Methods: A total of 80 patients with Type 1 diabetes mellitus under the age of 20, alongside 40 healthy individuals of comparable age serving as a control group were enrolled.

Results: The significantly elevated Dectin-1 levels in Type 1 Diabetes Mellitus (T1DM) patients compared to controls, indicating a potential biomarker for the disease. Glycaemic variations were notable, with higher HbA1c and fasting blood glucose levels in T1DM patients. Lipid profiles showed increased total cholesterol, triglycerides, and low-density lipoprotein, alongside decreased high-density lipoprotein, suggesting heightened cardiovascular risk. Additionally, renal function markers like urea and creatinine were elevated, indicating metabolic changes. Correlation analyses demonstrated strong links between Dectin-1 and various metabolic markers, with Dectin-1 showing excellent diagnostic capability for T1DM, highlighting its role in treatment and immunity.

Conclusion: Dectin-1 significantly increased in T1DM patients. It is significantly correlated with HbA1c, renal markers, and lipid profile. The results of statistical analysis of ROC curve suggest that Dectin-1 could be considered as a excellent biomarker,

Key Words: Type 1 DM, Dectin-1, Autoimmune Disorder, Glycemic Control, Metabolic marker

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INTRODUCTION

Diabetes mellitus (DM), also known as diabetes, is a chronic metabolic disorder characterised by hyperglycemia, or elevated blood glucose levels.[1] This illness results from either the body's inability to produce insulin or the ineffective utilisation of insulin, a hormone generated by the pancreas that is essential for regulating blood sugar levels. Diabetes affects multiple bodily systems, resulting in significant long-term health complications that extend beyond mere blood sugar rise.[2,3]

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Type 1 diabetes mellitus (T1DM) is a complex metabolic disorder resulting from the interplay of environmental, genetic, and immunological factors. This condition primarily occurs when the immune system attacks and destroys pancreatic beta cells, influenced by various environmental factors, including toxic agents and viral infections such as mumps and coxsackie B4. The susceptibility to disease and its course are significantly influenced by genetic predisposition. Recent research challenging the traditional belief that insulin resistance is confined to type 2 diabetes have demonstrated the significant role insulin resistance plays in type 1 diabetic mellitus (T1DM).[4,5]

A phenomenon termed "double diabetes" indicates that approximately 35% of Type 1 Diabetes Mellitus individuals exhibit insulin resistance. This resistance contributes to the metabolic strain on surviving beta cells and is evident even prior to a clinical diagnosis. Inadequate glycaemic regulation, heightened cardiovascular risk, and both microvascular and macrovascular complications, such as diabetic nephropathy and retinopathy, are the primary

repercussions of insulin resistance in type 1 diabetes mellitus.[5-8]

Dectin-1, a pattern recognition receptor in the C-type lectin receptor family, serves as a significant biological mediator linking metabolic dysfunction in insulin resistance to innate immunity.[9,10] This receptor, predominantly found on immune cells such as macrophages and dendritic cells, reacts to β-glucan structures by initiating complex inflammatory cascades that significantly influence insulin sensitivity. Dectin-1 initiates a complex signalling network involving spleen tyrosine kinase (Syk), which subsequently triggers many inflammatory pathways.[11] Pro-inflammatory cytokines encompass tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), which directly engage with insulin signalling pathways as a consequence of this activation. The molecular mechanism involves the disruption of insulin receptor substrate (IRS) phosphorylation and diminished translocation of glucose transporter 4 (GLUT4) in adipose and muscle tissues, thus resulting in the development of insulin resistance.[12-14]

METHODS

This study was conducted from 10th October 2024 to 31st December 2024, collecting 120 blood samples from type 1 diabetic patients under 20 years old at Baqubah and Al Batool Teaching Hospitals in Diyala Governorate. Additionally, 40 samples from healthy subjects of the same age group served as a control. Blood was drawn via venous puncture, with 5 ml collected in plastic syringes. The samples were divided: one part in EDTA tubes for HBA1C testing and another in gelatin tubes for serum extraction. The serum underwent centrifugation and was further divided for various blood tests, including FBS, TG, TC, HDL-C, uric acid, urea, and creatinine, while a portion was stored at -20°C for Dectin-1 level evaluation. Statistical analyses were performed using SPSS-25, employing T-tests for two data sets and one-way ANOVA for three or more groups, with a significance threshold set at $p < 0.05$.

RESULTS

The analysis highlighted notable differences between T1DM patients and a control group. The mean age of T1DM patients was 11.6 years, higher than the control group's 9.5 years, indicating a suitable age for paediatric diabetes. T1DM patients had a BMI of 21.83 kg/m², slightly lower than the controls at 22.45 kg/m², with no significant difference. However, fasting blood sugar levels were significantly higher in T1DM patients (271.5 mg/dl) compared to controls (84.95 mg/dl), indicating hyperglycemia. Additionally, HbA1c levels were significantly elevated in T1DM patients (8.14%) versus controls (4.58%), underscoring the need for effective glycemic management in juvenile diabetes (Table 1).

Comparing uric acid, creatinine, and urea levels between Type 1 diabetes mellitus (T1DM) patients and a control group, the mean uric acid level in T1DM patients was 3.335±0.261 mg/dl, slightly lower than the control group's 3.451±0.9100 mg/dl, with no significant difference ($p > 0.05$). However, creatinine levels showed a significant difference ($p<0.0001$), with T1DM patients averaging 0.915±0.092 mg/dl compared to 0.676±0.098 mg/dl in controls. Urea levels were also higher in T1DM patients (24.510±6.781 mg/dl) than in controls (16.600±3.081 mg/dl) [Table 2].

Type 1 diabetes mellitus (T1DM) patients exhibit significantly higher cholesterol and triglyceride levels compared to a control group, indicating a greater risk for hypercholesterolemia and cardiovascular issues. T1DM patients had mean cholesterol levels of 193.25 mg/dL and triglycerides at 131.68 mg/dL, while controls showed lower levels. Additionally, LDL-cholesterol levels were notably higher in T1DM patients (117.55 mg/dL) compared to controls (73.75 mg/dL), while HDL-cholesterol levels decreased in T1DM patients (49.95 mg/dL) versus controls (58.70 mg/dL). The variability in cholesterol levels among T1DM patients underscores the need for regular monitoring and management of lipid profiles in this population (Table 3). The comparative analysis of lipid profiles between T1DM patients and a control group. The data shows marked differences across all measured lipid parameters (Fig. 1).

In T1DM patients, the average Dectin-1 level was significantly higher (2027.368±180.61) than in controls (1185.34±65.32), with a mean difference of 1542.328. A high t-test score (20.99) and a p-value of 0.0001 indicate a strong statistical significance, suggesting a biological link between type 1 diabetes and elevated Dectin-1 expression (Table 4). The significant correlations between Dectin-1 levels and diabetes markers, specifically HBA1C% and fasting blood glucose (FBG) were noted. A strong positive relationship (correlation of 0.76) exists between Dectin-1 and HBA1C%, suggesting Dectin-1 may serve as a reliable biomarker for long-term glycaemic control in Type 1 diabetes patients (Table 5).

The significant positive correlations were found between Dectin-1 and urea ($r=0.485$, $p=0.003$) and creatinine ($r=0.59$, $p=0.001$), while no significant correlation was observed with uric acid ($r=0.02$, $p=0.95$). Notably, a strong correlation with cholesterol ($r=0.69$, $p<0.0001$) was identified, though triglycerides, LDL, and HDL showed no significant associations. These findings suggest Dectin-1's potential impact on kidney function and lipid profiles (Table 6).

The statistical analysis of the ROC curve for Dectin-1 in type 1 diabetes patients indicates remarkable diagnostic performance, with an AUC of 0.994, nearing perfection.

Table No. 1: Comparison of Age, BMI, FBS and HbA1C in T1DM Patients and Controls

Variable	T1DM Patients	Control	p-value
Age (Years)	11.600±2.062	9.500±2.99	P>0.05
BMI (Kg/m$^{2)}$	20.83±3.783	22.44±1.35	*P*=0.385
FSG (Mg/dl)	271.500±99.051	84.950±7.970	P<0.00001
HBA1C %	8.145±1.993	4.584±0.281	p<0.0001

Table No. 2: Comparison of urea, creatinine, and uric acid in studied groups

Variable	T1DM Patients	Control	p-value
Uric acid Mg/dl	3.335±0.261	3.451±0.910	0.38
CRE Mg/dl	0.915±0.092	0.676±0.098	< 0.001
URE Mg/dl	24.510±6.781	16.600±3.081	< 0.0001

Table No.3: Comparative of lipid profile in studied groups

Variable	T1DM Patients	Control	p-value
Total Cholesterol (mg/dL)	193.25±16.24	147.50±8.35	< 0.001
Triglycerides (mg/dL)	131.68±17.52	67.90±7.50	< 0.001
LDL (mg/dL)	117.55±14.76	73.75±6.48	< 0.001
HDL (mg/dL)	49.95±8.82	58.70±6.64	0.008

Table No.4: Presents a comprehensive statistical analysis of Dectin-1 expression levels comparing T1DM patients with a control group

Groups	Dectin-1 (ng/ml)	Mean difference	Standard error	t-test	p-value
T1DM Patients	2027.36±180.61	842.02	72.34	20.99	P<0.0001

Table No.5: Correlations of Dectin-1 with FBS and HBA1C in Type 1 diabetic patients

Variable	Serum Dectin-1 in type 1	
Fasting blood glucose	Correlation coefficient	0.56
	Significant value	P<0.001
HBA1C%	Correlation coefficient	0.76
	Significant value	P<0.001

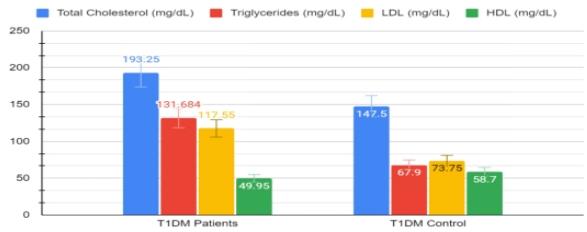

Figure No. 1: comparison of lipid profile between studied groups

Table No.6: Correlation of Dectin-1 with urea creatinine, uric acid and lipid profile parameters in Type 1 diabetic patients

Variable	Serum Dectin-1 in type 1	
Urea	Correlation coefficient	0.485
	Significant value	0.003^{x}
Creatinine	Correlation coefficient	0.59
	Significant value	0.001^{x}
Uric acid	Correlation coefficient	0.02
	Significant value	0.95
Cholesterol	Correlation coefficient	0.69
	Significant value	0.001^{x}
Triglyceride	Correlation coefficient	0.30
	Significant value	0.140
LDL	Correlation coefficient	0.29
	Significant value	0.231
HDL	Correlation coefficient	-0.23
	Significant value	0.338

Table No.7: The statistical analysis of receiver operating characteristic curve and AUC of dectin-1 in Type 1 diabetes patients

Area under the ROC curve (AUC)	0.994
Sensitivity	95.5
Specificity	90.2
Standard Error	0.00757
95% Confidence interval	0.891 to 1.000
Z statistic	65.232
Significance level P (Area=0.5)	<0.001

The test exhibits a sensitivity of 95.5% and specificity of 90.2%, supported by a low Standard Error of 0.00757. The 95% Confidence Interval ranges from 0.891 to 1.000, reinforcing its diagnostic reliability. A substantial P-value (P<0.0001) and a z-statistic of 65.232 strongly reject the null hypothesis, confirming Dectin-1's effectiveness in distinguishing between positive and negative cases. Overall, Dectin-1 shows promise as a highly reliable diagnostic tool for type 1 diabetes (Table 7).

DISCUSSION

A measurement of blood glucose levels following an overnight fast, fasting blood sugar (FBS) Dectin-1

expression was shown to be greater in T1DM patients with higher FBS levels in a 2024 publication.[9] Patients with FBS levels above 180 mg/dL had noticeably more Dectin-1 expression than those with FBS levels less than 180 mg/dL, according the results. Dectin-1 levels were also favourably associated, according another investigation, with FBS levels in T1 diabetic patients.[15,16] These outcomes line up with those of this study. A gauge of average blood glucose levels over the past two to three months is HbA1c. Higher HbA1c levels in T1DM patients raised Dectin-1 expression, according to a prior study.[19-21] Dectin-1 levels were also positively linked, according another study, with HbA1c levels in T1DM patients.[22] These findings also line up with those of this investigation. The processes behind the interactions between FBS and HbA1c with Dectin-1 in T1DM remain mostly unknown. Dectin-1 may be involved in the autoimmune response and the loss of pancreatic beta cells in T1DM.[20]

Urea is a waste substance expelled in the urine. Higher urea levels in T1DM patients raised Dectin-1 expression, according previous studies.[23] The urea levels in patients with T1DM were favourably linked with Dectin-1 levels.[24] These findings contradict those of this study in dosage. Produced by the muscles, creatinine is a waste product eliminated in the urine. Dectin-1 levels were higher in T1DM patients with higher creatinine levels according to a past study.[25] Another study found that creatinine levels in T1DM patients were favourably linked with Dectin-1 levels.[26,27]

Cholesterol content in the blood is total cholesterol. Dectin-1 expression was found to be higher in T1DM patients with higher total cholesterol levels.[28] One sort of fat present in blood is triglycerides. Dectin-1 expression was higher in T1DM patients with higher triglyceride levels.[29] Patients with triglycerides above 150 mg/dL showed notably increased Dectin-1 expression than those with triglyceride levels below 150 mg/dL. Because it lowers extra cholesterol from the bloodstream, high-density lipoprotein-cholesterol (HDL-C) is sometimes referred to as "good" cholesterol. Dectin-1 levels were inversely linked with HDL-C levels in individuals with T1DM.[10] Patients with HDL-C levels above 60 mg/dL had notably lower Dectin-1 levels than those with levels below 60 mg/dL, according the study findings. Often referred to as "bad," low density lipoprotein-cholesterol (LDL-C) can build up in artery walls and raise the risk of heart disease. Higher LDL-C levels in T1DM patients raised Dectin-1 expression.[30] Patients with LDL-C levels above 100 mg/dL demonstrated notably higher Dectin-1 expression than those with levels below 100 mg/dL, according to the study findings. The outcomes of this investigation matched those of lipids profile parameters presented above.[31]

Except Uric Acid, the results of correlations for dectin-1 with other T1DM parameters tested in this study FBG, HbA1C, urea, creatinine, and lipid profile parameters indicated substantial correlation.[32] These results are especially remarkable since they show that the Dectin-1 levels elevation in T1DM patients have a significant correlation with either immediate blood glucose levels or long-term glucose control as determined by HBA1C%. This implies that the rise of Dectin-1 in diabetic patients affects other features of the condition being directly connected to blood glucose control. Strong statistically significant link between Dectin-1 levels and the results among the investigated kidney indicators, urea and creatinine. This suggests a possible function of Dectin-1 in kidney function or disease development in type 1 diabetic patients. The strong link between urea, creatinine could imply that Dectin-1 has a special relationship with kidney function in some respects of renal physiology.

CONCLUSION

Dectin-1 emerges as a critical molecular mediator bridging immune function and metabolic health. Dectin-1 significantly increased in T1DM patients. Dectin-1 significantly correlated with FSG, HbA1c, renal markers, and cholesterol. Its dynamic interactions with these parameters underscore the receptor's significance in understanding complex physiological processes.

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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Original Article

Estimate the Level of MiR-125a-5p and IL-23 in Rheumatoid Arthritis Patients

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ABSTRACT

Objective: To evaluate the expression of miR-125a-5p, IL-23, and inflammatory markers in RA patients, assess DAS28 severity, and compare findings with healthy controls to support early diagnosis and treatment.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the College of Health and Medical Technology, Middle Technical University, Baghdad Iraq from 1st May 2024 31st October 2024.

Methods: One hundred and fifty blood samples, 100 rheumatoid arthritis patients (53 females, 47 males) and 50 healthy controls (28 females, 22 males) were enrolled. RNA was extracted using Trisol; cDNA synthesized and analyzed by real-time PCR using SYBR Green. IL-23 was measured via ELISA. Anti-CCP, RF, and Hs-CRP were analyzed using Cobas e 411, Abbott, and Roche analyzers.

Results: miR-125a-5p expression significantly increased in RA patients, severe 11.16±7.28, moderate 6.34±1.29, mild 5.75±2.46 and control 1.0±0.98. IL-23 levels also increased with disease severity

Conclusion: Elevated miR-125a-5p and IL-23 levels are linked to RA progression and can aid in diagnosis and disease monitoring.

Key Words: Interleukin-23, MiR-125a-5p, Anti-ccp, RF, Hs-CRP, DAS28

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INTRODUCTION

Rheumatoid arthritis is a systemic, inflammatory/ autoimmune, polygenic disease affecting millions of people worldwide. Its etiopathology is attributed to a crosstalk between genetic predisposition, autoimmunity and environmental factors. This heterogeneous disorder is characterized by chronic synovitis and a fluctuating clinical course that may result in long-term disability and reduced quality of life in many patients.[1,2] According to the 2010 Classification Criteria of the American College of Rheumatology (ACR), rheumatoid factor (RF) and/or antibodies against cyclic citrullinated proteins (anti-CCP) is needed for classification.[3-4] Anti-CCP are highly specific for RA and are detected in 60%–70% of RA patients; RF is also present in nearly 70% of patients with RA.[5,6]

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Nowadays, diagnostic tests for RA are not sufficiently accurate, and leading to the late diagnosis of the patients. Therefore, new biomarkers need to be identified to provide a rapid, simple, with high sensitivity and specificity for the diagnosis of RA.[7,8]

MiR-125a-5p located in 19q13.41, known to play a regulatory role in various physiological and pathological processes, including inflammation and immune responses[9] is involved in modulating the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, also IL-23 and IL-10 which are critical mediators of inflammation in RA 15. Its play a role in regulating the migration, proliferation and inflammatory responses of RA-synovial fibroblast, impacts the differentiation and functions of T cells, B cells, macrophages and influence the balance between Th17 cells and T cells (Tregs).[10]

IL-23 is a pro-inflammatory cytokine belonging to the IL-12 cytokine family. IL-23 is essential for the differentiation of Th17 lymphocytes, a subtype of T lymphocyte implicated in chronic inflammatory/ autoimmune mediated diseases, produced by mononuclear (dendritic cells and macrophages) cells in the synovial fluid of RA patients, promotes inflammatory responses by inducing IL-8 and IL-6 production from human fibroblast-like synoviocytes.[11] It plays a key role in both innate and adaptive immunity.[12] The ability of IL-23 to induce IL-17 provides a unique role in the development and the maintenance of autoimmune inflammation.[13]

METHODS

This cross-sectional study in Wasit Province included 150 blood samples (100 RA patients and 50 healthy controls) collected from 1st May 2024 31st October 2024. RA diagnosis was based on ACR criteria and confirmed by rheumatologists. Anti-CCP, RF, and CRP were measured using automated analyzers, IL-23 by ELISA, and miR-125a-5p expression by RT-qPCR normalized to U6**.** Autoimmune disease, malignancy, pregnancy and current infectious disease were included. All participants in this study were informed before collecting samples, and a verbal agreement was obtained from each of them. The subject data, permission form, and the study protocol were examined and approved by a local ethics committee.

Molecular detection of miRNA-221 was carried out according to Taq Man™ MicroRNA Assayfor 20 samples (5 mild, 5 moderate, 5 severe, and 5 control),

Total RNA was extracted from whole blood using the TRIzol® reagent kit following the manufacturer's instructions. Briefly, 500 µl TRIzol® was added to 250 µl blood, followed by 0.2 ml chloroform. After mixing and phase separation, samples were centrifuged, and the aqueous phase was transferred. RNA was precipitated with isopropanol, washed with 75% ethanol, air-dried, and dissolved in 50 µl of dissolving solution. RNA was stored at -70 °C and quantified using a Nanodrop spectrophotometer by measuring absorbance at 260/280 nm.

Amplifying of the has- miR125a-5p and housekeeping gene U6 were carried out using primers (Table 1). Primers have been diluted with 300 µl distilled water, then 10 µl from each primer added to 90 distal water to make concentration 100 in whole blood patients and control. These primers were provided by Macrogen Company, Korea:

MiRNA cDNA synthesis step for miRNA125a5p by using cDNA master mix was used. After that, these qPCR master mix component placed in qPCR premix standard plate tubes then the plate mixed by vortex centrifuge for 3 minutes, and placed in Real-Time PCR system. Thermocycler conditions convert RNA into cDNA (RT step) firstly the temperature 50°C for 1 hour and for heat inactivation 95°C for 5 min.

A RT Quantitative PCR master mix was prepared using the GoTaq® qPCR Master Mix (Promega), based on SYBR Green dye detection. The mix was dispensed into qPCR premix standard plate tubes, vortexed and centrifuged for 3 minutes, then loaded into the Real-Time PCR system. The thermocycling conditions were: initial denaturation at 95°C for 5 min (1 cycle), followed by 40 cycles of denaturation at 95°C for 20 sec, annealing at 56°C for 30 sec, extension at 72°C for 30 sec, and a final hold at 4°C.

The qPCR also used in quantification of housekeeping gene (U6) used in normalization of miRNA125-a-5p expression analysis. qPCR master mix was prepared according to kit .After that, these qPCR master mix component placed in qPCR premix standard plate tubes that contain the other qPCR SYBR green dyeamplification components, then the plate mixed by vortex centrifuge for 3minutes, then placed in Real-Time PCR system .

Statistical analysis was performed using SPSS-28.0. Anova and independent t-test were used to compare groups, and Pearson's correlation assessed relationships between variables. ROC analysis was used to determine cut-off values. DAS28 scores were interpreted based on EULAR and APLAR criteria: >5.1 indicates active disease, <3.2 indicates low activity, and <2.6 indicates minimal disease activity.

RESULTS

Table 2 revealed highly significant differences ($P<0.01$) between RA patients and healthy controls: Anti-CCP (43.8±19.7 vs. 8.09±1.94), Hs-CRP (15.9±5.2 vs. 0.91±0.33), ESR (45.79±17.7 vs. 12.06±1.84), and RF (27.0±11.1 vs. 5.2±1.67). Table 3 shows IL-23 levels significantly differed ($P<0.01$) among groups based on DAS28 severity: Control (107.8±23.6), Mild (129.8±10.8), Moderate (269.7±59.8), and Severe (751.5±94.7). Post hoc analysis indicated significant differences ($P<0.01$) between most groups, except between moderate vs. mild and mild vs. control ($P>0.01$), which were not statistically significant.

The findings demonstrated that the result, IL-23 level increases with development of the disease, as seen by considerably greater mean level seen in the patient samples compared to the control samples as seen in Figure1A.The current research successfully identified IL-23 level linked to RA. Figure 1B show the DAS28 classification of patient and compare with control. Patient suffering from RA were divided into four groups according to the DAS28 index. The ANOVA statistical test results showed that the mean level of IL-23 statistically varied between the four different subgroups in terms of disease activity. The levels of IL-23 were increasing by elevating the DAS28 index.

Table (3) also showed DAS28 classification of patient and compare with control for has-miRNA125-a-5p expression. The results showed that the mean folding of has-miRNA125-a-5p statistically varied between the four different subgroups in terms of disease activity. The levels of has-miRNA125-a-5p folding were increasing by elevating the DAS28 index

Figure 2A shows the comparison of has-miR-125a-5p expression between RA patients and healthy controls. The results indicate a significantly higher mean level of has-miR-125a-5p in RA patients, suggesting its upregulation with disease progression. Figure 2B presents the DAS28-based classification of RA patients into four subgroups, compared with the control group. ANOVA analysis revealed significant differences in IL-

23 levels among the subgroups, with levels increasing in parallel with DAS28 scores. Additionally, has-miR-125a-5p expression increased with higher disease activity. The Mean, SD and median of ΔCt, ΔΔCt and Fold change of has-miRNA125-a-5p in Patient compare with control (Table 4). To identify the changes that happened in the expression levels of the selected miRNAs in blood from patients with RA compared to healthy controls, real-time qPCR was implemented. To show the mean expression, the formula ΔCt=Ct (Reference gene) – Ct (miRNA of interest) was used. The overall analysis of the results showed a significant increase in the expression levels in patients compared to healthy controls ($p< 0.01$). Table 5 displays the Spearman correlation results, showing a strong positive correlation between miR-125a-5p and CRP, ESR, Anti-CCP, RF, DAS28-ESR, and DAS28-CRP, indicating its potential as a biomarker for RA severity.

Table No.1: Primer Sequence with their product size

Primers	**Sequence**		**Reference**
has-miR-125-a5p	RT primer	5′TGTCAGGCAACCGTATTCACCGTGAGTGGTTCACAG-'3	15
	has- miR-128b	5′-UCCCUGAGACCCUUUAACCUGUGA-'3	
	F	5′-TGTCAGGCAAGTATTCACC'3	
	R	5′-CGTCAGATGTCCGAGTAGAGG'3	
U6 (snRNA)	F	5′-CTCGCTTCGGCAGCACATAT -'3	16
	R	5′- TTGCGTGTCATCCTTGCG-'3	

Table No.2: Distribution of Biomarkers level according to studied groups

Test	**RA patient**	**Control**	**t test**	**p.value**
Anti-CCP	43.8±19.7	8.09± 1.94	17.97	HS (<0.01)
CRP	15.9±5.2	0.91± 0.33	28.25	HS (<0.01)
ESR	45.79±17.7	12.06±1.84	18.8	HS (<0.01)
RF	27.0±11.1	5.2±1.67	17.5	HS (<0.01)

Table 3: Serum levels of IL-23 and blood gene folding of has-miRNA125-a-5p according to disease severity in the studied groups

Groups	Sever group (F1) N= 40	Moderate group (F2) N=36	Mild group (F3) N= 24	Control (F4) N=50	ANOVA
IL-23 (Pg/ml)	751.5±94.7	269.7±59.8	129.8±10.8	107.8±23.6	HS (<0.01)
Post hoc test (P-Value)	F1 vs F2 ,F3,F4 =HS < 0.01 F2vs F3 = NS > 0.01		F2 vs F4 =HS < 0.01 F3 vs F4 = NS > 0.01		
has-miRNA125-a-5p	11.16±7.28	6.34±1.29	5.75±2.46	1.0 ±0.98	HS.<0.01
Post hoc test (P-Value)	F1 vs F2 ,F3 = HS < 0.01 F3 vs F4 = NS > 0.01		F2 vs F3, F4 = HS < 0.01 F4 vs F1 = HS < 0.01		

Table No.4: Mean, median and standard deviations (SD) of the studied miRNA PCR values (ΔCt, ΔΔCt and fold change)

has-miRNA125-a-5p				**P. value**
Groups		**Patient (No. = 15)**	**Control (No. = 5)**	
ΔCt	MEAN	0.40	3.13	< 0.01
	SD	0.77	2.40	
	MEDIAN	0.56	2.00	
ΔΔCt	MEAN	-2.73	0.00	< 0.01
	SD	0.77	2.40	
	MEDIAN	-2.57	-1.13	
Fold change (2^-ΔΔCT)	MEAN	7.75	1.74	< 0.01
	SD	5.29	1.10	
	MEDIAN	5.95	2.19	

Table 5: Correlation among studied parameters in RA groups

		MICRO RNA	DAS 28 CRP	DAS 28 ESR	CRP(mg\dL)	IL -23	RF	ANTI CCP	ESR
MICRO RNA	R	1							
DAS 28 CRP	R	.614**	1						

DAS 28 ESR	R	.593**	.967**	1					
CRP(mg\dL)	R	.659**	.958**	.975**	1				
IL -23	R	.565**	.887**	.865**	.830**	1			
Rheumatoid titer	R	.607**	.952**	.946**	.943**	.892**	1		
ANTI CCP	R	.554*	.939**	.968**	.951**	.891**	.947**	1	
ESR	R	.618**	.970**	.959**	.944**	.919**	.956**	.955**	1

**. Correlation is significant at the 0.01 level

Table No.6: Estimation of has-miRNA125-a-5p cut-off values, sensitivity, specificity in studied groups

TEST	Cut-off	SN (%)	SP (%)	AUR	P-Vales
NPT	3.44	85 %	95 %	0.90	<0.01

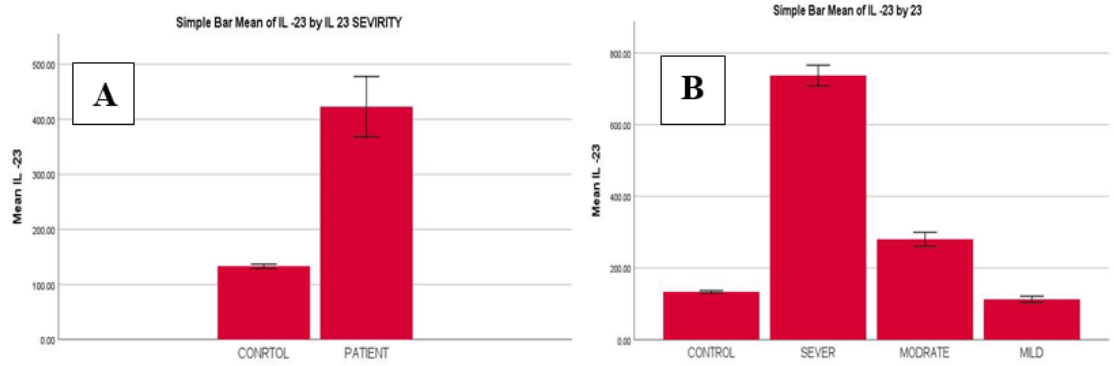

Figure No. 1: Comparison of levels of circulating IL-23 (A) Between rheumatoid arthritis (RA) patients and healthy controls. (B) Between severity group based on DAS28 classification into mild, moderate, severe and control

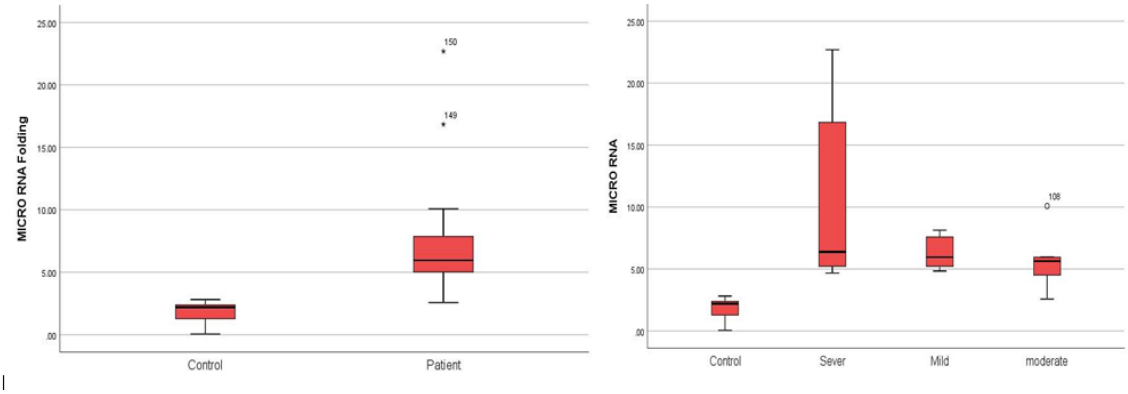

Figure No. 2: Comparison of levels of circulating miR-125a-5p between rheumatoid arthritis patients and healthy co

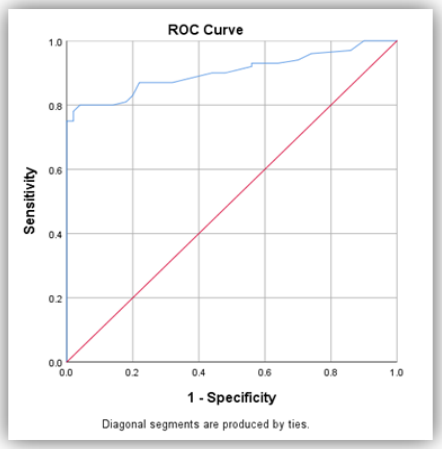

Figure No. 3: ROC Curve chart for the association has-miRNA125-a-5p Marker in the RA studied group

Receiver Operating Characteristic (ROC) analysis was used to identify the optimal cutoff value for the diagnostic test. Table 6 and Figure 3 illustrate the balance between sensitivity and specificity, along with the statistical significance of the test parameters. The ROC analysis demonstrated a high diagnostic accuracy of hsa-miR-125a-5p expression for RA, with an area under the curve (AUC) of 0.90 ($p < 0.01$). The optimal cutoff value was 3.44, with a sensitivity of 85% and specificity of 95%.

DISCUSSION

MIR-125a-5p were significantly elevated in RA patient compare with healthy control subjects, this result similar to that found an increase blood level of miR-125a-5p in RA patients.[16,17] Ormseth et al[18] showed there was a significant over expression miR-125a-5p, in

peripheral blood of RA patients compared to control. When analyzing the with previous studies[19,20] confirmed that the upregulation miR-125a-5p expression appears significantly up-regulated of RA patients compared with healthy controls these studies match with our study. On the other hand, the results of this study indicated that the levels of miR-125a-5p are positively correlated with CRP, ESR, Anti CCP, RF, DAS 28, IL-23, and DAS 28 CRP indices in RA patients, this finding match with other studies that found similar correlation.[19,20] These result is proposing the increase expression of mir-125a-5p is positively associated with the disease activity. The elevated mir-125a-5p expression is to increase the inflammatory process citrullination in RA patients according to(23) that found expression miR125a-5p with other microRNAs were higher in RA patients. Therefore, these findings suggest that the mir-125a-5p may be involved in the occurrence and progression of RA disease. IL-23 result in this study were significantly elevated in RA patient compare with healthy control subjects, these results are consistent with the previous studies that revealed a significant increase in the mean of IL-23 level among the RA patients as compared to the controls[23], other studies found the circulating IL-23 concentrations were significantly high in patients with RA compared with controls and there was a significant positive correlation between serum IL23 levels in patients with RA and individual disease activity parameters.[24,25] Furthermore, study revealed no correlation between IL-23 and disease activity measured by DAS 28 score despite of gradual increase in the median level of serum IL-23 in RA patients with remission, low activity, moderate activity and severe activity, this increased serum level failed to achieve a significant difference[26] which not corresponding with our study. IL-23 is crucial for the maintenance and expansion of Th17 cells. Zaky et al[26] showed that a negative correlation between miR-125a-5p expression and the percentage of Th17 cells in human CD4+ T cells as well as a positive correlation between miR-125a-5p expression and the percentage of T-regulatory in human CD4+ T cells. In summary, these data demonstrate that the up regulation of miR-125a-5p expression act as a compensatory mechanism to limit excessive inflammation that caused by high level of IL-23 in blood of RA patient.

CONCLUSION

Elevated miR-125a-5p expression in RA patients suggests its role in disease progression and potential as a diagnostic marker. Additionally, increased IL-23 promotes IL-17 production, contributing to systemic inflammation and elevated CRP and ESR levels.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Noor Mahdi Dakhil, Suhad Hassan Aubaid
Drafting or Revising Critically:	Raya Al-Saade, Sahar Adnan Shams Al-din
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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

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Original Article

Left Ventricle Dilatation: Early Marker of Structural Remodelling of the Heart in Obese People

Asaad Hasan Noaman, Falah Mahdi Dananah, Amina Abdul Baqi Khuthur, Shaymaa AH Jasim

ABSTRACT

Objective: Transthoracic echocardiography is used to examine whether or not left ventricular dilatation is an early and independent indicator of cardiac remodeling in obese people.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Al-Batool Hospital (bariatric surgery consultation), from January to October, 2024.

Methods: This cross-sectional study was consisted of 200 adults between the ages of 30 and 60 were split into two groups: non-obese (body mass index 22.4, n=80) and obese (body mass index ≥30, n=120). Left ventricular end-diastolic diameter left ventricular end-systolic diameter (LVESD), left ventricular mass index, and ejection fraction were assessed using standard 2D transthoracic echocardiography. Multivariate regression and Student's t-check were used for statistical comparisons.

Results: Compared to non-obesity controls, obese people had significantly higher mean left ventricular end-diastolic diameter (5.9±0.3 cm vs 5±0.4 cm, $p<0.001$) and left ventricular mass index (128±14 g/m² vs. 97±13 g/m², $p<0.001$). Left ventricular dilatation linked to body mass index ($r=0.6$, $p<0.001$). After indexing for age, sex, and hypertension, left ventricular dilatation still showed a correlation with body mass index ($r=0.622$, $p<0.001$).

Conclusion: Even in the absence of obvious clinical symptoms, LV dilatation is a unique and early echocardiographic indicator of structural heart transformation in obese people.

Key Words: Left ventricular dilatation, Obesity, Cardiac remodelling, Echocardiography, Body mass index, Heart failure, Structural changes, Cardiomyopathy

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INTRODUCTION

With its incidence nearly tripling globally over the past forty years, obesity has become a significant global health issue of the twenty-first century. In 2022, over 1.9 billion persons were overweight, with over 650 million of them classified as obese, according to the World Health Organization.[1] Because of its detrimental hazard on heart obesity is linked to increased cardiovascular morbidity and death.[2,3]

Cardiac remodeling is a complicated process that involves alterations in the heart's length, shape, and characteristics in response to long-term stressors such as obesity, metabolic syndrome, and hypertension.[4,5] These changes may initially be advantageous as well, but they frequently lead to maladaptive consequences, such as heart failure.[6,7] Increased LV mass, chamber dilatation, and left ventricular (LV) hypertrophy are characteristics of structural remodeling in obese people.[3,8]

Numerous investigations have reported concentric hypertrophy and elevated left ventricular mass in obese patients, frequently without any corresponding symptoms.[8,9] The early diagnosis of LV dilatation, that may occur before clinical symptoms and serve as an important indicator of early cardiac remodeling, has received less attention, though.[10,11] A reliable and non-invasive method for identifying such alterations before they progress to systolic dysfunction is echocardiography.[12]

In obese people without established cardiovascular disease, this study attempts to determine whether left ventricular dilatation, as measured by transthoracic echocardiography, can function as an early marker of structural cardiac remodelling.[13,14] Finding these early indicators may help prevent the development of heart failure and allow for prompt intervention.[11,15]

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METHODS

This observational, cross-sectional study was conducted at Al-Batool Hospital (bariatric surgery consultation), from January to October 2024. Two hundred adult contributors between the ages of thirty and sixty have been enlisted and divided into the following groups: 120 individuals with a BMI of 30 kg/m² are in the obese organization. The non-obese manipulate group consisted of 80 people with a BMI of 22.4±18 kg/m². Participants with significant valve disease, kidney disease, cardiac dysfunction, coronary artery disease, or pregnancy were not allowed to participate. Body weight and height were obtained for each person to determine their BMI. Lipid profile, fasting glucose, and blood pressure had also been noted. The presence of hypertension and diabetes mellitus was noted.

Vivid e9 system was used to perform two-dimensional transthoracic echocardiography. As stated by the recommendations of the American Society of Echocardiography, the parameters were measured.[12] Left Ventricular End-Diastolic Diameter (LVEDD), Left Ventricular End-Systolic Diameter (LVESD), Left Ventricular Mass Index (LVMI)Left Ventricular Ejection Fraction (LVEF) and LVEDD >5.6 cm in adult males and >5.2 cm in adult females are the criteria used to characterize LV dilatation

SPSS version 26 has been used to perform statistical analysis. T-test was utilized to analyze study groups. The relationship between echocardiographic indices and BMI was examined using Pearson's correlation. To identify the predictors of LVEDD, a multivariable linear regression was used. A p-value <0.05 was considered significant.

RESULTS

The mean age was 45.3±8 years in the control group and 46.6±8.4 years in the obese persons (p=0.3). 53% of the obese group and 47% of the control group were men (p=0.6). Participants who were obese had significantly higher rates of type 2 diabetes and systemic hypertension (Table 1).

Those who were obese had significantly larger LV dimensions and a higher LV mass index. LVEDD in the obese group measured 5.9±0.3 cm, while the controls measured 5±0.4 cm (p<0.001). 38.3% of obese participants had LV dilatation, compared to 5.0% in the control group (Table 2).

Regression charts showing the strong relationships between SBP and LVEDD (right) and BMI and LVEDD (left). Accounting for gender and age, multivariable regression analysis identified BMI (β=0.48, p<0.001) and SBP (β=0.25, p=0.002) as independent predictors of LVEDD (Fig. 1). There was a strong positive correlation between BMI and LVEDD (r=0.62, p<0.001). Multivariable linear regression revealed BMI (β=0.48, p<0.001) and systolic blood pressure (β=0.25, p=0.002) as independent predictors of LVEDD after accounting for gender and age.

Table No.1: Baseline characteristics of the patients (n=200)

Variable	Obese (n=120)	Control (n=80)	*P*-value
Age (years)	46.6±8.4	45.3±8	0.3
Male sex	53%	47%	0.6
BMI (Kg/m^2)	34.9±3.1	22.4±1.8	0.001
Hypertension	40%	10%	0.001
Type 2 diabetes	27%	7%	0.001

Table No.2: Echocardiographic findings

Variable	Obese (n=120)	Control (n=80)	*P*-value
LVEDD (cm)	5.9±0.3	5±0.4	0.001
LVESD (cm)	4.1±0.2	3.2±0.3	0.001
LV mass index (g/m^2)	128±14	97±13	0.001
LVEF (%)	58±7	62±4	0.08

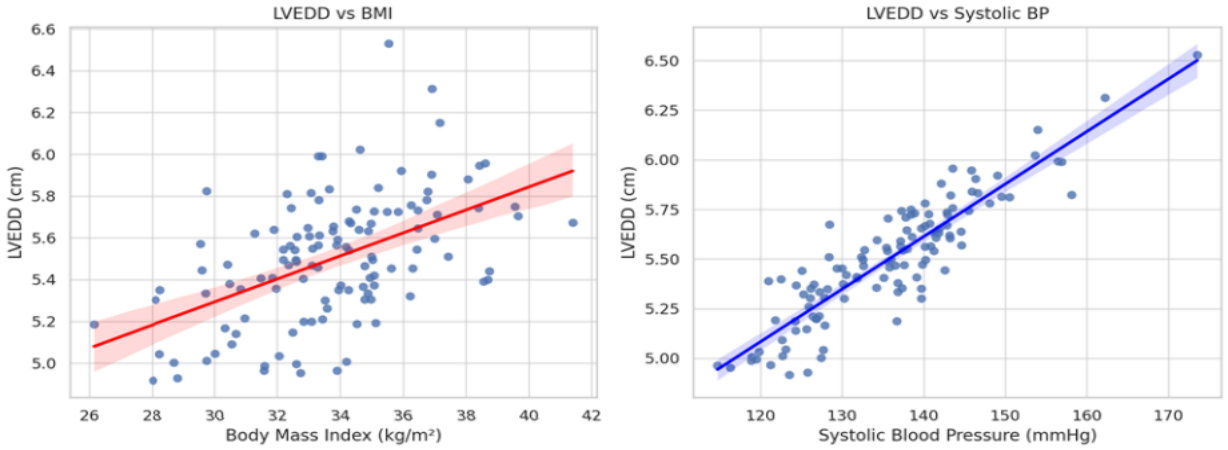

Figure No. 1: Predictors of LVEDD from multivariable regression

DISCUSSION

This study shows that in obese people, left ventricular dilatation is a significant early indicator of heart structural remodeling. Our results are consistent with previous autopsy and echocardiography-based research that suggest obesity lead to myocardial remodeling and volume overload even when there is no obvious cardiac condition.[3,8,16]

Obese people have a considerably higher mean LVEDD than non-obese controls. Additionally, more than one - third of obese individuals had echocardiography features of LV dilatation. These findings support the idea that obesity per se independent of diabetes or hypertension, may also contribute to early chamber remodeling, most likely as a result of adipose-mediated inflammation and prolonged preload.[4,5,11]

It's interesting to note that although LVEF was somewhat lower in the obese group, it was still within the normal range, supporting the idea that structural changes occur before functional damage. Because of this, LVEDD is a valuable early diagnostic sign that can be detected using non-invasive imaging.[6,10,12]

These findings have been supported by earlier reports by Alpert et al[2] and Cheng et al[3], which highlight how early identification of LV structural alterations might direct physicians in aggressive cardiovascular risk management. It has been demonstrated that weight loss and lifestyle changes reduce left ventricular mass and improve heart geometry, highlighting the need of early reputation.[11,15]

Our study is hindered by its cross-sectional design, which restricts the ability to infer causality. Furthermore, although adjustments were made for confounders, unmeasured variables such as physical activity and subclinical metabolic inflammation may also influence cardiac geometry.[14,17]

CONCLUSION

One common and quantifiable early sign of cardiac structural remodeling in obese people is left ventricular dilatation. To enable early intervention and stop the development of heart failure, echocardiographic examination of LVEDD should be considered in routine cardiovascular assessments of obese patients, including those who do not exhibit obvious symptoms.

Author's Contribution:

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Drafting or Revising Critically:	Amina Abdul Baqi Khuthur, Shaymaa AH Jasim
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Stature Estimation Using Percutaneous Tibial Length Between Male and Female Cadavers in Lahore

Correlation Coefficient Between Tibial Length and Body Stature

Riasat Ali, Ahmad Raza Khan, Fariha Tariq, Khalid Mahmood, Aatiqa Abbas and Noreen Kashif

ABSTRACT

Objective: The main objective of this study is to determine the correlation coefficient between tibial length and body stature (body length).

Study Design: comparative cross-sectional study

Place and Duration of Study: This study was conducted at the Forensic Medicine & Toxicology Department of KEMU, Lahore from August 2019 to February 2020.

Methods: In this study, percutaneous tibial length was measured along with body length in 64 dead bodies (32 male & 32 female). It was a comparative cross-sectional study. Non-probability consecutive sampling technique was used. It was carried out in the Forensic Medicine & Toxicology Department of KEMU, Lahore.

Statistical Analysis: Data was analysed by using SPSS version 26. Correlation coefficient was calculated between percutaneous tibial length and body length of dead bodies.

Results: Pearson correlation coefficient was 0.930 & 0.889 in males and females respectively. Very strong statistically positive and significant results were found.

Conclusion: The link of association of percutaneous tibial length with body stature is remarkably strong. This enables accurate estimation of height in deceased persons. Identification of cadavers was aided with the help of a developed regression equation for estimation of height.

Key Words: Stature Estimation, Percutaneous Tibial Length, Forensic Anthropology and Cadavers

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INTRODUCTION

The need to identify oneself as an independent individual is probably one of the primal instincts of human nature. It is an inborn desire to self-awareness, an innate sense of individuality to identify consciousness and personal experiences.[1] This individualization helps in interactive relationships including alliances of social network whether it be familial or work related.[2] Expanding the explorative need for identification opens up the civil as well as the medicolegal debate of why absolute identification is mandatory.[3] Medical care requiring blood group analysis, diagnosis and management of individual diseases is a cardinal aspect of identity establishment.[4]

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Social linkage demands identity as a requirement in civil cases like inheritance, inherent lineage and cultural heritage.[5] How and when can a person vote, what is the legal age for any job, what is the legal age of majority entitling someone for a capital punishment, how to track criminal activity, all these issues fall under the purview of an identification of a person. All this protocol builds up for prevention of any law and order scenario like impersonation, theft, fraud and tracking a murderer or a rapist in heinous crimes.[6] The phenomenon of corpus delicti can only be put in place through personalization, that is proving the crime committed by a specific criminal fellow. Besides this identification is a pivotal factor in cases of unknown dead bodies or in cases of mass disaster where at times only body parts are available, which need to be identified for not only provision of closure to the family but also to settle legal matters like solving a murder or issuance of monetary compensation regarding either insurance claims or consolation cash to the relatives of the deceased victims issued by the authorities. Hence either the unknown mutilated bodies, decomposed dead individuals, mass disaster related body parts either belong to a male individual or a female victim is the main objective of this research study project. How to differentiate male from female especially in cases of

body parts like upper or lower limbs only or from the human skeletal remains.[7]

Among all the biological parameters body stature has a significant role in identifying personal individuality and for the research purpose of this study Percutaneous Tibial Length is the variable to be studied for a stature calculative differentiation between a male and a female individual. Percutaneous tibial length is length of tibia measured from skin surface which has a significant positive correlation with stature (height) of a human being, meaning that as percutaneous tibial length increases so does stature tends to increase.[8]

Stature estimation is a fundamental aspect of forensic anthropology, aiding in the identification of individuals in cases involving fragmented or dismembered remains.[9] The tibia, being one of the most robust and accessible long bones, has been widely studied for its correlation with stature.[10] Percutaneous tibial length (PCTL) offers a non-invasive measurement method, making it particularly useful in forensic and medicolegal contexts. Research has consistently demonstrated that lower limb dimensions, including tibial length, are among the most reliable predictors of stature due to their strong correlation coefficients ($r > 0.8$) and minimal error margins.[11]

Population-specific formulas are crucial for accurate stature estimation, as hereditary and environmental factors significantly influence body proportions. Studies on Han populations in southern China have shown that regression equations developed for one group may not apply to others due to ethnic variations.[12] Similarly, secular changes and urbanization have impacted generational height trends, necessitating updated regression models tailored to specific regions.[13] Despite advancements in forensic methodologies, there remains a lack of contemporary data for South Asian populations, particularly in Pakistan. This study aims to address this gap by analysing sexual dimorphism in tibial-stature relationships among male and female cadavers from Lahore.

By developing localized regression equations based on PCTL measurements, this research seeks to enhance the accuracy of stature estimation protocols for Punjab's unique demographic profile. Such findings will contribute to forensic anthropology databases and improve identification processes in medico-legal investigations.

METHODS

It was a comparative cross-sectional study. The study was conducted in the Department of Forensic Medicine & Toxicology, KEMU, Lahore. The study was completed in one year after approval of the synopsis. 32 Males and 32 Females dead bodies aged between 20-50 years. Non-probability consecutive sampling was used. Dead bodies with healthy normal limbs without any deformity or disease (local skin disease, ulcer). Ages between 20-50 years were included in this study. Percutaneous tibial length is the total length of tibia significantly presenting the distance between the medial most superficial points on the upper border of the medial condyle to the superficial lower most point of medial malleolus of tibia. Both points were marked. By spreading caliper at these points length of tibia was taken in centimeters. Dead body length (body stature) was taking on the autopsy table. The dead was put on the autopsy table straight unbent legs and other parts of the body. Feet were put together. With a marker a line was made on the table at the top of the head. Another line was made on table at the heel. Then the distance between those lines was measured in centimeters.

Statistical Analysis: Data was analyzed by SPSS version 26. Descriptive data were expressed as maximum, minimum, mean and standard deviation. Pearson correlation coefficient for male, female and overall was derived between tibial length and dead body stature.

RESULTS

In the current study, 64 cadavers were brought to the mortuary of the Forensic Medicine & Toxicology Department of KEMU, Lahore. Descriptive data of body stature and tibial length of overall samples as shown in *Table 1*. Maximum, minimum and mean values of tibial length were 51, 29 and 43.41cm and the standard deviation was ± 7.59cm. Minimum, maximum and mean body stature were 179,133 and 159.23 cm and SD was ±19.77.

Table No.1: descriptive statistics results of study participants.

Variable	Minimum	Maximum	Mean±SD
Tibial Length	29	51	43.41±7.59
Body Stature	133	179	159.23±19.77

The correlation coefficient between tibial length and body stature in males was 0.930 with p value <0.001 and in females was 0.889 with p value of <0.001, as shown in Table 2.

Table No.2: Correlation coefficient between tibial length and body stature.

Variables	Male		Female	
Tibial Length & Body Stature	Co-efficient	p-value	Co-efficient	p-value
	0.930	<0.001	0.889	<0.001

The regression equation calculated between tibial length and the stature of the body was $63.12 + 2.34\ Tibial\ length\ (X)$.

DISCUSSION

Percutaneous tibial length is length of tibia measured from skin surface has a strong correlation meaning that as percutaneous tibial length increases so does height. Multiple studies across different regions of the world including both males and females show a consistent statistically significant, positive correlation between percutaneous tibial length and stature. Correlation coefficient (r) often ranges from 0.69 to 0.94 which is suggestive of moderate to strong interactive relationship and this correlation tends to be stronger in males compared to females within the same population, however this can vary significantly between different populations, ethnicities, and even geographical regions.[14]

The relationship between percutaneous tibial length and stature is often modelled using linear regression equations. Equation for estimation of stature is: Stature = a + b × (Percutaneous Tibial Length) where 'a' is a constant (intercept) and 'b' is the regression coefficient (slope). These constants and coefficients are determined empirically for specific populations and sexes.[15]

In forensic related cases where only skeletal remains or fragmented body parts are found, forensic doctors use these correlations and population-specific regression equations to estimate the stature of the deceased, aiding in identification.[16]

The current study required to establish a correlation between percutaneous tibial length and stature among cadavers aged 20 to 50 years from the Lahore population. Our findings indicate a strong positive correlation between tibial length and body stature, with correlation coefficients of 0.930 for males and 0.889 for females ($p < 0.001$ for both). The derived regression equation, Body Stature (Y) = 63.12 + 2.34 Tibial Length (X), underscores the predictive value of tibial length in estimating stature within this demographic.

These findings are in accordance with some others carried out in adjacent areas. Such as, a study conducted on Nepalese medical students found significant relationships between height and percutaneous tibial length, having regression equations of 104.80 + 1.81 Tibial Length for males and 93.58 + 1.91 Tibial Length for females.[17] Also, studies conducted with the Bengali ethnic group showed a strong correlation between standing height and tibial length, highlighting the importance of tibial measurements for estimating height.

Under the Mediterranean umbrella, an Italian population study created new regression models for estimating height from tibial length, noting the need for population-specific formulas because of differences in body proportions across regions.[18] Moreover, studies among Acehnese ethnic group in Indonesia showed strong (r = 0.81) correlation between tibial length and height, which further confirms the usefulness of tibial measurements for estimating height.[19]

With so many global studies available, surprisingly, not much attention has been paid to the Pakistani Population. This study aims to fill the gap by presenting accurate data that can be used anthropologically or forensically, without further analysis of larger samples representing different regions.[20]

In conclusion, the strong correlation between percutaneous tibial length and stature observed in this study reinforces the tibia's role as a reliable predictor of stature. These findings contribute valuable data to the field of forensic anthropology in Pakistan and underscore the need for continued research to refine and validate stature estimation models tailored to specific populations.

CONCLUSION

The importance of establishing uniqueness in the medicolegal system, emphasizing the estimation of an individual's height as a key method for positive identification. It highlights that various body parts can be used for estimating stature, and presents findings showing a strong positive correlation between percutaneous left tibial length and body stature. The study concludes that a regression equation can be developed to estimate height in deceased individuals based on tibial length.

The conclusive inference drawn from this research study is that there is a linear correlation between the length of tibia and the height of an individual and this is also specific to male and female gender respectively. These numerical calculations can be charted according to ethnicity, gender and geographical areas which can later be used for identification purposes in case of any mass disaster or where fragmented body parts are brought in by the police for post mortem examination and identification.[24]

Author's Contribution:

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Drafting or Revising Critically:	Khalid Mahmood, Aatiqa Abbas, Noreen Kashif
Final Approval of version:	All the above authors
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Original Article

Comparison of Intrathecal Bupivacaine with Additives Buprenorphine Versus Bupivacaine with Dexmedetamidine for Postoperative Analgesia in Lower Limb Surgeries

Salman Athar Qureshi, Faiqa Qurban and Muhammad Umair Aslam

ABSTRACT

Objective: This study compared intrathecal bupivacaine with additive buprenorphine versus bupivacaine with dexmedetomidine in lower limb surgery

Study Design: Randomized Controlled Trial study.

Place and Duration of Study: This study was conducted at the Department of Anesthesia, DHQ Teaching Hospital Gujranwala from for 12 months from 30-09-2021 to 29-09-2022.

Methods: After taking informed consent and demographic detail 60 patients were enrolled. Patients were divided randomly into 02 groups. Group 1, patients were given 60µg of buprenorphine with 2cc (15mg) of 0.75 % heavy bupivacaine. Group 2, patients were given 5µg of dexmedetomidine with 2cc (15mg) of 0.75 % heavy bupivacaine. The duration between start of spinal anaesthesia till the first dose of rescue analgesia recorded as duration of analgesia.

Results: From buprenorphine group the mean duration of analgesia of the patients was 234.67±13 minutes whereas in group dexmedetomidine the mean duration of analgesia of the patients was 275.17±29.77 minutes (p-value=<0.001). From buprenorphine, VAS score was 3.87±0.63 while with dexmedetomidine group VAS score was 3.90±0.66 (p =0.842). From buprenorphine group the mean rescue analgesia was 4.40±0.56 mg while from dexmedetomidine group the mean rescue analgesia was 4.33±0.55 mg (p-value=0.644).

Conclusion: These findings suggest that while dexmedetomidine may provide prolonged analgesia, both adjuvants effectively manage postoperative pain, offering viable options for spinal anesthesia in lower limb procedures.

Key Words: Intrathecal bupivacaine, Dexmedetomidine, Buprenorphine, Lower Limb Surgeries

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INTRODUCTION

Although bupivacaine is the most often used long-acting local anesthetic,[1] the addition of opioids may enhance the quality of anesthesia and analgesia by reducing the time it takes for sensory block to develop, extending the length of sensory block & hence the duration of analgesia into the postoperative phase.[2]

Bupivacaine is a widely used local anesthetic for spinal anesthesia, available in both hyperbaric and isobaric formulations.

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The choice between these two solutions remains a topic of debate, particularly regarding the predictability of the level of analgesia they provide.

Hyperbaric Bupivacaine, which contains added dextrose to increase its specific gravity, tends to follow gravitational movement in the cerebrospinal fluid (CSF), leading to a more controlled and predictable spread of anesthesia.[3]

In contrast, isobaric Bupivacaine, which has a density similar to CSF, exhibits a more variable distribution, as it is influenced by factors like patient positioning and CSF dynamics. While hyperbaric solutions offer greater control over the anesthetic spread, isobaric solutions may provide a more gradual onset and potentially longer duration of action. The addition of dextrose to local anesthetic solutions plays a crucial role in modifying their pharmacokinetic and pharmacodynamic properties, ultimately impacting the effectiveness and reliability of spinal anesthesia.[4]

The ideal intrathecal medication for spinal anesthesia should provide an optimal balance between effective

anesthesia, prolonged postoperative pain relief, and minimal side effects.[5] Bupivacaine, a long-acting amide local anesthetic, is widely preferred due to its reliable sensory and motor blockade, hemodynamic stability, and extended duration of action. Its prolonged analgesic effect makes it particularly suitable for procedures requiring sustained postoperative pain control, reducing the need for additional analgesics. When used alone, Bupivacaine offers adequate anesthesia, but combining it with adjuvants like opioids (e.g., fentanyl, morphine) or alpha-2 agonists (e.g., dexmedetomidine, clonidine) can enhance its efficacy, prolong analgesia, and reduce postoperative opioid consumption.[6]

In subarachnoid block, opioids have been used with Bupivacaine to extend the effect, increase analgesia quality, and reduce the need for post-operative analgesics. The reason for combining opioids & local anesthetics is that this combination will relieve pain by acting on two separate sites: local anesthetics on the nerve axon & opioids on the spinal cord receptor site.

Through literature, it has been noticed that dexmedetomidine with bupivacaine is more effective in improving duration of analgesia than buprenorphine. But not much work has been done in this regard. Moreover, no local evidence found in literature which could help us in implementing better drug to reduce postoperative pain and less analgesia consumption by improving duration of analgesia requirement. So, we planned to conduct this study to obtained more exact & reliable results which can be implemented in local setting. So, in future, the results of this study will help us to confirm the results of previous studies and will improve our practice and local guidelines.

METHODS

This study was conducted in the Department of Anesthesiology at DHQ Hospital, Gujranwala, over a 12-month period following the approval of the study synopsis by the institutional review board. The sample size was determined using a 95% confidence level and 80% power of the test, based on data from previous studies evaluating the mean duration of analgesia following spinal anesthesia.

Patients between the ages of 18 and 70 years who were scheduled to undergo lower limb surgeries under spinal anesthesia were considered eligible for inclusion in the study. Patients classified as American Society of Anesthesiologists (ASA) III or IV, those with a known allergy or hypersensitivity to the study drugs, or those with contraindications to spinal anesthesia were excluded from participation. Before enrollment, detailed informed consent was obtained from each participant. Additionally, baseline demographic and clinical data such as name, age, gender, body mass index (BMI), ASA classification, and the type of surgical procedure were documented systematically.

Participants were randomly assigned to one of two groups:

- Group 1 received 60 μg of buprenorphine along with 2 cc (15 mg) of 0.75% hyperbaric bupivacaine.
- Group 2 received 5 μg of dexmedetomidine in combination with 2 cc (15 mg) of 0.75% hyperbaric bupivacaine.

Before the administration of spinal anesthesia, standard preoperative monitoring was initiated, including electrocardiography (ECG), non-invasive blood pressure (NIBP) monitoring, and pulse oximetry (SpO_2). Under strict aseptic conditions, spinal anesthesia was administered to each patient in a sitting position at the L4-L5 interspace using a 25G Quincke spinal needle. After successful drug administration, the exact time of surgery completion was recorded. Following the procedure, all patients were transferred to the postoperative recovery unit, where they were closely monitored for 24 hours for any adverse effects or complications.

To evaluate the effectiveness of analgesia, pain intensity was assessed using the Visual Analogue Scale (VAS) at regular intervals. The time to first onset of significant pain (VAS score >4) was carefully documented. Once the pain threshold exceeded this level, rescue analgesia in the form of Nalbuphine (0.1 mg/kg) was administered. The total duration of analgesia was measured as the interval between the administration of spinal anesthesia and the first request for additional pain relief.

All relevant patient data, including pain scores, time to rescue analgesia, and any observed side effects, were meticulously recorded in a structured proforma for subsequent analysis.

Data analysis was performed using SPSS version 21. Continuous variables, such as age, BMI, and duration of analgesia, were expressed as mean ± standard deviation (SD), while categorical variables, including gender, ASA classification, and surgical procedure type, were presented as frequency and percentages. To compare the mean duration of analgesia between the two study groups, an independent sample t-test was applied.

To address potential confounding factors, data stratification was carried out based on age, gender, BMI, ASA status, and type of surgery. Following stratification, additional post-stratification independent sample t-tests were performed to ensure the robustness of the comparative analysis.

This comprehensive methodology ensured that the study adhered to scientific rigor and statistical accuracy, allowing for reliable assessment of the effectiveness of buprenorphine and dexmedetomidine as adjuvants in spinal anesthesia for lower limb surgeries.

RESULTS

Of total 60 patients, mean age was 46.97±11.78 years with minimum 25 and maximum ages 69 years. From buprenorphine group the mean age was 45.93±10.99 years &from dexmedetomidine group the mean age was 48.00±12.62 years.

In this study ASA I patients were 39(65%) whereas ASA II patients were 21(35%). In this study 21(70%) patients were from ASA I in buprenorphine group and 18(60%) patients were from ASA I in dexmedetomidine group. Similarly, 09(30%) patients were from ASA II in buprenorphine group and 12(40%) patients were from ASA II in dexmedetomidine group. Comparison of ASA between study groups showed insignificant difference statistically.
i.e. p-value = 0.417.
According to this study dynamic hip screw and inter-medullary surgical procedure were done in 12(20%) patients respectively, distal locking plate and external fixator tibia were done in 5(8.33%) patients respectively, k nail femur failure done in 9(15%) patients, femoral nail anti-rotation procedure done in 11(18.33%) patients and tension wire binding noted in 4(6.67%) patients.

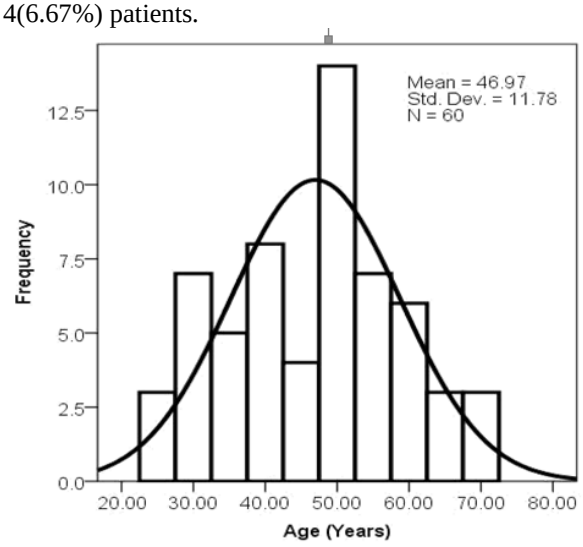

Figure No. 1: Distribution of Age (Years)

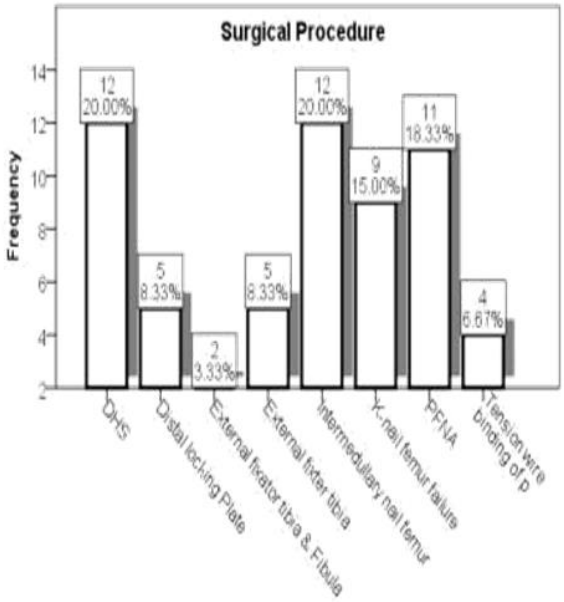

Figure No. 2: Distribution of Surgical Method

From buprenorphine group dynamic hip screw procedure was done in 5(16.7%) patients whereas from dexmedetomidine group the dynamic hip screw procedure was done in 7(23.3%) patients, p-value = 0.545.

Table No. 1: Comparison of Demographic and Clinical Variables between Study Group

Variable	Bup Group (n=30)	Dex Group (n=30)	p-value
Age (years) (Mean ± SD)	45.93 ± 10.99	48.00 ± 12.62	0.502
Gender			
Male	15 (50%)	14 (46.7%)	0.796
Female	15 (50%)	16 (53.3%)	
ASA Status			
ASA I	21 (70%)	18 (60%)	0.417
ASA II	9 (30%)	12 (40%)	
Surgical Procedure			
Dynamic Hip Screw	5 (16.7%)	7 (23.3%)	0.545
Distal Locking Plate	3 (10.0%)	2 (6.7%)	
External Fixator (Tibia & Fibula)	0 (0.0%)	2 (6.7%)	
External Fixator (Tibia)	3 (10.0%)	2 (6.7%)	
Intramedullary Nail (Femur)	6 (20.0%)	6 (20.0%)	
K-Nail Femur Failure	6 (20.0%)	3 (10.0%)	
Femoral Nail Anti-Rotation	4 (13.3%)	7 (23.3%)	
Tension Wire Binding (Patellar)	3 (10.0%)	1 (3.3%)	
Duration of Analgesia (minutes) Mean ± SD	234.67 ± 13.00	275.17 ± 29.77	<0.001
Pain on VAS (Mean ± SD)	3.87 ± 0.63	3.90 ± 0.66	0.842
Rescue Analgesia (mg) (Mean ± SD)	4.40 ± 0.56	4.33 ± 0.55	0.644

The mean duration of analgesia of the patients was 254.92±30.59 minutes with minimum and maximum duration of 209 & 323 minutes respectively. From buprenorphine group the mean duration of analgesia of the patients was 234.67±13 minutes whereas in

dexmedetomidine group was 275.17±29.77 minutes. Comparison of duration of analgesia (minutes) between study groups showed significant difference statistically. i.e. p-value < 0.001. The average pain on VAS was 3.88±0.64 with minimum and maximum pain scores of 3 & 5 respectively.

From buprenorphine group the mean pain on VAS score was 3.87±0.63 while from dexmedetomidine group the mean pain on VAS score was 3.90±0.66. Comparison of pain on VAS between study groups showed insignificant statistically. i.e. p-value=0.842. The average rescue analgesia was 4.37±0.55 mg with minimum and maximum rescue analgesia of 3 & 5 mg respectively.

Comparison of rescue analgesia between study groups showed insignificant statistically. i.e. p-value=0.644. In patients having age ≤ 50 years; in buprenorphine group the mean duration of analgesia of the patients was 235.50±14.16 minutes and in dexmedetomidine its mean value was 273.59±35.63 minutes (p-value=<0.001).

In patients having age > 50 years; in buprenorphine group the mean duration of analgesia of the patients was 233±10.79 minutes and in dexmedetomidine its mean value was 277.23±21.00 minutes (p-value=<0.001). In male patients; in buprenorphine group the mean duration of analgesia of the patients was 233.53±12.94 minutes and in dexmedetomidine its mean value was 280.07±24.95 minutes (p-value=<0.001). In female patients; in buprenorphine group the mean duration of analgesia of the patients was 235.80±13.42 minutes and in dexmedetomidine its mean value was 270.87±33.64 minutes (p-value=0.001).

DISCUSSION

Multiple studies have demonstrated that intrathecal dexmedetomidine provides a significantly longer duration of analgesia compared to buprenorphine when used as an adjuvant with hyperbaric bupivacaine for lower limb surgeries. Recent studies have directly compared the analgesic efficacy of buprenorphine versus dexmedetomidine when added to intrathecal bupivacaine for lower limb surgeries, providing valuable insights into their relative performance. These investigations have focused on parameters such as duration of analgesia, onset of sensory and motor blockade, and hemodynamic stability.[7]

A study by Rajni Gupta et al. demonstrated that dexmedetomidine as an intrathecal adjuvant to bupivacaine significantly prolonged both sensory and motor block duration compared to fentanyl. The mean time for sensory regression to S1 was notably longer in the dexmedetomidine group (D) (476 ± 23 minutes) than in the fentanyl group (F) (187 ± 12 minutes), indicating a more sustained analgesic effect with dexmedetomidine. (P<0.001).[8]

A study by Al-Mustafa et al. reported that the higher doses of DEX with bupivacaine resulted in a more extended sensory and motor block, contributing to better postoperative analgesia.[9] The duration time of analgesia with dexmedetomidine is proportionate to its dosage, according to a study by Eid et al.[10]

Rajan US et al resulted in their study across the two groups, the average time of start of sensory & motor block, 2 segment regression & the length of motor block were comparable or not significant statistically. When comparing Buprenorphine to Nalbu-phine with Bupivacaine, the postoperative analgesia duration was considerably longer with Buprenorphine (p0.05).[7] Soumya Samal et al used intrathecal Buprenorphine and Dexmedetomidine for post-operative analgesia & observed that intrathecal Buprenorphine lasts longer than intrathecal Dexmedetomidine without causing significant hemodynamic alterations.[11] Another randomized trial reported that mean duration of analgesia was 210±22.4minutes with buprenorphine and 240±30.2 minutes with dexmedetomidine (p<0.0001).[12]

This discussion highlights the dose-dependent efficacy of Buprenorphine and Dexmedetomidine as adjuvants for prolonged analgesia. Buprenorphine at higher doses (50 µg) provides 6–15 hours of pain relief, while Dexmedetomidine (5 µg) offers ~13.7 hours, making it comparable to mid-range Buprenorphine doses. However, Buprenorphine at ≤50 µg/kg may provide even longer analgesia than Dexmedetomidine. Both agents extend the duration of analgesia beyond that of plain Bupivacaine, but Dexmedetomidine may be associated with fewer side effects in some studies.[13] Given the variability in outcomes, dose selection and surgical context are crucial in optimizing postoperative pain management. Research indicates that dexmedetomidine prolongs analgesia by approximately 40-70% longer than buprenorphine, while also offering a faster onset of sensory and motor blockade.[14-18] Both adjuvants have been shown to effectively extend postoperative pain relief compared to bupivacaine alone, making them valuable choices for optimizing spinal anesthesia outcomes in orthopedic procedures. Additionally, dexmedetomidine has been associated with more stable hemodynamics and fewer side effects, further supporting its potential as a preferred intrathecal adjuvant for prolonged analgesia in surgical settings.[19-21]

CONCLUSION

These findings suggest that while dexmedetomidine may provide prolonged analgesia, both adjuvants effectively manage postoperative pain, offering viable options for spinal anesthesia in lower limb procedures. However, in terms of pain relief and the need for rescue analgesia, both groups demonstrated comparable efficacy.

Limitations of the Study

1. **Short Follow-Up Duration** – Postoperative analgesia was assessed for only 24 hours. Long-term pain relief and potential delayed complications were not evaluated.
2. **Subjective Pain Assessment** – The study relied on the **Visual Analogue Scale (VAS)** for pain assessment, which is inherently subjective and may vary based on individual pain tolerance and perception.
3. **Potential Confounding Factors** – Although data were stratified by age, gender, ASA status, and surgical procedure, other factors such as individual pain thresholds, opioid tolerance, and comorbidities were not accounted for.
4. **Limited Dose and Drug Combinations** – Only one fixed dose of **buprenorphine (60 µg)** and **dexmedetomidine (5 µg)** was used. Different doses or combinations with other adjuvants might yield different outcomes.
5. **No Long-Term Adverse Effect Analysis** – The study focused only on **analgesic duration and pain scores** without assessing potential long-term side effects such as **neurological deficits, respiratory depression, or hemodynamic instability**.
6. **Rescue Analgesia Standardization** – The same rescue analgesia (**Nalbuphine 0.1 mg/kg**) was given to all patients, but individual variations in analgesic requirements were not explored.
7. **Exclusion of ASA III & IV Patients** – The study excluded high-risk patients (ASA III & IV), limiting its applicability to patients with significant comorbidities who might experience different analgesic outcomes.

Author's Contribution:

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Drafting or Revising Critically:	Salman Athar Qureshi, Muhammad Umair Aslam
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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Original Article

Ferritin Relationship with Clinical Changes Nutrient Elements, Vitamins D & C and Liver, Kidney Functions in β-Thalassemia Major Patients

Jamal Harbi Hussein Alsaadi

ABSTRACT

Objective: The current study was carried out to study ferritin levels and their effect on nutrient and Vitamins C&D levels in patient with beta thalassemia major.

Study Design: Case series study

Place and Duration of Study: This study was conducted at the Department of Biochemistry, Thalassemia Center for Genetic Blood Diseases in Thi Qar University, southern Iraq from November 2023 to April 2024.

Methods: Current study studied 48 cases of beta thalassemia major and 44 healthy as controls, aged range (8-22) years. Biochemical and hemotograte ferritin, calcium, potassium, iron, sodium, D and C vitamins. Enzymes were measured: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, urea, creatinine and total Bilirubin levels.

Results: The study found highly statistically significant in ferritin (1624.81 µg/L) and a significant decrease in hemoglobin (Hb) levels (7.205 g/dL) vitamins D and C recorded 17.319 µg/L, 1.202mg/dl respectively, also a significant increase in AST (39.38 IU/L) , ALT (58.71 IU/L)and TSB (2.52 mg/dL), and a decrease in ALP(73.90IU/L) ,urea (27.01mg/dL) and Creatinine (0.80mg/dL), also calcium (3.03 mg/dL), (P > 0.05) and potassium (3.08mmol/L) and a significant increase in sodium (139.14 mmol/L) and iron (164.47µg/dL) in patients compared to healthy group.

Conclusion: The present study showed differences in ferritin, calcium, potassium, sodium, ions, and vitamins D and C content in the serum of beta thalassemia patients compared with the control group

Key Words: Nutrients, Ca, K, Na, Iron, Vitamins D & C.

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INTRODUCTION

β-Thalassemia major an inherited disease that is transmitted from parents to children through genes. It is a defect in biosynthesis of hemoglobin leads to inactive of erythropoiesis hence leads to severe anemia, β-Thalassemia is often diagnosed in the first six months of life. The newborn, and it may be fatal if the patient does not receive appropriate treatment[1]. Main cause of death is heart failure, because iron overload. The patient's symptoms of thalassemia appear on patient in first months of life because a defect and rapid breakdown of erythrocytes[2]. Symptoms of strong anemia are as follows: yellowing of skin color with pale, delayed growth, poor appetite, and infections.[3] As anemia continues, other pathological symptoms are observed, change in bone shape, especially the cheeks bones and face.[4] Regular blood transfusions and iron chelation therapy It led to a significant improvement in survival and quality of life in beta thalassemia patients dependent on blood transfusion, but it led to the emergence of multiple complications, including the kidneys Abnormalities and diseases of the liver and heart and deficiency of some important nutrients for the body[5]. A significant increase in low molecular weight proteinuria or decreased levels of some important nutrients and biochemical variables that are signs of other diseases.[6] The current study was carried out to determine effect of increasing ferritin levels in serum of patients with β- thalassemia major on enzymes, biochemical liver and kidney function parameters and some essential nutrients, sodium, potassium, and iron compared to healthy subjects Vitamin D is a fat-soluble vitamin found in many natural products. It can be synthesized in the body with the help of skin exposure

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to sunlight. Vitamin D is an essential factor for calcium metabolism[7]. Low levels of vitamin D in children and adolescents with beta-thalassemia cause multiple bone problems. Vitamin C, or ascorbic acid, is a water-soluble vitamin. Vitamin C easily reaches the body's tissues but is not stored well. .Vitamin C is a good antibacterial and antifungal, and is important antioxidant that can neutralize harmful free radicals.[8]

METHODS

48 patients with beta thalassemia 29 males and 19 females and 44 healthy people as a control group 22 males and 22 females. Referring to Thalassemia Center for Genetic Blood Diseases in Thi Qar province, southern Iraq, during from November 2023 to April 2024. Consent was provided by all Volunteers in study Diagnosis of disease was made on the basis of clinical features, Hb-electrophoresis, and frequent blood transfusion.

Exclusion of study: Patients with thyroid dysfunction, diabetes, kidney failure diseases, genetic diseases other than beta thalassemia major.

Sample Collection: Blood samples were collected early in the morning . Where 5 ml divided into two parts, whole blood for measured complete blood count. The other part is put in clean, dry test tubes for five minutes, then serum was separated at 4000 rpm, frozen serum is stored and frozen at temperature -80°C Determination of hemoglobin.by a Coulter LH 750 auto analyzer

Determination the ferritin

Determination Calcium, Sodium, Potassium, iron and D, C vitamin

Determination AST, ALT and TSB

Determination Serum Creatinine and Urea[9]

Statistical analysis: The statistical method ANOVA was used and Pearson correlation coefficients, p-value level of $p < 0.05$ and $p < 0.001$. Conduct statistical analysis using SPSS statistical program, version 23.0.3.

RESULTS

Demographic and clinical of Studied Groups: 92 volunteers in study, age range from 8 to 22 years, were divided into two groups: patients group, (48) and the healthy group (44) as control. Table 1.

Serum ferritin and hemoglobin levels: A highly statistically significant difference P≤0.01 in ferritin between patients (1624.81 µg/L) and control 59.29 a significant decrease at P≤0.01 hemoglobin (7.205 g/dL) in patients compared with (12.85 g/dL) control. Table 2.

Table No.1: β- thalassemia patients and controls

β- thalassemia (n = 48)	Controls (n = 44)	Parameter
Age (years): Mean ± SD	15.22727± 5.102867	14.0625± 5.051428
Sex (%): Male Female	22(50%) 22(50%)	29 (60.42%) 19 (39.58%)

S.D. standard deviation, n : number of subjects.

Table No.2: Ferritin and hemoglobin levels

Parameter Groups	Ferritin (µg/L) Mean ± SD	Hb (g/dL) Mean ± SD
Control (n=44)	12.85a±1.03	59.29 a±20.74
β- thalassemia (n=48)	7.205b±1.82	1624.81b±149.60

Hb: hemoglobin, (a , b) Means having different letters in the same column differed significantly (P≤0.01)

A negative correlation, between ferritin and Hb at β- thalassemia group with correlation coefficient, r= -0.06434. Figure No. 1.

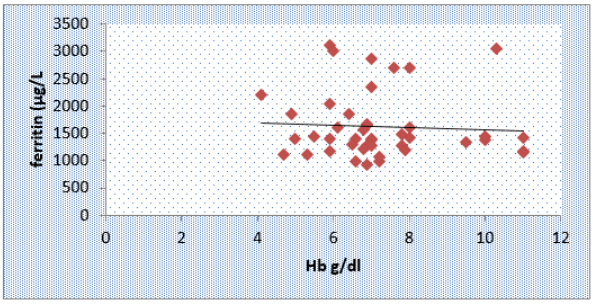

Figure No.1: Ferritin correlation with Hb

Serum Nutrients emements Levels: Increases statistically significant differences in Fe (109.77 µg/dL) and Na (139.14 mmol/L) levels, while decreases statistically significant in Ca (3.03 mg/dL) and K (3.08 mmol/L) at (P>0.05) and (P≤0.01) Comparison with control. Table 3.

Correlations between ferritin in patients with Ca (r= 0.169), a positive correlation, while ferritin with Na (r = 0.0288), a moderate positive correlation, also ferritin with K (r = -0.20695) its a moderate negative correlation and ferritin with Fe (r = -0.00091) a moderate positive correlation. Figure 2.

Table No.3: Nutrient elements levels

Parameter Groups	Ca (mg/dL) Mean ± SD	Na (mmol/L) Mean ± SD	K (mmol/L) Mean ± SD	Fe (µg/dL) Mean ± SD
Control (n=44)	6.63^{a}±2.09	130.74^{a}±6.74	4.144^{a}±1.00	109.77^{a}±11.25
β- thalassemia (n=48)	3.031^{b}±1.02	139.14^{b}±7.60	3.08^{c}±1.21	164.476^{b} ± 21.7

Fe: iron, **Ca:** calcium **Na:** sodium, **K:** potassium, **SD** standard deviation , **n** umber of the subjects

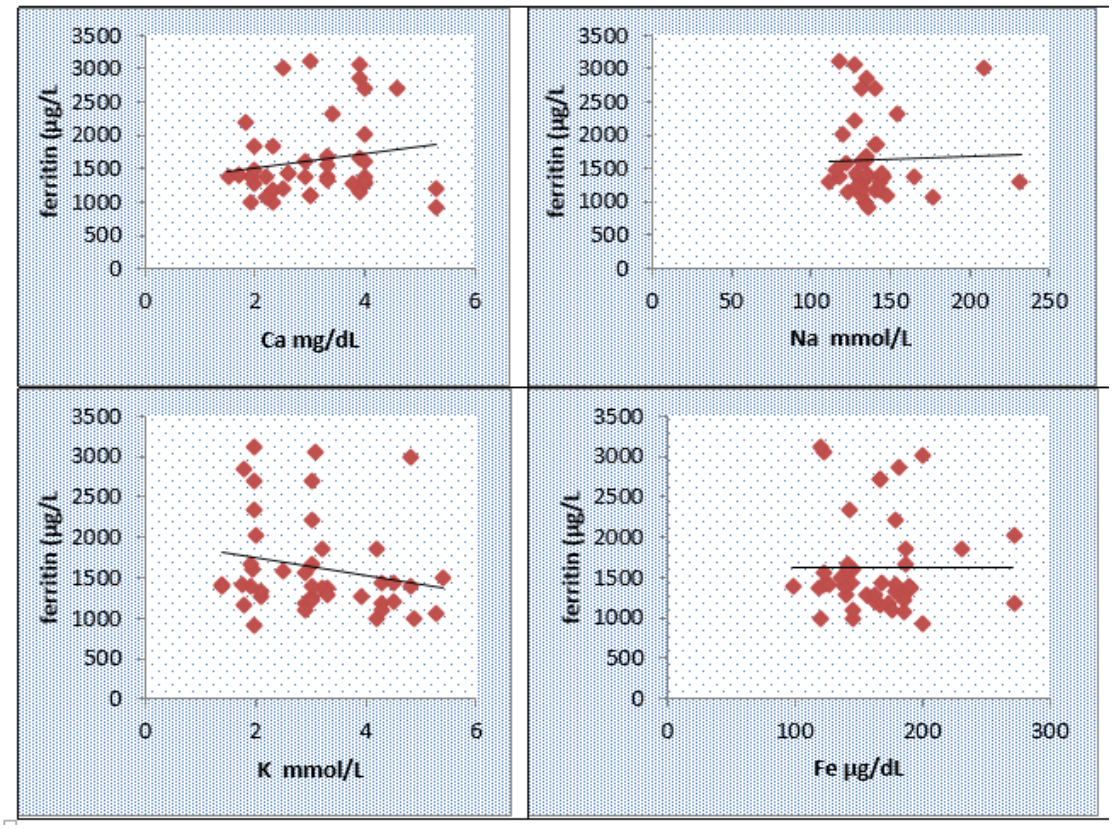

Figure No.2: Ferritin correlation with Ca,Na, K and Fe.

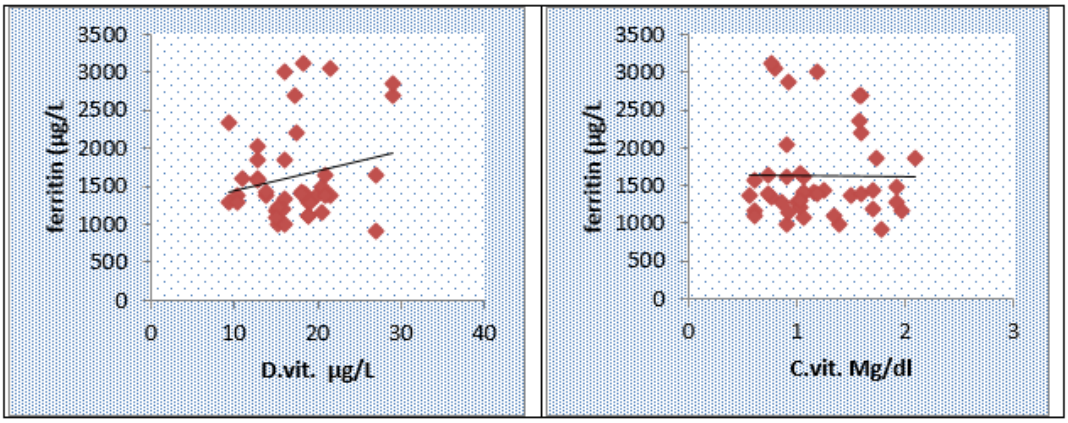

Figure No.3: Ferritin correlation with Ca,Na, K and Fe

Vitamin D, with an average of 17.319µg/L. and vitamin C 1.202 mg/dL in patients compared to controls. see table 4.

Correlation between ferritin in patients with Vit.D a positive correlation (r= **0.2112)**, while ferritin with Vit.C. a negative correlation (r = - 0.0028). Figure 3.

Table No.4: D.vit and C.vit levels

Parameter Groups	**D.vit (µg/L) Mean ± SD**	**C.vit (mg/dL) Mean ± SD**
Control (n=44)	33.82[a]±6.49	1.81 [a]±0.51
β- thalassemia (n=48)	17.31905 [b]±4.74	1.202[c]±0.46

Serum Liver Function parameters Levels: Increases significant of AST, ALT and TSB at (P< 0.01) and high decreases significant of ALP at (P< 0.01) in β- thalassemia patients compared with control. Table 5.

Correlations between ferritin in patients with AST (r= 0.057), a positive correlation , while ferritin with ALT (r = 0.0153), a weaken negative correlation , ferritin with ALP (r = -0.1707) its a moderate negative correlation and ferritin with TSB (r = 0.232631) a weaken negative correlation. the Figure 4.

Table No.5: Kidney Function parameter levels

Parameter Groups	AST (IU/L) Mean ± SD	ALT (IU/L) Mean ± SD	ALP (IU/L) Mean ± SD	TSB(mg/dL) Mean ± SD
Control (n=44)	25.94[a]±8.60	24.86[a]±10.04	90.83[a]±49.83	0.724[a]±0.45
β- thalassemia (n=48)	39.38[b] ± 15.57	58.71[b]±32.67	73.90[b]±27.10	2.52[b]± 1.313

ALT: Alanine transaminase, **ALP:** Alkaline phosphatase, **AST:** Aspartate transaminase, **TSB** : Total Serum Bilirubin

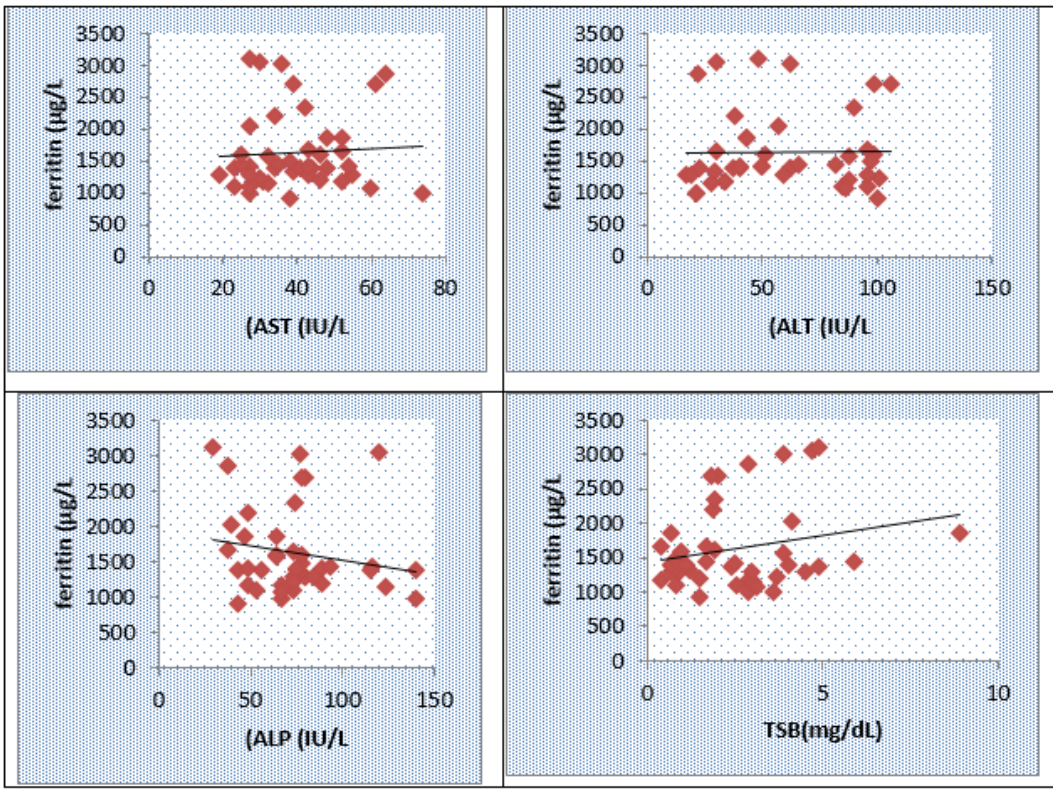

Figure No.4: Ferritin correlation with AST, ALT, ALP and TSB

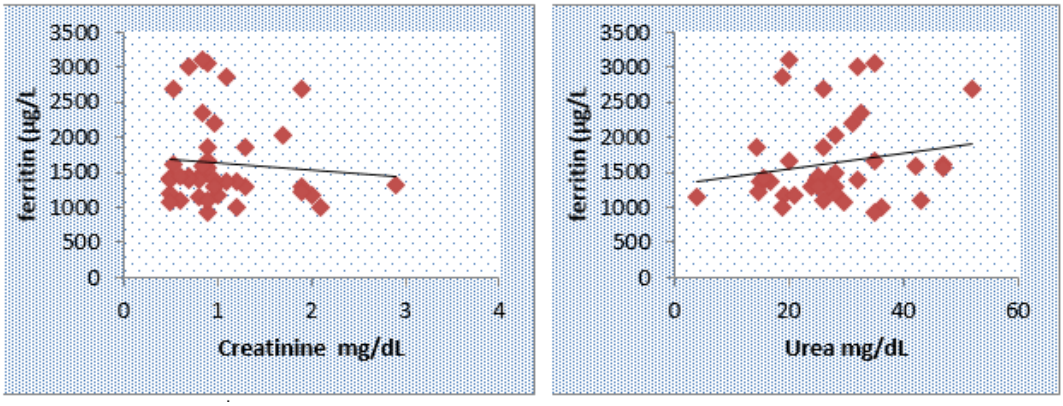

Figure No.5: Ferritin correlation with Creatinine and Urea

Serum kidney Function parameters Levels: A statistically significant increase at (P< 0.01) of creatinine (27.01 mg/dL), while no significant differences at (P< 0.01) in urea (0.80 mg/dL) in β- thalassemia patient compared with control. Table 6. Correlations between ferritin in patients with Urea r=0.179617,while ferritin with Creatinnine (-0.08805). Figure 5.

Table No.6: Urea and Creatinine

Parameter Groups	Urea (mg/dL) Mean ± SD	Creatinine (mg/dL) Mean ± SD
Control (n=44)	12.01[a]±3.83	0.67[a]±0.23
β- thalassemia (n=48)	27.01[b] ± 13.19	0.80[a]±0.17

DISCUSSION

Beta thalassemia is one of the most common genetic diseases in world general and special of Asia. Thalassemia is a disease the formation of dysfunctional red blood cells. The presence of these abnormal erythrocytes stimulates increased iron absorption from small intestine, causing an increase blood iron levels[10]. Iron metabolism, showed thalassemia carriers have a higher levels of iron and ferritin compared to healthy people. thalassemia patients with H63D mutations. Many clinical studies have confirmed low hemoglobin concentration are accompanied a decrease in number of red blood cells and a decrease in values of their specific indicators (MCV, MCH, HCT)[11]. The current study found an increase, iron and ferritin levels in serum beta- thalassemia patients, this results consistent with many other studies. High ferritin content is directly related to accumulation of reactive iron in tissues with beta thalassemia patients. Iron overload leads to another pathological mechanism causes oxidative damage to red blood cell membranes, which is called the second disease.[12] Thalassemia is also accompanied by metabolic dysregulation. Lack of oxygen in cells ,cell damage and all physiological changes lead to ineffective erythropoiesis, hemolysis and anemia. Patients with thalassemia depend on blood transfusion and bone marrow ,transplantation for their survival[13]. A significant increase in sodium (Na), and iron, (Fe) in the studied group, and decrease of calcium (Ca) and potassium the results showed a slight, non-significant increase in Na conc. in beta-thalassemia, patients compared with control group. at ($P > 0.05$). These results are similar to the results of some previous studies[14]. Also decrease in potassium may be because they suffer from hemolysis of erythrocytes, (R.B.C). It occurs in blood has been stored for time periods and is transfused to patient because potassium tends to leak from stored blood. High sodium level in the beta thalassemia group caused damage renal tubules by excess iron levels[15]. Most scientific explanations point to the most important causes of these diseases, which are associated with toxic effects of iron overload associated with blood transfusion[16]. Results are consistent with other studies Dhale et al and Al-Rubae[17] there are several factors cause low concentrations of vitamin D in the serum of beta-thalassemia patients. The most important of these are malnutrition, insufficient exposure to sunlight, bone disorders and liver dysfunction.[18] Some studies have also confirmed that dysfunction of some endocrine glands that affect metabolism in the body, such as the thyroid, causes a deficiency in vitamin D levels. 19 Vitamin C is essential for maintaining, along with vitamin E, an essential role in the antioxidant activity in the body .Vitamin D and calcium are major factors in bone metabolism and play an important role in bone growth and maintenance. Previous studies have shown that patients with β-thalassemia suffer from a significant decrease in vitamin D levels due to excessive iron absorption, which leads to a significant decrease in calcium absorption. Vitamin D deficiency is considered a major cause of bone disease in patients with β-TM and thus anemia and skeletal dysfunction. a significant increase in ALT, AST and decrease ALP and increase in TSB, results are consistent, with studies conducted Navadia et. al.[19] This is due to secondary injury to liver cells and the occurrence of fusion with hepatocytes to deposit iron in the liver. High levels of AST and ALT and low levels of ALP in thalassemia shows muscle and liver dysfunction, studies on kidney health in thalassemia patients have been enhanced. Previous studies confirmed the presence of renal disease in 1.8% of TDT patients and classified renal dysfunction as the fourth most common cause of morbidity. Beta thalassemia, the most serious risk of which is high iron levels due to regular blood transfusion, leads to iron deposition in renal microtubules, glomeruli, and interstitium, leading to renal atrophy, glomerulosclerosis, and interstitial fibrosis[20]. Also, hypoxia and chronic severe anemia lead to an increase in the generation of free radicals of the type ROS, RNS, and the occurrence of oxidative stress, the main cause of many diseases in the body, including tubular cell dysfunction. In addition, iron chelate toxicity can lead to glomerular dysfunction.[21]

CONCLUSION

Current study were conclude increased ferritin in beta thalassemia patients have indirect influence on levels of serum calcium potassium, sodium, ion content, Vitamins D, C, liver enzymes and kidney function parameters.

Significant decrease in values of necessary nutrients in body leaded complications and disorder in kidney and liver with thalassemia patients.

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Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Jamal Harbi Hussein Alsaadi
Drafting or Revising Critically:	Jamal Harbi Hussein Alsaadi
Final Approval of version:	All the above author
Agreement to accountable for all aspects of work:	All the above author

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

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Ethical Approval: No. 7/54/1280 Dated-27.04.2023

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