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Original Articles

Editorial

Families Play an Important Role in Helping OCD Patients

Dr. Mohsin Masud Jan
Editor

Obsessive Compulsive Disorder (OCD) is not a fatal mental condition but it is said to be highly disabling. What are the early signs of it? Both obsessions and compulsions are part of OCD, and most people with the disorder have both, it is simpler if one looks at them separately.

Obsessions are unwanted thoughts, images and/or feelings that come again and again in a person's mind. The nature of these thoughts is such that they cause fear, shame, and/or intense anxiety. Most of the time, even though they may try very hard, people who have OCD are unable to get rid of their obsessions. Sometimes obsessions have been called hiccups of the mind because the brain seems to get stuck on a particular thought or urge and does not stop even though the person desperately wants them to.

Obsessions vary from person to person. Some people have repeated thoughts about getting sick or being contaminated by germs. Others get images of hurting a loved one. Others are frightened and ashamed of negative thoughts they may have about religion or their brain may get stuck with the idea that everything must line up 'just right.' Some people get obsessed that they may lose something important.

Whatever the content of their obsession, the important thing to remember is that they are unwanted and are severe enough to cause intense anxiety and discomfort.

Behavior therapy helps patients deal with and manage the anxiety arising from their obsessions as well as to reduce or eliminate compulsive rituals.

Compulsions are strong urges to act or think in a way to reduce or undo the discomfort that is caused by the obsession. Compulsions, like obsessions, also vary from person to person. A person who has obsessions about being contaminated with germs or being unclean may spend hours in the shower, use strong cleaning products or a whole bar of soap at one time, wash their hands excessively to the point of making them bleed all in an attempt to get rid of the obsession. Another person may refuse to shake hands with anyone or touch household items. Some people may spend hours checking and rechecking stoves, or locks. Others may feel they have to place things in a specific pattern and if they do not do that, they fear something catastrophic may happen.

Some compulsions are mental and involve reciting prayers or a word or phrase repeatedly. At times, people with OCD can spend several hours trying to finish one prayer because each time they say it, they fear that they have not said or done it 'right'.

It is important to keep in mind that obsessions and compulsions are very different from everyday worries, superstitions or cautious habits that many people have. People who have OCD spend a lot of time on their obsessions and compulsions, to the extent that these symptoms interfere with their day-to-day life, including their relationships.

The early signs of OCD will vary according to the nature of the obsessions and compulsions. When the onset is in childhood, parents are often able to observe the compulsions. A child may erase their homework repeatedly to the extent of tearing the paper in order to do the work 'perfectly'. They may stay up till late at night checking and rechecking the door and window locks of their house. Often, younger children will ask their parents the same question repeatedly seeking reassurance again and again. However, as children get older they will often start hiding their obsessions or compulsions because they become aware that they are not normal.

In adults the onset can be gradual but with time, these fears and rituals grow to become OCD.

There seem to be several factors that can cause OCD. Although genetic factors do play a part and we see a higher prevalence of the disorder in children and first-degree relatives of people who have OCD, these factors are not the only cause. There is some evidence that childhood OCD can be caused by certain kinds of infections, such as strep throat. Several studies suggest that people who develop OCD have some abnormalities in specific areas of the brain. Other studies point to changes in certain neurotransmitters or chemicals in the brain. Some temperamental factors such as, low self-esteem or a tendency towards guilt or shame can also make a person more vulnerable to OCD as can a history of physical and sexual abuse in childhood or other stressful or traumatic events.

So far, there are no specific blood or other objective medical tests to diagnose OCD. However, there is a lot of research with brain imaging and scanning techniques that, at some point, may prove to be useful diagnostic tools. At this time, mental health professionals rely on the patient's history and their clinical judgment to make the diagnosis. Many times the patient's family can provide very useful information. In addition, there are standardized manuals, checklists, and rating scales, which help clinicians not only to diagnose but also to evaluate the severity of the obsessions and compulsions.

Although there are other mental health conditions that may be confused with OCD, it is not an exceptionally

difficult disorder to diagnose. At the same time, because fighting this disorder requires so much trust and cooperation between patient and clinician, it is very important that if a person feels uncomfortable for whatever reason, they try and seek a second opinion.

Patients here, in general, when they go to a psychiatrist, are almost always, not aware that what they are suffering from is OCD. Instead, the reason for seeking help is because they are unable to function or are experiencing intense distress.

The most important thing that people who suffer from OCD can do for themselves is to realize that it is not a weakness, nor is it their fault.

OCD is a brain disorder just like diabetes is a disorder of the pancreas. Most people, although they feel distressed or unhappy about having diabetes, do not feel ashamed or embarrassed of having this condition. People who suffer from OCD also must not feel shame or embarrassment about having this disorder. It is hard enough to have OCD — they must not make the burden heavier for themselves by feeling that they have to hide it from others or not get help for it.

Sometimes because the obsessions and compulsions are so unreasonable and excessive, many people start thinking of themselves as "crazy" — this stops them from talking about their condition. Educating themselves about this disorder will empower them to deal with it. They will also realize that they are not alone and there are many others who also suffer like them. This is important because many times people who have OCD feel very alone.

Whether they manage their condition themselves or with the help of mental health professionals, the crucial thing is that they know that there are treatments available for this disorder and they must not give up.

There are currently two forms of treatments that are considered to be effective for OCD.

Behavior therapy helps patients deal with and manage the anxiety arising from their obsessions as well as to reduce or eliminate compulsive rituals. This sort of treatment requires a lot of trust and cooperation on the part of the patient, as it is something they themselves do with the guidance and support of a trained professional.

The other form of treatment is with medications. These medications specifically affect the chemicals in the brain, which are thought to contribute towards obsessions and compulsions. Many times, both forms of treatment are used in combination to get the best result.

Families can also play a very important role in helping patients who are struggling with OCD. Often family members, because they do not understand the disorder, will end up being infuriated and frustrated with the patient or they may become part of the rituals that are demanded by the patient just because that seems like the easier thing to do rather than to argue or resist. Both of these reactions are not helpful to the patient in the long run.

Original Article

Dengue Fever

Evaluation of Upper Right Abdominal Pain in Patients of Dengue Fever

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ABSTRACT

Objective: To Evaluate patients with upper Right abdominal pain in dengue fever.
Study Design: Prospective descriptive study
Place and Duration of the Study: This study was conducted at Mamji Hospital Karachi between August 2012 to October 2014.
Materials and Methods: The patients presented with confirmed dengue fever with upper right abdominal pain. The study was conducted in Mamji Hospital F.B area. The data was gathered and analysed on SPSS version 15.
Results: Total cases were 113.Males were 62 (55%) and 51(45%) were females. The mean age was 27 $\pm$ 9. With the range from 18 to 36 years. Males were slightly more than females. The causes of abdominal pain were acalculus cholicystitis in 57 cases(50%), hepatitis in 17 cases(15%) , pancreatitis in 5 cases(4.4%) and no cause was detected in 34 cases(30%).Total leukocyte count was not high and thrombocytes were low in every case. SGPT was mildly(Less than 100) raised in 71 cases (63%), moderately raise (more than 200) in 27 cases (23%) and severely raised in 12 cases (10.6%) (more than300) and in 03 cases (3%) the SGPT was normal. Ultrasound finding of acute acalculus cholecystitis were enlarged gallbladder with thickened wall in 57 cases (50%) while 9 cases (8%) had gall stones without inflammatory signs. Perihepatic fluid in 35 cases (31%). Swollen pancreas were noted in 5 cases (4.4%). Amylase were raised in 13 cases (11.5%) and serum lipase were raised in 5 cases (4.6%).
Conclusion: The acalculus cholecyctitis is one of the the commonest cause of upper abdominal pain in dengue fever. It is also consider as an early sign of severe infection.
Key Words: Dengue fever, upper abdominal pain, ultrasound abdomen

Citation of article: Alvi H. Rehan M, Talib A. Evaluation of Upper Right Abdominal Pain in Patients of Dengue Fever. Med Forum 2015;26(12):3-6.

INTRODUCTION

Dengue fever[1 2] also known as breakbone fever. It is a tropical disease spread by mosquito ades aegypti and the virus is dengue Skin rash is similar to measles. Symptoms include fever, headache and body ache. The disease may progress to dengue hemorrhagic fever that manifest as bleeding from different sites. Majority of the infection is milder usually passed unnoticed. The virus has four different types, and one infection gives lifelong immunity to that particular infection and partial to others remaining types. A second infection may be more serious. There is no vaccine available so the prevention of the mosquitos and public protective measures are more appropriate. Now in Pakistan it is also one of the commonest infectious disease.

The dengue fever was milder like upper respiratory tract type fever in 80% of the cases while in the remaining there was a course of the disease and from uncomplicated to complicated even dengue hemorrhagic syndrome to toxic shock syndrome. The incubation period is 4 to 7 days but may prolong to 14 days. The travelers after returning to home develop fever 14 days later then dengue should not be consider. The characteristic symptoms of dengue are sudden-onset fever, headache (typically retro-orbital), muscleache and a rash. The course of infection is divided into three phases: febrile, critical, and recovery. In febrile phase there is high grade fever (103-105 °F), and is associated with backache and headache, Nausea and vomiting may also occur. A rash occurs in 50–80% of cases as flushed skin, as "if you press the area the impression of your hand or fingers was visible (blanches when pressed). The fever was classically biphasic. This phase was last for one week and as the fever subsides patient may complain right upper abdominal pain[4 5 6]. Some time it was during the febile period but mostly after fever subsided. The commonest cause of upper abdominal pain related to disease may be acalculus cholecystitis[7], acute hepatitis[8], pancreatitis and gastritis. This may include in critical phase, the disease proceeds to a critical phase as fever resolves during this period, there is leakage of plasma from the blood vessels, typically lasting one to two days[9]. This may result in fluid accumulation in the chest and abdominal cavity as well as depletion of fluid from the circulation and decreased blood supply to vital organs. At this point there is few petechiae that may proceed to bleeding. There may also be organ dysfunction and severe bleeding, typically from the

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gastrointestinal tract[10]. In less than 5 % of the cases because of the less intravascular volume and frank bleeding there will be chances of shock and lead to dengue shock syndrome. Then the last recovery phase it is with resorption of the leaked fluid into the bloodstream. This usually lasts two to three days, sometimes it may continue with severe itching. Some patients may complain fatigue even after weeks of cure of illness.

Dengue may involves others systems of the body. A decreased level of consciousness occurs in 0.5–6% of severe cases, which may be due to brain or liver and it is the result of inflammation by virus or by indirectly as a consequences of the vital organs. Other rare[11] presentation of dengue are transverse myelitis,[11] Guillain-Barré syndrome, acute liver failure[12] heart, skeletal muscles and dengue shock[13].

MATERIALS AND METHODS

This is a prospective descriptive study conducted in Mamji Hospital Karachi a private Hospital. This is a very busy Hospital and covered a large area of Sorab goth, Federal B area, North Karachi and Nazimabad. The average OPD is more than 300 /day of different specialities and 20-30 admission per day. All the patients included were adult and the duration of the study is from Aug 2012 to Oct 2014. The patients included were positive cases of dengue fever and presented with upper abdominal pain during the course of illness or as the first symptoms.

Detailed history and clinical examination were done in every case, and basic biodata were recorded in preset proforma. The symptoms and sign were recorded and laboratories finding were recorded on the day first and on daily basis. The data were analysed on SPSS version 15.

Inclusion Criteria:

1) Patients were adults
2) Resident of Karachi
3) Dengue serology was positive
4) Upper abdominal pain
5) Ultrasound confirm the diagnosis
6) Duration of disease greater than five days.

Exclusion Criteria:

1) Age less than 12
2) Negative Ultrasound finding
3) Negative dengue serology
4) Other etiology like appendicitis, renal stone basal pneumonia and liver abscess.

RESULTS

Total cases were 113.Males were 62 (55%) and 51(45%) were females. The mean age was 27 ± 9. With the range from 18 to 36 years. Males were slightly more than females. The causes of abdominal pain were acalculus cholicystitis in 57 cases(50%), hepatitis in 17 cases(15%), pancreatitis in 5 cases(4.4%) and no cause was detected in 34 cases(30%).Total leukocyte count was not high and thrombocytes were low in every case. SGPT was mildly(Less than 100) raised in 71 cases (63%), moderately raise (more than 200) in 27 cases (23%) and severely raised in 12 cases(10.6%) (more than300) and in 03 cases (3%) the SGPT was normal. Ultrasound finding of acute acalculus cholecystitis were enlarged gallbladder with thickened wall in 57 cases(50%) while 9 cases (8%) had gall stones without inflammatory signs. Perihepatic fluid in 35 cases (31%). Swollen pancrease were noted in 5 cases (4.4%). Amylase were raised in 13 cases(11.5%) and serum lipase were raised in 5 cases (4.6%).

The platelets and haematocrit were done daily and incremental pattern in haematocrit and decremental pattern in platelets were sign of seriousness. The increasing haematocrit was showing the dehydration status of the patients. The blood pressure and pulse were checked frequently and good hydration was maintained. Hepatosplenomegaly was noted in 17 cases(15%). All the results were shown in different tables as following.

Table No 1. Bio data

Total cases	113
Male	62 (55%)
Female	51 (45%)
Mean age	27± 9 YEARS
Range of age	18-36 YEARS
Abdominal pain	113 (100%)
Fever	73 (64.6%)
Nausea	113 (100%)
Vomiting	78 (69%)
Jaundice	34 (30%)
Flushing/bleeding	113 (100%)

Table No. 2: Abdominal Etiology

Acalculus cholicystitis	57(50%)
Hepatitis	17 (15%)
Pancreatitis	05 (4.4%)
No causes detectred	34 (30%)

Table No. 3: Laboratory Finding

Test	Mean	Range
Hemoglobulin	11 g/dl	9-14 g/dl
Hematocrit	44	39-47
Tlc	3100	2700 -5100
Platelets	67000	31000-97000
Total bilirubin	2.7mg/dl	2.1-3.7 mg/dl
Sgpt	163	63-509

Table No. 4: Ultrasound Finding

ultrasound finding	No of cases
hepatosplenomegaly	17 (15%)
acalculus cholicystitis (tickened wall)	57 (50%)
gall stone	09 (8%)
swollen pancreas	05 (4.4%)
perihepatic fluid	35 (31%)

DISCUSSION

Dengue fever also known as breakbone fever. It is a tropical disease spread by mosquito ades aegypti and the virus is dengue Skin rash is similar to measles. Symptoms include fever, headache and bodyache. The disease may progress to dengue hemorrhagic fever that manifest as bleeding from different sites. Majority of the infection is milder usually passed unnoticed. Dengue is the one of the commonest infectious disease in Pakistan.The patients with prolong disease may present differently even if they were afebrile like abdominal pain, nausea and vomiting and extreme weakness[14]. Among the causes of abdominal pain the acalculus cholecystitis, viral hepatitis and acute pancreatitis were the commonest. Whenever a patient with dengue present in OPD with upper abdominal pain the first thing was to had an ultrasound abdomen[15] done that showed gall bladder wall thickness, gall stones, hepatosplenomegaly swollen pancrease and ascites. The pathogenesis was either the direct effect of the virus or the increase permeability caused by the virus so there may be capillary leakage.The upper abdominal symptoms were quite alarming after the fever subsided and they should be managed properly as they are the complications of the disease.The abdominal pain that was not localized to the right upper side may had acute appenditicitis in the differential. The first thing was to hospitalized the patient. The management was not very difficult. It was basically symptomatic stomach rest hydration and electrolyte balance.

In our study only those patients were included which either present with abdominal pain or developed abdominal pain during the course of illness. Among 113 patients 57 cases were diagnosed as acalculus cholecystitis [16] almost half of the cases, while 17 cases had viral hepatitis and 05 had acute pancreatitis. The raised SGPT level and low platelets were very common in dengue fever the raised SGPT were responsible for the upper gastrointestinal symptoms[17]. But with the course of illness the acalculus cholecystitis was also the main cause of the upper right abdominal pain the ultrasound showed gall bladder wall thickness. In 34 cases no cause was detected so it was difficult to say that they had GERD or gastric erosions or ulcers as upper G.I. endoscsopy was not performed because of low platelets, later patients were managed so the OGD was postponed As the cases were positive for dengue serology but IgM of HAV and HEV were send in those patients in which the SGPT was raised and ultrasound showed increased echogenicity of liver but they all come negative. No patient was alcoholic. Other studies[16] done on same topic the commonest cause was acalculus cholecystitis in dengue fever of upper right abdominal pain. Diarrhoea[18] is also caused by dengue fever so it aggravates the dehydration

In our view the abdominal pain might be the early sign of the dengue shock syndrome and keep a real eye on the rising haematocrit and it will give you an idea that how much patient was dehydrated and if not properly managed[19] patient might gone into shock. As we took good care of the patient in this vulnerable period and no patient was discharged prematurely so there were no further complication were recorded. Therefore in the patients of dengue fever and dengue hemorrhagic fever[20] the upper abdominal pain and persist upper gastrointestinal symptoms will be alarming and should need urgent abdominal ultrasound and proper management.

CONCLUSION

The acalculus cholecyctitis is the commonest cause of upper abdominal pain in dengue fever. It is also consider as an early sign of severe infection.

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

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Original Article

Sterilization

Effectiveness of Sterilization and Disinfection of Extracted Human Teeth for Institutional Use: A Case Control Study conducted at a Tertiary Care Hospital

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ABSTRACT

Objectives: To determine the most effective method of sterilization and disinfection of extracted human teeth for use in dental colleges..

Study Design: Case Control study

Place and Duration of Study: This study was conducted at OMFS & Microbiology Department, KMDC, Karachi from June 2013 to December 2013.

Materials and Methods: Freshly extracted human teeth (n=50) were obtained and sent for bacteriological processing. Teeth were dividing into 5 groups; samples were taken pre and post treatment. A platinum wire loop was flamed in red heat and cooled; sample was inoculated in enrichment media. Plates were placed in incubator at 37℃ for 48 hours. Colony count was noted to observe the quantity of microorganism, which determines the efficacy of the sterilizing method.

Results: The results of the study revealed that the autoclave, hot air oven had shown no growth. While 5 % sodium hypochlorite, hydrogen peroxide and normal saline had shown positive growth of microorganisms.

Conclusion: Autoclave and Hot air oven are effective methods of sterilization of extracted human being teeth for use in dental college in preclinical settings.

Key Words: Sterilization and Disinfection, Preclinical, Microbiology, Extracted teeth, Institution

Citation of article: Kashif M, Khan MI, Iqbal N, Mazhar L, Mahrukh S, Arshad M, et al. Effectiveness of Sterilization and Disinfection of Extracted Human Teeth for Institutional Use: A Case Control Study conducted at a Tertiary Care Hospital. Med Forum 2015;26(12):7-10.

INTRODUCTION

Extracted teeth used customarily in dentistry preclincal settings in dental colleges to teach and to build the technical and preclinical skills of the students. Some endodontic procedures can be teach using models, plastic blocks, and typodont tooth models for practicing in the beginning of the course and before entry to the clinical environment. However, there are instances when no substitute is available and students uses extracted teeth for examination, restorative and endodontic preparations or for research purpose.[1]

Human extracted teeth used in dental institutes to teach the clinical methods for patient's treatment. More over these teeth were used for various purposes such as preparing ground section for histological study and for learning endodontic procedures such as cavity preparation, root canal treatment, developing and testing various restorative materials, for crown preparation, and inlay.[2]

In recent years, Guidelines for the control of infection in dental institutes had revised due to likelihood of fractious infectivity from extracted teeth. Commands by American Dental Association (ADA) and Centre for Disease Control (CDC) called for removal of any organism capable of transmitting diseases from non-disposable items/instruments that comes in direct contact with blood stream and may transmit diseases like Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV) used in patient care. The materials might be expose to blood or saliva. These body fluids were associated with extracted human teeth that are used in dentistry to setup system and skills.[3] In a recent study conducted at Institute of Dental Sciences, Uttar Pardesh, India, it was evident that formalin, hypochlorite, and autoclaving was the choice of sterilization of extracted human teeth for institutional use and extracted teeth are hazardous and should handled carefully.[4]

Extracted human teeth most commonly used in many institues of Pakistan and after in depth search of data

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scant or no data availbale in Pakistan to determine the effectiveness of various methods of sterilization and disinfection on extracted teeth. Hence, this study was palnned with the rationale that to determine the most effective method of sterilization and disinfection of extracted human teeth for institutional use.

MATERIALS AND METHODS

This study was conducted at Department of Oral and Maxillofacial Surgery and Department of Microbiology, Karachi Medical and Dental College (KMDC) from June 2013 to December 2013. In this case control study the teeth of the patients i.e. cases and controls were recruited through non-probability, convenience-sampling method. The inclusion criterion was intact freshly extracted human permanent molars from both mandibular and maxillary arches. The exclusion criterion was third molars from both arches, teeth having amalgam filling and broken down roots or teeth and patients with known contagious disease.

Crudely extracted human teeth (n=50) were obtained from the Department of Oral and Maxillofacial surgery of Karachi Medical and Dental College (KMDC) and bacteriological process was carried out in the Department of Microbiology, Karachi Medical and Dental College and Abbassi Shaheed Hospital.: Total 50 teeth randomly divided into 5 groups each group having 10 teeth.Group 1 i.e. Autoclave is a control group while group 2 -5 are identified as Case groups. The teeth were kept in a container immersed in distilled water and then treated as follows.

Group – 1: Teeth were autoclaved at 121 degree Centigrade at 16 Ibs pressure for 20 minutes duration as Control group.

Group – 2: Teeth were kept in hot air oven.

Group –3: Teeth were engrossed in 10ml of 5 % sodium hypochlorite in bottle for 7 days.

Group –4: Teeth were engrossed in vinegar for 7 days.

Group - 5: Teeth were engrossed in Normal Saline for 7 days.

A sample taken before treatment and after treatment from the container group wise. A platinum wire loop was flamed in red heat in burner and cooled. A loop full sample inoculated in simple, selective, enrichment media like Nutrient agar, Blood agar, Macokonkey agar. The plates placed in incubator at 37°C for 48 hours. After 48 hours of incubation, growth in different types of colony, its size, consistency, lactose fermentation haemolysis etc observed carefully. Based on colony morphology, Grams and Biochemical reaction the organisms identified. The colony count noted to observe the quantity of microorganisms.

Efficacy of the sterilizing method was judged by assessing the colony count. No or minimal growth was considered as most effective method of sterilization.

RESULTS

The results of the study revealed that the autoclave, hot air oven had shown no growth. Sodium hypochlorite 5%, hydrogen peroxide, and normal saline had shown positive growth. (Table- I and figure I).

Table No. I: Showing colony count and growth of bacteria after using different disinfection and sterilization methods

Type of Disinfection/ Sterilization	Duration	No. of Teeth	Efficacy of different sterilization methods and disinfectant solutions/ Colony count (10)	Growth of Bacteria
Autoclave at 121 C, 16Ibs pressure(Control)	20 min	10	0	No growth
Hot air oven at 170 C	1hour	10	0	No growth
5 % Sodium hypochlorite	7 days	10	6	Growth positive
Vinegar	7 days	10	8	Growth positive
Normal Saline	7 days	10	10	Growth positive

Table No.2: Infection control guidelines for use of extracted teeth in dental educational settings[18]

- Extracted teeth used for the education of dental health care workers should be considered infective and classified as clinical specimens because they contain blood.
- All persons who collect, transport or manipulate extracted teeth should handle them with the same precautions as a specimen for biopsy.
- Before extracted teeth are manipulated in dental educational exercises, the teeth first would be cleaned of adherent patient material by scrubbing with detergent and water or by using an ultrasonic cleaner.
- Teeth should then be stored, immersed in a fresh solution of sodium hypochlorite (household bleach 1:10 with tap water) or any liquid chemical germicide for clinical specimen fixation.
- Persons handling extracted teeth should wear gloves. Gloves should be disposed off properly and hands washed after completion of work activities. Additional personal protective equipment e.g. face shield or surgical mask and protective eyewear should be worn if mucous membrane contact with debris or spatter is anticipated when the specimen is handled, cleaned or manipulated.
- Work surfaces and equipment should be cleaned and decontaminated with an appropriate liquid chemical germicide after completion of work activities.

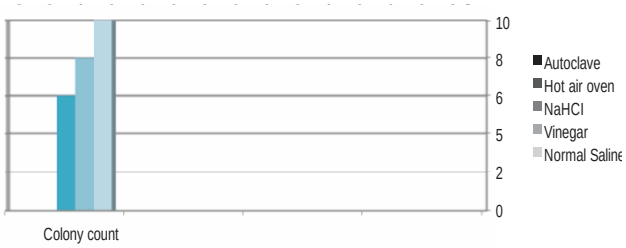

Figure No.1: colony count with different methods of disinfection and sterilization

DISCUSSION

Sterilization embrace absolute demolition of the entire forms of microbial existence together with bacteria viruses and spores on the surface of an entity or in a fluid to thwart disease spread linked with the use of that item. Whereas the disinfection process is proposed to considerably, diminish the quantity of pathogenic microbes on instruments by removing and/or killing them[5]. Extracted teeth serve for educational tool in teaching institute in particular to dental students. It was documented HIV, HBV, HCV, aerobic and anaerobic bacteria were present as pathogenic and non-pathogenic state in pulp, radicular and periradicular tissue of extracted human teeth.[6,7]

It was evident from the results of our study that sterilizing human extracted teeth within autoclave and hot air oven revealed no growth of microorganisms after sterilization. This is in consistent with the study, which showed autoclave as effective and recommended method of sterilization.[8,9,10] The use of autoclave is simple, willingly accessible, contemptible and appropriate technique of sterilization. It does not modify the "sense" and cutting characteristics of teeth. This finding supported by earlier studies on functional characteristics of extracted human teeth. Further, it could effectively destroy and kill all types of microorganisms. There is an apprehension regarding using it for sterilization of extracted teeth with amalgam restorations as it may liberate mercury fumes in the air through exhaust remaining mercury pollution of autoclave and hot air oven [11,12]. The thermal cycling may cause teeth with amalgam restorations to fracture due to their differences in co-efficient of thermal expansion. This is the reason we kept amalgam restorative teeth in exclusion criteria.

Sterilization with 5 % sodium hypochlorite showed little growth of microorganisms. This is not in consistent with the study conducted at India that revealed that sodium hypochlorite 5% could be efficiently used method for sterilization as it showed no growth of microorganism on culture media. [10] Another study conducted at University of Mosul revealed that NaOCl and autoclave prevented the growth completely in all types of the bacteria that were used to infect the teeth i.e. Proteus species, Escherichia coli, Kelebsiella species, Staphylococcus aureus, Streptococcus mutans the type of bacteria that were inoculated inside the pulp chambers.[10]

In this study Vinegar has shown growth of microorganisms which is contrary to the study published in 2014 that showed 100% efficacy of vinegar in preventing the growth of microorganisms after immersing teeth in vinegar for 7 days.[16]

Normal saline though an isotonic solution, revealed growth of microorganisms which is in consistent with the study on extracted human teeth which showed that teeth engrossed in normal saline revealed ed positive development of microorganisms on culture media.[15]

The dissimilarity in efficacy of the methods experienced may possibly be due to:

- reduced infiltration of agents into the pulp space
- Inactivation of disinfectants by the macrobiotic substances present in the teeth.[12]

The advantage and important aspect of study is that institutional guidelines can be formulated and implemented regarding sterilization and disinfection of extracted teeth for preclinical students. In the light of study results cross infection control guidelines can also be implicated for preclinical students as autoclave and hot air oven are readily available, cost effective and less time consuming.

The limitations of the study were this is a single centre study thus findings cannot be generalized and small sample size.

CONCLUSION

It has been concluded that autoclave and hot air oven are the proper methods of sterilization for preclinical student. Hence, the goal of the study is to develop an awareness in students and dentists to handle the extracted human teeth with care as non sterilized extracted teeth can be the source of life threatening cross infections.

Recommendations: It recommends that further research and longitudinal studies should be conducted to evaluate the effect of the use of autoclave, hot air oven, vinegar and sodium hypochlorite on the physical property of the teeth. It also recommends that students should be discouraged to use amalgam-filled teeth for preclinical use and if used these teeth should not be sterilized by hot air oven due to spillage of mercury vapors and contamination. It also recommends that students must follow Center for Disease Control and Prevention (CDC guidelines) for the routine handling of extracted teeth in dental institutions. [18] (Table – 2).

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

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Original Article

Impaired GTT in TB

Frequency of Impaired Glucose Tolerance Test in Patients with Active Tuberculosis

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ABSTRACT

Objective: To determine the frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis in tertiary care hospital, Karachi.

Study Design: Cross sectional study.

Place and Duration of Study: This study was conducted at Dow University Hospital (Dow University Health Sciences Karachi) from 1st June 2014to 30th November 2014.

Materials and Methods: A total of 110 diagnosed adult cases of active pulmonary tuberculosis on the basis of sputum smear positivity were included in this study. Blood samples were drawn for investigations and sent to the same reference laboratory to minimize bias. Data was collected on a pre-tested self administered Performa.

Results: There were 59.1% were male and 40.9% were female. Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis was observed in 20.9%. Rate of impaired glucose tolerance was highly associated with above 40 years of age patients and with family history of diabetes.

Conclusion: In Pakistan DM is on the rise and TB has one of the highest incidence in the world. There is emerging evidence that one disease is fuelling the other. The interest in diabetes and TB is mounting rapidly, so the clinician & researchers should prepare themselves to meet the challenges of the two disease combined.

Key Words: Impaired glucose tolerance, pulmonary tuberculosisand diabetes mellitus

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INTRODUCTION

Diabetes is one of the major public health problems of this century. The increased prevalence of tuberculosis in diabetic patients (four times higher than non-diabetics)[1] has been well documented while conversely the prevalence of diabetes mellitus in tuberculosis patients has received scant attention[2]. People with impaired glucose tolerance and impaired fasting glycemia are at high risk of progressing to type 2 diabetes; although this is not inevitable. Diabetes depresses the immune response, which in turn facilitates infection with Mycobacterium tuberculosis and/or progression to symptomatic disease. Underlying impaired glucose tolerance or diabetes is a recognized cause of inadequate response to chemotherapy. So it is important that In patients attending Chest Clinic for treatment of pulmonary tuberculosis belonging to the age group 45 years and above routine investigations should include exclusion of diabetes as 80% of unknown cases of diabetes could be diagnosed in this group[3].

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It was initially believed that the tuberculous patients do not develop diabetes with any greater frequency than non-tuberculous. This view held sway till Nichols (1957) shattered this belief when he revealed that 5 % of his tuberculous patients had diabetes mellitus and a further one third had an abnormal screening test. Subsequent studies showed glucose tolerance increases with age, with significant increase after 40 years[4, 5, 6]. Prevalence of IGT is significantly more in males (18.67%)[3]than in females (12.90%)[3] and they are more prone to complicated diseases[6,7,8].Lower socioeconomic group is more affected, possibly because of malnutrition [8,9].Incidence of complications such as cavitory lesions, spontaneous pneumothorax followed by hydro and pyo-pneumothorax is also high in patients with IGT[8, 9,10,11].

The high prevalence of I.G.T. in patients of pulmonary tuberculosis observed in studies probably reflects an increased association between Tuberculosis & Diabetes mellitus[12, 13].

Operational Definition

Impaired Glucose Tolerance: OGTT have done to assess impaired glucose tolerance. Glucose tolerance will be considered impaired when blood glucose levels 2 hours after ingestion of 75gm of glucose are ≥140mg/dl[14, 15].

Interpretation of Results: Results of Oral glucose tolerance test interpreted according to WHO Diabetes Criteria[15].

1999 WHO Diabetes criteria – Interpretation of Oral Glucose Tolerance Test

Glucose levels	NORMAL		impaired fasting glycaemia (IFG)		impaired glucose tolerance (IGT)		Diabetes Mellitus (DM)	
Venous Plasma	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs
(mmol/l)	<6.1	<7.8	≥6.1 & <7.0	<7.8	<7.0	≥7.8	≥7.0	≥11.1
(mg/dl)	<110	<140	≥110 & <126	<140	<126	≥140	≥126	≥200

Pulmonary Tuberculosis: Patients who are smear positive for mycobacterium tuberculosis with or without classic symptoms of chronic cough with blood-tinged sputum, fever, night sweats and weight loss were considered having active pulmonary tuberculosis[16].

MATERIALS AND METHODS

The cross sectional study was conducted at the Dow University Hospital (Dow University Health Sciences Karachi) for a period of six months from 1st June 2014 to 30th November 2014.

110 sample size of non probability purposive sampling were taken.

Inclusion Criteria: Recently diagnosed adult cases of both gender (within a month of diagnosis) of active pulmonary tuberculosis on the basis of sputum smear positivity.

Exclusion Criteria: All already diagnosed cases of type1 and 2 diabetes.

Patients with significant co-morbidities like chronic renal failure, hepatobiliary disease, thyroid disease, Cushing syndrome and pregnancy.

Data Collection Procedure: A total of 110 patients presenting to the CHEST OPD& DUE (Ojha Campus)fulfilling the inclusion criteria assessed by consultant were included in the study. The purpose, procedure risks and benefits of the study were explained to the patient and a written informed consent was taken from each patient for inclusion in the study. Blood samples was drawn for investigations by a trained phlebotomist (more than one year experienced) and sent to the same reference laboratory to minimize bias.

Data was collected on a pre-tested self administered Performa after taking permission from ethical committee of hospital. The socioeconomic data including age, sex, occupation, family monthly income was recorded. BMI was calculated by measuring height and weight of patient. Patients were interviewed regarding the family history of tuberculosis and diabetes .Confounding variables were controlled by strictly following exclusion criteria. The patients fulfilling inclusion criteria were instructed to fast for 8-12 hours prior to the test. A baseline blood sample was drawn for fasting blood glucose level. Then patient was given a measured (75 grams) dose of glucose solution to drink within 5 minutes time frame. Blood sample was drawn at 2 hour interval for measurement of glucose level with auto analyzer method. Blood samples was drawn for investigations by a trained phlebotomist (more than one year experienced) and sent to the same reference laboratory to minimize bias. The results of oral glucose tolerance test were interpreted according to 1999 WHO Diabetes criteria. Glucose tolerance was considered impaired as per operational definition. All data was collected by the researcher on structured Performa attached with synopsis.

Data Analysis Procedure: The data was analyzed with the help of SPSS Program version 16.0. Mean and standard deviation were computed for numerical variables like age groups, height, weight whereas frequency and percentages were employed to assess the categorical variable like gender and presence or absence of impaired glucose tolerance. Results was described and also presented in the form of tables and graphs. Stratification was done with regard to age, gender, family history of DM, BMI to control the effect modifier through Chi-Square test. P-value ≤0.05 was taken as significant.

RESULTS

A total of 110 diagnosed adult cases of active pulmonary tuberculosis on the basis of sputum smear positivity were included in this study. Most of the cases were 31 to 60 years of age as presented in figure 1. The average age of the patients was 43.41 ± 5.41 years. Similarly average weight, height and BMI of the patients were also presented table 1. There were 59.1% were male and 40.9% were female (figure 2). Family history of diabetes was observed in 42(38.2%) cases (figure 3) and family history of tuberculosis was 45.5% cases as presented in figure 4.

Table No.1: Descriptive statistics of the patients
n=110

Variables	Mean ± SD	Max-Min
Age (Years)	43.41 ± 5.41	70-20
Weight (kg)	52.45 ± 7.84	65 – 35
Height (cm)	159.48±8.95	152-185

Table No.2: Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis

Age Groups	Impaired glucose tolerance		Total
	YES n=23	NO n=87	
20-30 Years	1(5.6%)	17(94.4%)	18
31-40 Years	2(8%)	23(92%)	25
41-50 Years	8(22.2%)	28(77.8%)	36
51-60 Years	9(36%)	16(64%)	25
>60 Years	3(50%)	3(50%)	6

Chi-Square =11.636 p=0.020
Linear by linear association = 10.922 p=0.001

Table No. 3: Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis with respect to gender

Gender	Impaired glucose tolerance		Total
	YES n=23	NO n=87	
Male	15(31%)	50(76.9%)	65
Female	8(14.7%)	37(82.2%)	45

Chi-Square =0.452 p=0.502

Table No.4: Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis with respect to family history of diabetes

Family History of Diabetes	Impaired glucose tolerance		Total
	YES n=23	NO n=87	
Yes	13(31%)	29(69%)	42
No	10(14.7%)	58(85.3%)	68

Chi-Square =4.144 p=0.042

Table No.5:Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis with respect to family history of tuberculosis

Family History of Tuberculosis	Impaired glucose tolerance		Total
	YES n=23	NO n=87	
Yes	12(24%)	38(76%)	50
No	11(18.3%)	49(81.7%)	60

Chi-Square = 0.53 p=0.467

Table No.6: Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis with respect to BMI of patients

BMI	Impaired glucose tolerance		Total
	YES n=23	NO n=87	
Under weight	8(32%)	17(68%)	25
Overweight	5(16.7%)	25(83.3%)	30
Normal	8(17.8%)	37(82.2%)	45
Obese	2(20%)	8(80%)	10

Chi-Square =2.458 p=0.48

Regarding categories of body mass index, 27.3% cases were overweight, 9.1% obese, 20.7% were under weight and 40.9% were normal as shown in figure 5. Abnormal GTT was observed in 26.36% (29/110) cases in which 1.8%(2/110) with impaired fasting glycemia and 3.6%(4/110) were frankly diabetic while frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis was observed in 20.9% (23/110) cases as presented in figure 6. Frequency of impaired glucose tolerance was high in above 40 years of age and significant difference was observed in percentage of IGT among the age groups (p=0.001) as shown in table 2.Rate of IGT was31% (15/65) in male 14.7% (8/45) in female but statistically significant difference was not observed between male and female (table 3). Rate of IGT was high in those patients who had family history of diabetes than those who had family history of diabetes (31% vs. 14.78%; p= 0.042) while rate of IGT was not significant in those patients who had family history of tuberculosis than those who had not. (24% vs. 18.3% p=0.467) as shown in table 5. Rate of IGT was high in underweight but not statistically significant with other BMI groups as presented in table 6.

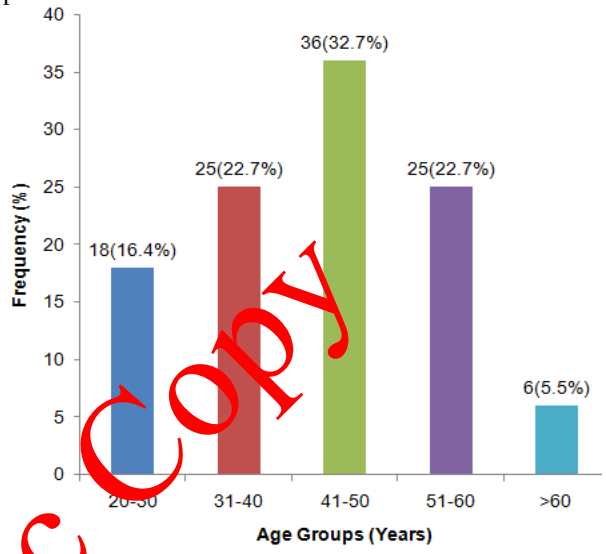

Figure No.1: Age Distribution Of The Patients

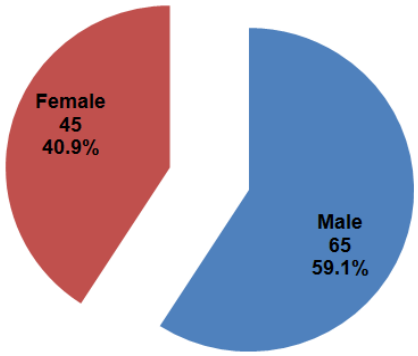

Figure No.2: Gender distribution of the patients n=110

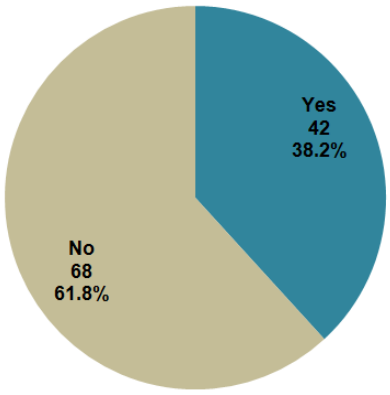

Figure No.3: Family history of diabetes of the patients n=110

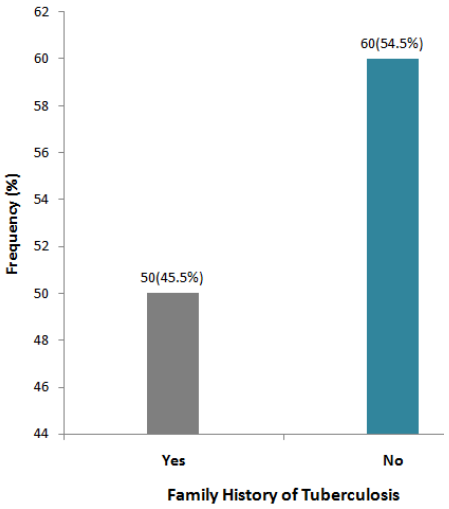

Figure No.4: Family history of tuberculosis of the patients n=110

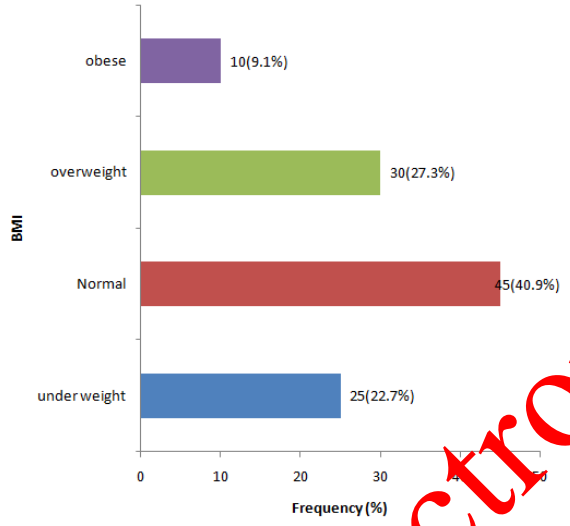

Figure No.5: Distribution of body mass index of the patients n=110

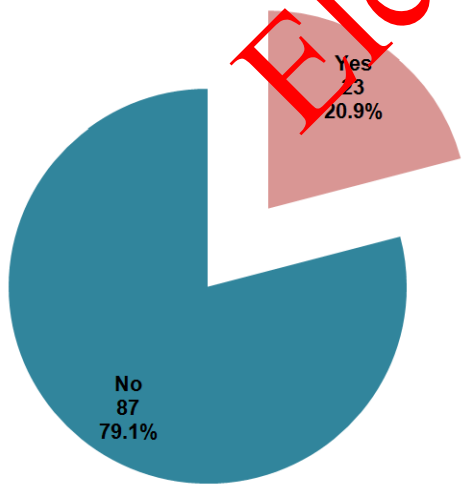

Figure No.6: Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis

DISCUSSION

Diabetics mellitus had noticed with a higher than usual risk of developing tuberculosis[17]. The frequency of tuberculous patients having diabetes concurrently tends to increase, and the relative risk of diabetics having tuberculosis is also high, a three to four times higher prevalence of tuberculosis had been observed in diabetic patients compared with non-diabetics[18]. Conversely, it is also possible that TB can induce glucose intolerance and also deteriorate glycemic control in subjects with diabetes[19]. The present study showed that there were 59.1% were male and 40.9% were female. Family history of diabetes was observed in 38.2% cases and family history of tuberculosis was 45.5% cases. Frequency of impaired glucose tolerance in patients presenting with active pulmonary tuberculosis was observed in 20.9% (23/110) cases. It was also observed in another study[5] that the glucose intolerance was detected in 52 (49%) patients, 31 Impaired Glucose Tolerance (IGT), 21 Diabetes Mellitus (DM).

In Jain et al[3] study the prevalence of abnormal GTT result was 18 (16.98%) which included 2 (1.88%) with impaired fasting glycemia, 11(10.34%) with impaired glucose tolerance and 5 (4.7%) were frankly diabetic. This result was statistically significant and compares to those found in the studies of Kishore et al[20] 20.9%, Singh et al[21]22.0 %, Mugusi et al[9] 19 % and Yamagishi et al[6]&Parez JC[22]14.1%.The present study revealed that while with the increasing age the number of tubercular patients declined, the prevalence of IGT increased. Frequency of impaired glucose tolerance was high in above 40 years of age and significant difference was observed in percentage of IGT among the age groups (p=0.001). The earlier exposure to pulmonary tuberculosis in our country and the development of resistance to the disease in later life accounted for involvement of younger population from tuberculosis. The higher prevalence of impaired glucose tolerance in the elderly was also observed by Kishore et al[20], who found that the prevalence of IGT was higher among patients aged 40 years or more. Yamagishi et al[6,22] and Roy choudhary and Sen[5] also had similar observations. In some recent studies done by Basugloet al[4] and Lin et al[7] a higher prevalence of IGT was found among the elderly. Patel at al[23], showing that 40-60 year old age group is the major affected group with about *57%* of that study sample *(251* diabetic patients with pulmonary tuberculosis) were 40-60 year old[24]. many other studies showed that relative risk of having pulmonary tuberculosis is higher among diabetics aging less than 50 year old, specifically 30-49 year old with a relative risk of 9.88 and 4.72 in the 30-39 year and 40-49 year age group respectively, compared to somewhat lower relative risk in older age groups, 1.76 relative risk in those over 49 year old[24].In this study rate of IGT was

31% (15/65) in male 14.7% (8/45) in female but statistically significant difference was not observed between male and female. In another study the prevalence of IGT was significantly more in males (14/75-18.67%) than in females (4/31-12.90%). Out of the 18 patients with IGT majority i.e.14 (77.78%) were males. Yamagishietal[6]found the complication twice in males than in females. Fernandez et al[8] found the prevalence in 6.2% in males and 3% in females. In present study the rate of IGT was high in underweight but not statistically significant with other BMI groups. Fernandez et al[8]in another Indian study found than BMI was lower in both the IGT and Normal Glucose Tolerance Test (NGTT) groups, while Zack et al[24] and Mugusi et al[9] found no significant differences in BMI in the two groups.Several theories have been put forward to explain why tuberculous patients develop glucose intolerance. Bloom[25] suggested that occult glucose intolerance predisposes to diabetes. Zack et at[24] suggested that glucose intolerance was not merely a reaction to acute tuberculous infection but rather a pre diabetic state.Hadden[26]suggested malnutrition in tuberculosis as a possible cause. Acute severe stress, fever, inactivity and malnutrition stimulate the stress hormones epinephrine, glucagon and cortisol which raise the blood sugar level[27]. Roychoudhary and Sen[5] suggested tuberculosis of pancreas as the possible cause. Similarly, higher incidence of chronic calcific pancreatitis occurs in patients of diabetes and pulmonary tuberculosis leading to absolute or relative insulin deficiency state. Clinical and subclinical hypoadrenalism has been described in these patients[28]. Plasma levels of IL-1 and TNF- a are also raised in severe illness, which can stimulate anti-insulin responses. Age, co-existing illness and alcoholism also influence the host response[6].Physician giving diagnosis and treatment of diabetes mellitus should understand that diabetics belong to the high risk group of developing tuberculosis and perform chest X-ray examination periodically[16;29]. There is evidence that diabetes is associated with a progressive shift of male predominance pulmonary tuberculosis. Perez et al[30] showed that in the non-diabetic population, male incidence of about 51%, compared to 75% frequency of tuberculosis among male diabetics.

Nakamoto found that 13 of 19 patients included in his study (68.4%) had poor glycemic control[31]. This study revealed that 8 patients (32% of the sample) had previous history of tuberculosis which is a percentage that can be considered as being higher than what is seen in non diabetic patient[32]. Diabetes is associated with high risk of recurrent tuberculosis. The important idea is that many of those with recurrent tuberculosis will have infection with resistant strains of mycobacterium. Studies conducted in regions with dual burden had reported that the prevalence of DM ranged from 14–40%[33]. A case control study conducted in Bangalore, South India, during 2001– 2003 reported that chronic disease particularly diabetes was a significant risk factor for developing TB. The prevalence rates of diabetes in TB and non-TB subjects were 22.2% and 15.9% respectively[34]. Based on secondary analysis of countrywide data, another research group estimated that 18.4% of subjects with PTB also have DM in India. A retrospective analysis of 2 years data on TB subjects from Saudi Arabia in 1998 evealed that 27% had DM[33]. Another study from Taiwan reported 16.9% of DM among TB patients[35]. All these reports indicate that routine screening for DM among TB patients should be encouraged in areas with high TB burden. Alisjahbanaet al[36]reported prospective data from a cohort of patients with TB in Indonesia, where the prevalence of confirmed DM among patients with TB is 14.8% compared with 3.2% in general population. A nationwide INDIAB study[37] conducted in the general population of Tamil Nadu, South India, showed that the prevalence rates of diabetes and pre-diabetes were 10.4% and 8.3% respectively, substantially lower in comparison with the estimated prevalence of DM and pre-diabetes in the current study among TB patients conducted in the same period.

CONCLUSION

In Pakistan DM is on the rise and TB has one of the highest incidence in the world. There is emerging evidence that one disease is fuelling the other. The interest in diabetes and TB is mounting rapidly, so the clinician & researchers should prepare themselves to meet the challenges of the two disease combined.

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

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Original Article

Effects of Thirty Minutes Regular Brisk Walk on Blood Pressure in Hypertensive Subjects

Effect of Walk in BP

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ABSTRACT

Objective: The present prospective study was conducted to observe the physiological effects of brisk walk on the systemic blood pressure in hypertensive subjects.

Study Design: Observational study

Place and Duration of Study: This study was conducted at Outpatient Department, NICVD- Jinnah Postgraduate Medical Center (JPMC) / Physiology department, BMSI, Karachi from December 2001 to May 2002.

Materials and Methods: A sample of 30 diagnosed cases of mild uncomplicated systemic hypertension and 30 healthy controls were selected as per study criteria. Aerobic exercise was explained as of doing regular brisk walking of 30 minutes on alternate days for 60 days. Systolic blood pressure (SBP), diastolic BP (DBP), heart rate (HR) and respiratory rate (RR) were checked at baseline and after 60 days of aerobic exercise. Consent of subjects and approval of ethics committee of institute was observed. Data was analyzed on the SPSS 10.0. Continuous and categorical data was analyzed by student`s t- test & Chi square test respectively as 95% confidence interval.

Results: Baseline systolic BP, diastolic BP, HR and RR were raised in hypertensive subjects compared to controls (p=0.001). After 60 days aerobic exercise, the systolic BP, diastolic BP, HR and RR were reduced in hypertensive subjects compared to controls (p >0.05).

Conclusion: The present study concludes that the aerobic exercise improves Systolic blood pressure (SBP), diastolic BP (DBP), heart rate (HR) and respiratory rate (RR) in hypertensive subjects.

Key Words: Aerobic Exercise, Systemic Hypertension, Heart Rate, Respiratory Rate

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INTRODUCTION

Ischemic heart disease (IHD) ranks as leading cause of morbidity and mortality the World over.[1] Similar to World scenario, a rising trend of IHD is reported for Pakistan and also related burden of mortality. In the United States, the mortality rate has greatly decreased during last two decades, has been reported. This reduction is reported due to a favorable trend of physical exertion as risk factor modifier.[2] Because the physical inactivity is a contributing risk factor due to its predisposition for the co morbidities of rise in systemic blood pressure, physical obesity, and a reduction in good cholesterol (HDL-C).[3] American Heart Association (AHA) has reported the "Physical inactivity" is a proven risk factor for coronary ischemic heart disease (CHD) independent to other risk factors.[4] Increased physical activity as during brisk walk, increases the respiratory rate, fastens the heart rate and increases the muscle contraction.[5] Thus brisk walk is one of the recommended way of aerobic exercise. During brisk walk, the body mobilizes various food stuffs- carbohydrates, good and bad cholesterol and fats, and moreover improves renal functioning and thus improved water & electrolyte balance of body. This brings a favorable change in systemic blood pressure and facilitates the body weight loss.[6]

Previous studies had reported favorable effects of aerobic exercise on the physiological factors regulating the systemic blood pressure and a reduction in cardiovascular mortality.[7-8]. The National High Blood Pressure Education Program Coordinating Committee (NHBBEPCC) recommended six approaches for primary prevention of systemic hypertension; viz. moderate physical activity, maintenance of body weight, avoidance of alcohol consumption, a reduction in sodium intake, adequate potassium intake and consumption of vegetables, fruits, low saturated oils/fats and low dairy products.[9] Moderately tailored exercise as determined by exercise testing helps to regulate systemic blood pressure within physiological limits.[10] The present study was planned to evaluate effectiveness of physical aerobic exercise in regulating systemic blood pressure in hypertensive subjects.

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MATERIALS AND METHODS

The present observational study was conducted at the outpatient department, NICVD- Jinnah Postgraduate

Medical Center/ Physiology department, BMSI, Karachi from December 2001 to May 2002. A sample of 30 diagnosed cases of T2DM subjects and 30 healthy, age and gender matched, controls were selected according to pre defined study criteria. Study participants were divided into Group A- controls and Group B- diagnosed cases of systemic hypertension. Hypertension was diagnosed as per criteria set out by JNC criteria. Uncomplicated systemic hypertensive cases not yet taking any medicament were included. Subjects suffering from concomitant systemic disease such as diabetes mellitus, cardiac failure, etc were excluded from study protocol. Physical examination was carried out. Subjects were informed about purpose of study, and willingness to participate. Volunteers were briefed about the merits and demerits of study. Volunteers were informed that they are free to withdraw from study protocol at any stage if they feeling problems. Willing subjects were asked to sign the consent form. Aerobic exercise was explained to participants. Subjects were asked to do brisk walking of 30 minutes duration regularly on alternate days for 60 days. Systolic blood pressure (SBP), diastolic BP (DBP), heart rate (HR) and respiratory rate (RR) were checked at baseline and after 60 days of aerobic exercise. Consent of subjects and approval of ethics committee of institute was observed. Data was collected in pre structured proforma. Data was analyzed on the SPSS 10.0. Continuous and categorical data was analyzed by student`s t- test & Chi square test respectively at 95% confidence interval.

RESULTS

Baseline SBP, DBP, heart (HR) and respiratory rates (RR) were found elevated in cases compared to controls (p=0.001). Table 1 shows the details of study variables of controls and cases at baseline. After 60 days aerobic exercise, the SBP, DBP and respiratory rate (RR) were reduced hypertensive subjects compared to controls (p >0.05). Heart rate showed significant differences p<0.05. Table 2 shows the details of study variables of controls and cases after 60 days of aerobic exercise.

Table No.1: Systolic and diastolic blood pressure, heart and respiratory rates of controls and cases before aerobic exercise

	Parameters			
group	**Blood pressure (mmHg)**		**Heart rate**	**Respiratory rate**
	Systolic	**Diastolic**	**per minute**	**per minute**
A (controls)	132.70± 0.50	87.30± 0.50	69.60± 0.61	16.30± 0.30
B (Cases)	166.80± 3.70	111.50± 1.30	98.50± 3.10	19.80± 0.50
P value	P=0.001	P=0.001	P=0.001	P=0.001

Table No.2: Systolic and diastolic blood pressure, heart and respiratory rates of control and cases after aerobic exercise

	Parameters			
Group	**Blood pressure (mmHg)**		**Heart rate (bpm)**	**Respiratory rate (bpm)**
	Systolic	Diastolic		
A (controls)	121.90± 1.20	82.30± 1.10	70.10± 0.40	16.00± 0.20
B (Cases)	124.50± 1.00	83.20± 1.10	73.30± 0.90	16.10± 0.30
P value	P>0.05	P>0.05	P<0.05	P>0.05

DISCUSSION

Modern life style, urbanization and industrialization are major menaces for the human health, as they have reduced the physical activity. Reduced physical activity of modern life has increased the incidence and prevalence of furious disease such as systemic hypertension, DM, and metabolic abnormalities of lipids and lipoproteins.[11] Previous reports had pointed that increasing the physical activity reduces risk factors for development of metabolic disorders and produces favorable changes in systemic blood pressure, blood glucose levels, lipids and lipoprotein fractions. [8,12]

The observations of present confirmed the findings of above cited studies, as we had observed favorable effects of aerobic exercise on the systemic systolic and diastolic BP in present prospective study. Our results are also in keeping to a previously cited report.[8]

A recent meta analysis had reported the effects of aerobic exercise in normotensive subjects. It was reported that from meta analysis of many RCTs that the aerobic exercise reduced the SBP and DBP by 4 mmHg in normotensive subjects. The observations of above study[13] are in agreement to our present study in terms of SBP and DBP. 25.3% reduction in SBP and 25.4% reduction in DBP were observed after aerobic exercise in hypertensive subjects in our present study, this much reduction in BP is a valuable clinical finding in reducing the cardiac and vascular diseases, and associated morbidity and mortality.

Aerobic exercise increases the integrity of vascular endothelium, and increase synthesis and release of NO/EDRF which improves the circulation, is one of the postulated underlying mechanism in reducing the systemic BP.[12] The findings of our present study are in agreement to above cited study as regards clinical observation of BP reduction, however, the finding of NO/EDRF of above study is incomparable as we could not measure the NO levels in our study subjects due to issues of funding and methodology.

On the basis of above discussion and available evidence, it is suggested that the aerobic exercise is very good for health as it improves normal circulation and blood pressure. Endothelium dependent vasodilatation is a proposed mechanism. Whatever, the underlying mechanism, the present study reports a

blood pressure normalizing tendency by aerobic exercise in mild hypertensive subjects.

CONCLUSION

Aerobic exercise improves systolic blood pressure (SBP), diastolic BP (DBP), heart rate (HR) and respiratory rate (RR) in hypertensive subjects. Future recommendations are suggested to find the underlying mechanisms of how the systemic blood pressure is reduced by aerobic exercise.

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

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Original Article

A Study of Correlation of Child Birth Weight with Maternal Body Mass Index

Correlation of CBW with MBMI

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ABSTRACT

Objective: The objective of this study was to determine correlation of child birth weight with maternal body mass index (BMI).

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at Department of Obstretics and Gynaecology Lady Aitchison Hospital Lahore and was completed in 14 months.

Materials and Methods: Data was collected using random sampling from 1000 females during post partum period. All pregnant females aged 18-35 years were taken and babies with intra uterine growth retardation (IUGR) were excluded. Data was managed in SPSS and was analyzed using same software.

Results: In this study, the average maternal weight, height and BMI were 63.50 ± 9.47 Kg, 154.10 ± 4.85 cm and 26.77 ± 4.03 respectively. The mean babies' weight on delivery was 2.83 ± 0.58 kg. There was significant positive correlation of fetal weight with maternal weight (r=0.072, p-value = 0.02), and maternal BMI (r=0.086, p-value = 0.007). This correlation was stronger in females who had normal vaginal delivery.

Conclusion: Birth weight has a significantly positive correlation with maternal weight and BMI. Being modifiable risk factors, controlling these may considerable reduce the risk of low birth weight and associated complications for both mother and newborn.

Key Words: Pregnancy, birth Wight, Maternal Weight, BMI

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INTRODUCTION

Low birth weight (LBW) has been defined by the World Health Organization (WHO) as weight at birth of less than 2500 g. LBW be the outcome of either preterm birth (before 37 weeks of gestation) or retarded fetal (intrauterine) growth. In 1976, the 29th World Health Assembly agreed on the following definition; Low birth weight (LBW) is a weight at birth of <2,500 gm (upto and including 2,499 g) irrespective of gestational age. [1]

Low birth weight is a very common, however potentially preventable problem particularly in developing countries. It has significant contribution in neonatal morbidity and mortality.[2] It is responsible of 60% of neonatal deaths during first year of their life and carries 40 folds higher risk of death for neonates in first month of their life.[3] Also it impacts infant health with various complications during later stages of life. Birth weight can also indicate maternal health and nutritional status before as well as during the pregnancy. [4]These complications include compromised cognitive and overall growth of child and increased risk of cardiovascular and metabolic diseases in adulthood.[5]

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Remarkable geographical differences have been reported in prevalence of low birth weight around the globe particularly in Asia. The highest percentage of low birth weight has been reported in South Asia and lowest prevalence in East Asia. In East Asia, Thailand has the highest percentage of 36% while the rest of the countries have 5-10% prevalence of low birth weight. However, South Asia has alarmingly high prevalence of as much as 50%. Almost 25% of neonates births in Pakistan are classified as Low Birth Weight (LBW).[4]

A number of risk factors, both maternal as well as fetal, contribute to development of low birth weight. Maternal risk factors are major, and are influenced by both social and biological factors. Where some non-modifiable maternal factors like parity, maternal age and birth order are considerable factor, most modifiable factors like malnutrition, antenatal visits, daily iron supplement intake and BMI are also significant contributors of the disease. One Pakistani study showed that maternal BMI<19 taken as malnutrition, low socio-economic status and low education were all significantly associated risk factors with Low Birth Weight (LBW).[6] Another Pakistani study, from Hyderabad Sindh showed that maternal biological and social factors such as teenage, illiteracy, lower social class and malnutrition taken as BMI<19 had significant association with low birth weight.[7] Similarly, pre-pregnancy body weight and during pregnancy weight of mother contributes significantly on birth weight on

neonates.[8] It is therefore mandatory to take account of such factors to be able to assess their role, control them and improve feto-maternal health outcomes. So this study was designed to determine correlation of child birth weight with maternal weight and BMI

MATERIALS AND METHODS

This Cross sectional study was conducted at department of Obstretics and Gynaecology Lady Aitchison Hospital Lahore and was completed in 14 months.

Sampling: Simple random sampling was used to collect the data

Sample size: A total of 1000 females in their post partum period were taken and their baby's weight was also measured at time of birth.

Sample Selection Criteria: All pregnant females aged 18-35 years were taken with live birth and no congenital anomalies

Exclusion Criteria: Babies with intra uterine growth retardation (IUGR) were excluded

Data Collection Method: This study was conducted at department of Obstretics and Gynaecology Lady Aitchison Hospital Lahore. Data was collected using random sampling from 1000 females after birth. All pregnant females aged 18-33 years during their in their post partum period were taken. Data was managed in SPSS and was analyzed using same software. All data was entered and analyzed using SPSS version 22. Qualitative data like frequency of maternal body mass index and babies low birth weight was used. Mean ± S.D, median, mode and other relevant statistics were calculated for maternal age, weight, height and BMI. Pearson correlation was used to measure strength of relationship between maternal weight, BMI and baby's birth weight.

RESULTS

In this study, the average maternal weight, height and BMI were 63.50 ± 9.47 Kg, 154.10 ± 4.85 cm and 26.77 ± 4.03 respectively. According to their BMI classification, 2(0.20%) females were under weight, 346(34.6%) had normal weight, 409(40.9%) were overweight and rest of 228(22.8%) were obese. The mean babies' weight on delivery was 2.83 ± 0.583 kg with prevalence of very LBW = 18(1.8%) and LBW were 175(17.5%). There was significant positive correlation of fetal weight with maternal weight (r=0.072, p-value = 0.024) and maternal BMI (r=0.086, p-value = 0.007). This correlation was stronger in females who had normal vaginal delivery. Moreover there was association between maternal BMI and Mode of delivery, p-value = 0.03.

Table No.1: Descriptive Statistics of Maternal weight, height, BMI and Fetal weight

	Maternal age (years)	Maternal Weight (kg)	Maternal Height (cm)	Maternal BMI	Fetal weight (kg)
Mean	26.76	63.50	154.10	26.77	2.830
Median	26	63	154	26.45	2.900
Mode	22	60	154	29.52	3.00
Std. Deviation	1.78	9.47	4.85	4.03	0.583
Minimum	18	33	126	14.57	1.00
Maximum	35	90	170	37.95	5.00

Table No.2: Correlation between fetal weight with maternal weight and BMI

		C-Section		Normal Delivery		Overall	
		Maternal Weight	Maternal BMI	Maternal Weight	Maternal BMI	Maternal Weight	Maternal BMI
Fetal Weight	**Correlation**	-0.032	-0.013	0.136**	0.143**	0.072	0.086
	p-value	0.580	0.820	0.000	0.000	0.024	0.007
	N	294	294	691	691	985	985

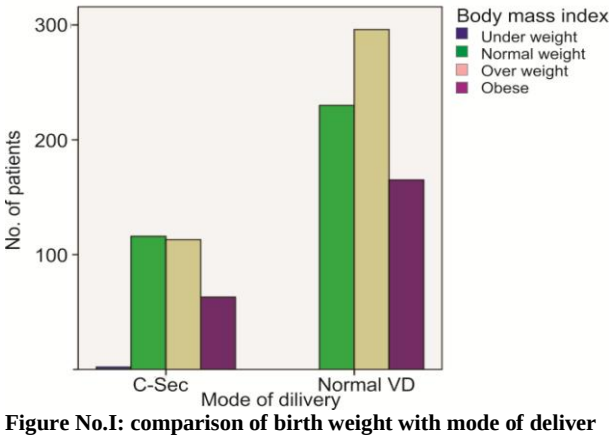

Figure No.I: comparison of birth weight with mode of deliver

DISCUSSION

Low birth weight remains to be a major public health issue causing neonatal deaths as well as serious morbidities. Furthermore, it results in long term poor health outcomes in low birth weighted infants in later stages of life. These problems include to diabetes, coronary heart disease, immune dysfunction and impaired cognitive development. [9]It is, hence important to address the factors associated with this problem; mostly being maternal. Maternal BMI is one of major maternal causes of low birth weight. A number of studies, both local and international have accused low BMI and/or maternal malnutrition to be a statistically significant risk factor for not only low birth weight but also of poor health outcomes of both mother and the infant. [3,10-12]

Therefore our objective to conduct this study was determining correlation of child birth weight with maternal weight and BMI. We found that average maternal weight, height and BMI were 63.50 ± 9.47 Kg, 154.10 ± 4.85 cm and 26.77 ± 4.03 respectively. According to their BMI classification, 2(0.20%) females were under weight, 346(35.13%) had normal weight, 409(41.52%) were overweight and rest of 228(23.15%) were obese. In study by Memon Y. only 1% of mothers having LBW babies were having body weight more than 55kg quite contrarily to our mean age i.e. 63.5 kg. However, consistent to our results they showed a highly significant association between low BMI and low weight with low birth weight.[7]

Bhatti A. also showed a significantly higher proportion of low BMI (<18.5) in mothers of cases (babies with LBW) than controls.[13] An Indian study showed significantly higher prevalence (80.96) of low birth weight in mothers having body weight of less than 40 kg. [1]In our study too, there was significant positive correlation of fetal weight with maternal weight (r=0.072, p-value = 0.024) and maternal BMI (r=0.086, p-value = 0.007). These results reveal importance of maternal BMI on health of newborn. Also these results suggest that if modifiable maternal factors, especially mother's weight and BMI are controlled, the risk of low birth weight and hence, mortality and lifelong complications related to LBW can be minimized. Further in depth studies are suggested in this regard as well.

CONCLUSION

Birth weight has a significantly positive correlation with maternal weight and BMI. Being modifiable risk factors, controlling these may considerable reduce the risk of low birth weight and associated complications for both mother and newborn.

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

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Original Article

Chromium Toxicity

Comparative Study of Chromium Toxicity with Hypertension and End Stage Renal Failure Cases

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ABSTRACT

Objective: To assess the association between hypertension and chronic renal failure with lethal effects of chromium on the beginning of the Chronic Kidney disease.
Study Design: A prospective cross sectional study**.**
Place and Duration of Study: Jinnah post graduate medical centre and Kidney centre Karachi during December 2012 to December 2013.
Materials and Methods: The present study was conducted on a total of 150 patients (age > 40 years) divided into three groups. Patients in Group I included 50 patients with hypertension whereas Group II included 50 patients with hypertension associated with chronic renal failure (CRF) and Group III consisted of 50 healthy controls.
Results: Results showed that levels of fasting blood sugar and HbA1c in both group I and group II were significantly higher as compared to controls. The levels of serum urea (**132.0 ± 28.2**) and creatinine (**7.8 ± 1.38**) in group II patients were significantly high as compared to group I and group III patients. The Creatinine clearance (**55.1 ± 9.61**) in group II patients were significantly less as compared to group I and group III patients. Serum chromium levels were significantly high in group II patients(**59.6 ± 6.73)** as compared to group I and controls.
Conclusion: Serum chromium level has significant correlation with glycemic index in both group I and group II patients compared to controls, while correlation with renal failure was significant only in group II patients.
Key Words: Hypertension, Serum chromium, Chronic renal failure, Creatinine

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INTRODUCTION

Kidney damage are common problems. The causes of nephropathies are different and some time life threatening (Sabolic 2006)[1].
Researchers found that derangements in serum creatinin level are some time fatal.(Chawla et al 2011) [2]. Chronic renal disease is a community health crisis. National Kidney Foundation on the source of clinical practice recommendations, found that twenty million subjects in the USA have ESKD, with eight million of these classified as having moderate or severe renal disease (Weiner DE et al 2004)[3].
Chronic kidney diseases may leads to several other diseases CVS disease and renal failure, also known as CKD, need treatment with dialysis or a kidney transplant for continued existence. (Jennette et al 2010)[4].
The main functions of the kidneys are regulation of water and electrolyte balance, excretion of hormones and many foreign substances, specifically drugs and regulation of arterial blood pressure and to eliminate waste products and excess water from the blood,. Disturbance of kidney functions leads to various health harms, such as bone disease , hypertension, anemia and hypercholesterolemia (Guttmann et al 2008)[5]. Some time mild failures in kidney function may leads to death, prolonged span of stay, and increased costs. The sign and symptoms of ESKD are mostly, GIT, CVS, blood and CNS symptoms. Dyspnia is a result of hypervolaemia, metabolic acidosis and anaemia (Hsu et al 2008)[6]. Numerous well-known as well as minor known relations exist between ESKD and both environmental causes and conditions, such as diabetes mellitus, hypertension, heavy metals, industrial chemicals, elevated ambient temperatures, and infections(Das and Singh 2011)[7].
Systemic hypertension and increased glomerular filtration lead to progressive nephron damage. Effective blood-pressure control delays the advancement of renal disease in adults with chronic kidney disease(Iyer et al 2010)[8].
High blood pressure is frequent in older patients is a risk factor for event of cardiac failure and CKD (Wohl et al 2009)[9].
Chromium is [Cr (VI)] a strong oxidizing agent, is carcinogenic, mutagenic, potent inducers of tumors in experimental animals; neurotoxic, immunotoxic, genotoxic reproductive toxic, and can cause DNA

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damage, sister chromatid exchanges gene mutations, and chromosomal aberrations (Soderlalnd et al 2010)[10]. Cr(VI) and Cr(III) are the predominant stable oxidation states. Many Cr(VI) compounds are highly soluble. Cr(VI) can readily cross the skin, and is transported into cells via an anion carrier (cr3). Most of Cr(III) compounds are generally insoluble and do not easily cross cell membranes Toxic effects of Cr(VI) result from its cellular activation via one- or two-electron reduction processes. (Singh SK et al 2012)[11].

MATERIALS AND METHODS

The present study is a cross sectional prospective included 150 patients. The patients are divided into three groups.

Group I included 50 patients (age > 40 years) having hypertension Group two comprises of fifty subjects (age more than forty years) having hypertension with end stage kidney diseases and Group three comprises of fifty normal subjects (age more than forty years). Experimental groups which treated to heavy water contamination by the poisonous metals were particular for this study.

Written approval was taken from all subjects and total examination and history was taken. The exclusion criteria are patients having liver disease, endocrine disease, pregnancy and females using oral contraceptive pills.

Patient's blood was collected for examination the serum level of urea, Creatinine, Creatinine clearance and chromium level (Owiredu et al, 2013)[12]. The data was analyzed on SPSS. Less than 0.05 p-value considered to be significant.

RESULTS

One hundred and fifty samples of blood were examined, who were divided into three groups having different age and gender were examined.

Results showed that group two subjects having high blood pressure and end stage renal diseases almost analogous with group one subjects with high blood pressure . Thus no obvious changes were noted in group one subjects when compared with group one subjects. FBS and HbA1c in both Group one and Group two were not significant as compared with normal subjects shown in Table 1.

Table No.1: Hypertension with end stage kidney diseases and controls

Glycemic index	(Group I) Hypertension (n=50)	(Group II) Hypertension with CRF (n=50)	(Group III) Controls (n=50)	p-Value
Fasting Blood Sugar (mg/dl)	100.5 ± 14.48*	101.3 ± 14.65	100.8 ± 15.47	0.001
HbA1c (%)	5.5 ± 0.88	5.6 ± 0.90	5.5 ± 0.94	0.001

* As compared to controls p<0.01

Table No.2: Relationship of kidney function in subjects with Hypertension , Hypertension with end stage kidney diseases and normal subjects

Renal function and serum chromium levels	(Group I) Hypertension (n=50)	(Group II) Hypertension with CRF (n=50)	(Group III) Controls (n=50)	p-Value
Urea (mg%)	24.2 ± 8.73	132.0 ± 28.2	22.3 ± 8.49	0.001
Creatinine (mg%)	1.15 ± 0.22	7.8 ± 1.38	1.12 ± 0.23	0.001
Creatinine Clearance (ml/min)	115.3 ± 13.91	56 ± 9.51**	108.8 ± 14.36	0.001
Chromium (ug/dl)	1.95 ± 0.64	59.6 ± 6.73	1.5 ± 1.07	0.001

* As compared to controls p<0.01. **As compared to Type II diabetic p<0.01

Table No.3:- Relationship between glycemic index, Chromiu and kidney parameters in groups.

		FBS	HbA1c	Urea	Creatinine	Creatinine clearance
Chromium (Pearson Correlation)	Control &HTN	.075	.051	.001	.073	.085
	Control & HTN with CRF	.126	.087	.340**	.358**	-.388**

** Relationship is significant at the 0.01 level (2-tailed)
* Relationship is significant at the 0.05 level (2-tailed)

The results showed that levels of serum urea (132.0 ± 28.2) and creatinine (7.8 ± 1.38) in group II patients (Diabetes with CRF) were significantly elevated (p<0.01) as compared to group I (Hypertension) and group III (Controls) patients. However Creatinine clearance (55.1 ± 9.61**) in group II patients (Diabetes

with CRF) were significantly less ($p<0.01$) as compared to group I (hypertension) and group III (Controls) patients. Serum chromium levels were considerably high ($p<0.01$) (59.6 ± 6.73) in group II patients (hpertension with CRF) as compared to group one (hypertension) and controls (group III) as shown in Table 2.

Table 3 shows the correlation between Chromium with FBS, HBA1c and renal parameters like Urea, Creatinine and Creatinine clearance in hypertension and hypertension with end stage kidney diseases subjects. Results have shown that lead have no significant correlation with glycemic index ($P<0.01$) in each. HTN and HTN with CRF, kidney parameters are significant ($P<0.01$) only hypertension with end stage kidney diseases subjects. Correlation between lead and creatinine and HBA1c were presented respectively in figure 5 (a,b) and figure 6 (a,b). in HTN patients (fig 5a and 6a) and in HTN with CRF patients (fig 5b and 6b).

DISCUSSION

The present study showed that in metropolitan city like Karachi, particularly in industrial area , hypertension is very common and most of hypertensive patient are associated with high serum chromium level and chronic renal failure. We also found that the majority of the subjects were normal, comparable results are also noted by Chawla et al in 2010 and weiner DE at al in 2004.

In our study we also found that there is a strong correlation between heavy metal poisoning with diabetes mellitus and hypertension when compare with control and similar finding was observed by Soderland et al in 2010 and wohl et al in 2009.

In our study we do not find that accumulation of chromium is the only factor causing chronic renal failure , there are other factors involve as well, other researchers found that chronic renal failure may occur with or without exposure of chromium.

In present study the results show that in patients with hypertension and CRF increased level of FBS and HBA1c also increase the level of urea, creatinin and creatinine clearance.

The main finding of our study was that chromium exposure is one of the aggravating factor causing chronic renal failure with or without hypertension , but other factors are involve.

CONCLUSION

It is concluded that increase serum chromium level is strongly correlated with hypertension and chronic renal failure.

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

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Original Article

Needle Aspiration

To Assess the Accuracy of Fine Needle Aspiration Cytology (FNAC) for Diagnosis of Malignancy in Solitary Thyroid Nodule at Civil Hospital Karachi

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ABSTRACT

Objective: To assess the accuracy of fine needle aspiration cytology (FNAC) for the diagnosis of malignancy in solitary thyroid nodule.

Study Design: Observational / Descriptive study

Place and Duration of Study: This study was conducted in the department of otolaryngology and Head and Neck Surgery at Civil Hospital Karachi from January 2007 to July 2008.

Materials and Methods: This study comprises of 70 cases in one and half years. We have included all cases of solitary nodule of either sex more than 10 years and excluded those patients who were exposed to radiation or underwent any sort of neck surgery previously. All Patients with solitary thyroid nodule were investigated with routine hematological and biochemical tests, thyroid profile, thyroid Scan, ultra sound neck and FNAC in outpatient department. At admission all risks/benefits of surgical procedures were explained to patients. Postoperative histopathological report of specimen was compared with preoperative fine needle aspiration cytology.

Results: In our 70 cases study 59 patients were diagnosed with benign and 11 with malignant disease. Sensitivity, specificity, accuracy was also recorded which were 72.72%, 99.20% and 94.20% respectively. Papillary carcinoma was found most common tumor in 63.63%.

Conclusion: FNAC is valuable investigation for the preoperative assessment of solitary thyroid nodule and also has high diagnostic accuracy in its evaluation.

Key Words: Solitary thyroid nodule, FNAC, Papillary carcinoma

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INTRODUCTION

Thyroid nodules are common in clinical practice. The importance of STN lies in increasing risk of malignancy in various studies from 5% to 20%.[1] STN were seen in both sexes, but four to six times more commonly in females.[2] Papillary carcinoma is more common histological type of thyroid cancers followed by Follicular, Medullary, Anaplstic, non-Hodgekin lymphoma and unclassified tumors in order of frequency[3]. Number of investigations including thyroid function test, thyroid ultrasound and thyroid scan are being used to distinguish between benign and malignant STN but none of them is found to be very sensitive, and specific.[4] Fine-Needle Aspiration biopsy is considered to be the "gold standard" in evaluation of STN.[5] If this is performed with perfection can obviate a lot of unnecessary surgery in thyroid lesion[6]. FNAC also provide knowledge of cancer cell type which aid in the planning of surgical procedure.[7] FNAC can easily be performed and accepted by patients and has low cost benefit ratio. If the sample is not diagnostic it can easily be repeated.[8] FNAC is also very safe and highly accurate in evaluation of thyroid nodule in childhood.[9]Now a days, FNAC has been adopted as initial test for diagnosing thyroid nodules and has reduced the use of imaging studies and has substantially decreased the cost of thyroid nodule management.[10]

The main purpose of this study is to know the accuracy of FNAC in evaluating solitary thyroid nodule so the surgical procedure can be planned accordingly.

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MATERIALS AND METHODS

This is a descriptive study and conducted in the department of otolaryngology and Head and Neck Surgery at Civil Hospital Karachi from January 2007 to July 2008. In the one and half year 70 patients were admitted with STN. We have included all cases of solitary nodule of either sex more than 10 years and

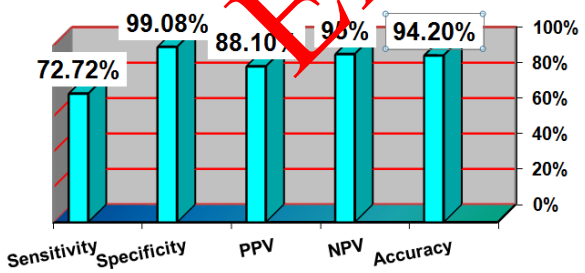
excluded those patients who exposed to radiation or any sort of neck surgery previously. Thyroid profile, thyroid Scan, ultra sound neck and FNAC was carried out in outpatient department. At admission risk/benefit were explained to the patient and informed written consent was taken regarding whole management. Postoperative histopathological report of specimen was compared with preoperative cytology.

Data analysis was done using SP10.0 version. No inferential test will be applied as it is a descriptive study. Sensitivity, specificity, probability value (PPV), non-probability value (NPV) and accuracy have been calculated for FNAC in diagnosis of solitary thyroid nodule as malignant or benign by taking histopathology as a gold standard.

RESULTS

In our 70 cases study 12 were males and 58 were females. Female to male ratio was 4.8:1. Age range for patients was observed between 12 to 70 years. Mean patient age was 36.6 years. Along with thyroid profile, thyroid ultrasound, thyroid scan, fine needle aspiration cytology was also performed in all the cases where 61 patients (87.14%) were reported with benign disease, 8 patients (11.4%) with malignant and 1 patient with suspicious cytology. Sensitivity, specificity, positive predictive value (PPV), Negative predictive Value (NPV) and accuracy of FNAC was also recorded which is shown in Graph. Histopathological results revealed that 59 patients (84.28%) were having benign nodular disease and only 11 patients (15.71%) with malignant disease.

In this study malignancy was noticed in 2 male patients and 9 female patients. Of the 2 males, 1 patient was having medullary carcinoma and other with papillary carcinoma. Among 9 females patients 6 patients had presented with papillary, 2 had follicular and 1 patient had hurthle cell carcinoma. None of patients presented with anaplastic carcinoma in this series.



Results of FNAC by Graphic presentation

Table No.1; Results of FNAC

S. #	Type of lesion	No of Patients	Percentage
1	Benign	61	87.14
2	Malignant	08	11.4
3	Suspicious	01	1.42

Table No.2: Results of histopathology

S. No	Types of lesion	No of patients	Percentage
1	Benign	59	84.28
2	Malignant	11	15.71

DISCUSSION

The management of solitary thyroid nodule is always challenging and remains controversial. Fine needle aspiration cytology is widely accepted as the most accurate, sensitive, specific and cost-effective diagnostic procedure in the assessment of thyroid nodule and help to select the patients preoperatively for surgery[11, 12]. The sensitivity of thyroid FNAC ranges from 65% to 99% and its specificity from 72% to 100%. In this study, sensitivity for cytological diagnosis of FNAC is 72.72%, specificity 99.08% positive predictive value 88.1%, negative predictive value 95% and diagnostic accuracy 94.2%. Which is in contrast of Lumachi F et all study[13]. In contrary to our results, Morgan A et al study showed overall sensitivity of FNAC detecting thyroid neoplasia was 55.0%, specificity 73.7% and accuracy 67.2%[14]. By Comparison to a local study Bukhari MH et al, reported sensitivity of FNAC as 90%, specificity 87.5%, and accuracy 87%, while positive predictive value (PPV) was 93% and negative predictive value (NPV) was 79.5%.[15] Our study testifies the results of study done at Aga khan university by Afroze N et al, where they have reported sensitivity 61.9%, specificity 99.31 and accuracy index of 94.5%.[16]

False negative FNAC results occurred in 3 (4.2%) of our patients. This is also consistent with reports in the literature that suggest a false negative rate of 2% to 7%.[17]

False positive cytology results were found in only 1(1.4%) patients in this series. This finding is consistent with other recent reports that cited an incident of false positive FNA cytology results ranging from 0% to 9%.[18, 19]

On histopathology we found 69 patients (84.28%) were having benign nodular disease and only 11 patients (15.71%) had malignant disease. Almost similar results are reported in an international study comparing 606 patients, showing a definitive histology of 82.5% benign nodules and 17.5% carcinomas[16]. Our study contradict to a local study, Ahmed M et al where malignancy was seen in only 4.4% of patients.[20]

Frequency of malignancy is also calculated in this study where we noticed papillary carcinoma in 7 patients (63.6%), follicular carcinoma in 2 patients (18.8%), medulary carcinoma in 1 patient (9%) and huthle cell carcinoma in 1 patient (9%). Similar results were reported by Virk MA et al, where papillary carcinoma was seen in 62.5% cases.

Fine needle aspiration cytology performed as a routine diagnostic procedure in patient with head and neck

masses has advantage of indicating the nature of disease to surgeon prior to surgery. A definite plan for surgery can be formulated. Similarly it avoids unnecessary operation such as in anaplastic carcinoma and lymphoma of thyroid where chemotherapy and radiotherapy are the treatment of choice.[21]

CONCLUSION

We can conclude that in the initial management of solitary thyroid nodule fine needle aspiration cytology is extremely safe, inexpensive and relatively accurate procedure. This unique investigation can be done along with other diagnostic modalities but its cost-effectiveness, accuracy and safety increases its worth and it has become widely accepted nowadays. In our study, sensitivity of fine needle aspiration cytology came out to be 72.7%, specificity 99.08%, and diagnostic accuracy was found to be 94.2%. Other studies in literatures also support the accuracy of FNAC in solitary thyroid nodule up to certain extend in preoperative patients and also helps to plain extend to thyroid surgery.

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

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Original Article

Celiac Disease

Frequency of Celiac Disease in Children with Iron Deficiency Anemia at the Children Hospital and The Institute of Child Health Multan

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ABSTRACT

Objective: To determine the frequency of celiac disease in patients presenting with iron deficiency anemia"

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at Department of Pediatric Gastroenterology, Children's Hospital & the Institute of Child Health; Multan lasted from December 2014 to October 2015.

Materials and Methods: Total one hundred patients were enrolled after fulfilling criteria. Non Probability consecutive sampling technique was used for sample collection. Patients were included in study fulfilling age one year to 14 years of both gender and diagnosed as iron deficiency anemia on serum ferritin level less than 15ng/ml.

Results: One hundred patients with iron deficiency anemia were enrolled in this study. The mean age of patients was 4.48±2.733. Fifty three(53%) male and forty seven (47%) were female. There was a significant difference between groups 1 and 2 in term of gender ($p<0.05$). The frequency of celiac disease was 21% in children with iron deficiency anemia.

Conclusion: Screening of celiac disease should be done as a routine investigation in children with iron-deficiency anemia.

Key Words: Celiac Disease (CD), Anti-Tissue Transglutaminase Antibody IgA (tTG-IgA), Iron Deficiency anemia (IDA)

Citation of article: Sheikh MA, Hussain S, Aziz MT. Frequency of Celiac Disease in Children with Iron Deficiency Anemia at The Children Hospital and The Institute of Child Health Multan. Med Forum 2015; 26(12):29-31.

INTRODUCTION

Celiac disease is an immune mediated enteropathy due to gluten sensitivity characterized by damage of small intestine resulting in villous atrophy, hypertrophy of crypts and increased intraepithelial lymphocytosis.[1] It occur due to intake of precipitant such as gluten in genetically susceptible individuals. Celiac disease manifestations vary from age to age, ranging from typical gastrointestinal symptoms to atypical like short stature and anemia.[3] It may behave like an iceberg presenting as infertility and recurrent iron deficiency anemia. Celiac disease prevalence is about 1% in Europe and USA, but it is rare and/or under diagnosed in asia.[4] Celiac disease is about 1:310 in india.[5] Celiac disease is associated with HLA DQ2/DQ8 but only 4% develop celiac disease after introduction of gluten.[6]

Iron deficiency anemia in children is a public health problem worldwide.[7] Iron deficiency causes cognitive impairments such as attention span, intelligence and sensory perception functions but can affects emotions and behavior as well.[8] In European children prevalence of iron deficiency anemia is 2-6%.[8] Anemia in celiac disease occur due to deficiency of iron, vitamins, macronutrients and micronutrient.[9] Anemia can be presenting feature of celiac disease.[10]

In a study by Çekın et al., prevalence of celiac disease in iron deficiency anemia is 7.14%.[11] In another study by Ertekin et al., prevalence of celiac disease in iron deficiency anemia is 21.3%.[12]

The Children's Hospital and the Institute of Child Health Multan is a tertiary care center providing services to the children of south Punjab. Many children with persistent or recurrent iron deficiency anemia are referred to Gastroenterology / Hepatology unit for evaluation and management. This study will probe to determine the frequency of celiac disease in iron deficiency anemia. And because there is difference in the prevalence of celiac disease in iron deficiency anemia in different studies my study will help to know the prevalence of celiac disease in iron deficiency anemia in our population. This study will also pave the way for health care providers for early screening of celiac disease.

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MATERIALS AND METHODS

A case control study was conducted at department of pediatric Gastroenterology Children's Hospital & the Institute of Child Health; Multan lasted from December 2014 to October 2015. Total one hundred patients were enrolled in a study group.

Non Probability consecutive sampling technique was used for sample collection. Patients were included in study fulfilling age one year to 14 years of both gender and diagnosed as iron deficiency anemia on serum ferittin level less than 15ng/ml.

Patients were excluded with recurrent bleeding e.g. peptic ulcer, haemorrhoids, already on gluten free diet, with congenital malformations (down syndrome, malrotation of gut etc) and suffering from co-morbid conditions like chronic liver disease, renal diseases etc.

Study was started after taking permission from the institutional ethical committee. Anemic children were recruited in study from hematology OPD after fulfilling the inclusion and exclusion criteria. After explaining risks and benefits of study, written informed consent was taken from the parents/guardians. In anemic children Hemoglobin and serum ferittin level were checked from laboratory of Children's Hospital & the Institute of Child Health, Multan. Iron deficiency anemia was diagnosed when Hemoglobin less than 9 Gm% and serum ferritin level <15ng/ml. Then serum sample for anti tissue transglutaminase (tTG) IgA was sent. IgA anti-tTG assays by ELISA seem to be highly sensitive (90-98%) and specific (94-97%) for diagnosis of CD. Level >90u/ml was considered strong positive while level ranging from18-90u/ml,upper GI endoscopy was planned to confirm the celiac disease. Level less than 18 was considered negative for celiac disease. A Performa was designed consisting of Patient name, age, registration number, anti-tissue transglutaminase IgA level, biopsy result, hemoglobin level and serum Ferritin results. The outcome variable that is presence of celiac disease was noted in the order of frequency.

Data analysis: Data analyzed statistically using SPSS (statistical Package for social sciences) version 16. Outcome variables were exposed as mean + standard deviation). The Chi-square test was used for categorical variables. A P.value<0.05 was considered statistically significant.

RESULTS

One hundred patients with iron deficiency anemia were enrolled in this study. The mean age of patients was 4.48±2.733. Fifty three(53%) male and forty seven (47%) were female. There was a significant difference between groups 1 and 2 in term of gender (p<0.05). The frequency of celiac disease was 21% in children with iron deficiency anemia.

Table No. 1: Descriptive Statistics of Patients' age, serum ferritin Level and Anti Tissue Transglutaminase IgA

	Age of the Patients	Serum Ferritin Level	Anti Tissue Transglutaminase IGA
Mean	4.4895	8.4050	63.5813
Std. Deviation	2.73025	3.52785	162.51910
Range	11.10	14.00	843.80
Minimum	.90	1.00	20
Maximum	12.00	15.00	844.00

Table No. 2: Group wise frequency of Anti-t TG-IgA

Anti-tTGIGA	Frequency	Percent
< 18	79	79.0
18-90	4	4.0
90 >	17	17.0
Total	100	100.0

DISCUSSION

Celiac disease is a systemic disease, which is associated with a number of hematologic manifestations.[13] Individuals can present with hematological abnormalities even prior to the diagnosis of celiac disease. Anemia, especially IDA, is a frequent feature in CD and may be the only presenting symptom. Increased prevalence of CD has been found in patients with IDA.[14,15] An early identification of CD in patients with IDA has great importance, since a strict adherence to a gluten- free diet not only provides management of anemia but also prevents the severe complications such as ulcerative jejunoileitis, intestinal lymphoma and neoplasm.[16] Using a highly sensitive screening test (tTG antibody test) and duodenal histological examination, we confirmed that IDA may be the only presenting symptom of CD.

Celiac disease has a wide clinical spectrum including GI and extra-GI findings, which can be diagnosed at any age from childhood to the elderly. Classical or typical form of CD is associated with features of malabsorption; however, a substantial number of CD patients have atypical manifestations, including hematologic, endocrinologic, renal, neurologic, psychiatric, dermatologic, and cardiovascular symptoms.

This study shows the high prevalence of 21% in children with iron deficiency anemia while none was found positive in healthy children. In the literature, there are some studies in which the prevalence of CD was investigated in newly diagnosed IDA, with different results. Corazza et al. reported the prevalence of CD as 5%.[17] In another study by Çekın AH et al., prevalence of celiac disease in iron deficiency anemia is 7.14%.[11] In study of Ertekin et al., prevalence of celiac disease in iron deficiency anemia is 21.3% that is very

close to our study.[12] Ayhan Gazi Kalayci et al[18] reported 4.4% while Umaprasanna S.et al[19] found occult celiac disease as 2.8%.

The study does have certain short comings; the study has been conducted on a smaller number of patients. In order to achieve validity, more prospectively designed, multi center involvement, larger sample sizes are required to know the exact prevalence of celiac disease in this part of the world.

CONCLUSION

Screening of celiac disease should be done as a routine investigation in children with iron-deficiency anemia. Biopsy may be suggested in patients with iron-deficiency anemia who have positive celiac disease serology.

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Original Article

A Survey to Assess the Food Habits of School Going Children from High Socioeconomic Background

Food Habits of Children

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ABSTRACT

Objectives: To assess food habits of school going children from a high socio-economic background, Establish food frequency of commercially available foods and to determine the risk factors and to suggest the way to control these risk factors.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted at the Department of Community Medicine, Al-tibri Medical College, Isra University, Karachi from 31st May to 30th July, 2015.

Materials and Methods: This survey was carried out on 200 students age of 6-12 years enrolled in KAS school belong to high socioeconomic group, randomly selected for interviews through an administrated structured food frequency questionnaire. This survey was conducted in three days during 8am to 3pm. All students who visited the health office for any reason were asked to participate in the study.

Results: The mean age of respondents was 12.7±3.7 years. Thirty-nine reported missing at least one meal a day. Averagely the students consumed 4.2meals per day. Most frequently missed meal was evening tea/snack (n=17), followed by midday snack (n=12). Few students reported missing lunch (n=3) or dinner (n=2) during the week.

Conclusion: Students need to be encouraged to take healthy foods, by creating opportunities for better choices. Health education strategies can be effective implemented in a school setting through innovate means can ensure better outcomes.

Key Words: Food habits, School-age Children, Commercially available foods, Risk factors

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INTRODUCTION

Internationally there has been little impetus for nations to develop food and nutrition policies since the calling for a marriage between agriculture and health by the being of nations in the 1940s. The relative complacency of the 1950s and 1960s toward nutrition was rocked by the global food crisis in 1973.[1,2] In late 1974 the Food and Agriculture Organization convened a World Food Conference and called on nations to develop food and nutrition policies. Norway was the only country to respond, adopting a National Nutrition Policy in 1976.[3] Toronto City Council is working with Boards of Education to establish nutrition programs in all Toronto schools[4] (Toronto Food Policy Council). In Pakistan, Agha Khan Project (Taranna Project) also has started working on school nutritional programs.

Improvements in the community's food intake and nutritional status only will occur it efforts are concentrated on making "healthy choices - easy choices".[5] Changes in the food supply may be required. However, any decisions made regarding interventions at particular points in the food and nutrition system must be based on knowledge of the whole system - objectives, inputs, outputs and linkages.[6] Knowledge of the food system is obtained by the collection of data, which then can be used as a guide for monitoring the system. In particular, it enables decisions to be made which enhance the food system effectiveness and ultimately improve the population's nutritional status.

Nutrition is the study of nutrients in food and how the body handles them including eating, digestion, absorption, storage and excretion. Nutrition is essential for a healthy physical and mental development. Meeting the nutritional requirements of Children 6-12 years of age takes larger amount of the same foods needed by preschool aged children. Children in this age group are the period where growth is slow but steady, in contrast intellectual and social growth is rapid. School children have long day and mentally demanding day followed by some very active periods. Leaning about food, growth and health is an important part of this age group. Good nutrition is based on variety, moderation, balance and regularity. Healthy life involves more than just eating. Children should be

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encouraged to get involved in physical activities regularly in order to have a good maintenance. The health and wellbeing of children is closely linked with that of the mother. The giving or life and support for survival of that life. Hence it is essential to develop effective strategies for improving the nutrition habits of school children so that they can go on to develop as healthy adults. Malnutrition can cause stunting, physical and mental retardation.

MATERIALS AND METHODS

This study consisted of 200 students age of 6-12 years enrolled in KAS school belong to high socioeconomic group. This cross sectional study was conducted at the Department of Community Medicine, Al-tibri Medical College, Isra University, Karachi from 31st May to 30th July, 2015. 200 students were randomly selected for interviews. They were an administrated structured food frequency questionnaire. All students who visited the health office for any reason were asked to participate in the study. The collection of dietary intake data from a community can he used to determine the proportion of the community that is receiving an excessive or inadequate intake of various nutrients. These data also can reveal relationships between dietary intake and nutritional status. Since dietary intake data are an indirect measure of nutritional status, they need to be considered along with the biomedical data when assessing the nutritional status of a community. This allows identification of the proportion or a community who may he malnourished with respect to one or more nutrients. The methods that have been developed to more dietary intake differ according to the type of information that is sought, the way in which the information is collected and the time frame of the study. There are advantages, disadvantages and limitations to each method, with no single method of dietary assessment being accepted as entirely satisfactory or universally the best.

Dietary Intake Methodologies: 24-hour recall: The respondent is interviewed and asked to recall all food and beverages consumed during the preceding 24 hours. After obtaining a description of the food and beverages consumed and the method of preparation, the interviewer records this intake. Accurate reporting of the portion size by the participant can be improved by showing household measures and food models and getting the respondent to compare their portion sizes to these.[8]

Food record: This method involves the respondent measuring and recording all food and beverages as they are consumed. The food items consumed may be measured using household measures (e.g. 3 tablespoons of rice) or by weighing the food when it is ready to eat[8]. Leftover food also must be measured and recorded. Food records are usually kept for three days (not necessarily consecutive), but may be kept for longer periods of a week, a month or even one year.[9]

Food frequency questionnaire: The questionnaire may be self-administered or completed by the interviewer. Respondents are required indicate how frequently they consume each food item listed[10] (as number of times consumed per day, week, month or year). Portion sizes are included for each food item. Although a food frequency questionnaire may specifically on food or beverages containing a particular nutrient, the food items listed should reflect the food preferences of the community being studied.

Diet history: This method of obtaining dietary intake data is usually only used in one on one clinical setting, due to the length of the interview (1-2 hours) and therefore the high cost involved.[11] The respondent is interviewed to determine their habitual food intake over a preceding, specified time period (e.g. three months or one year). The semi-structured interview incorporates several styles of dietary data collection, such as a food frequency and 24-hour recall. The respondents' psychosocial history with respect to food intake often is included.

Store turnover: Turnover of foodstuffs from a store is determined using store invoices for all food items delivered to the store during a preceding, specified time period. Total quantities of each food supplied are tabulated and the average daily supply is calculated. Apparent per capita consumption of food and nutrients per day is then calculated by dividing mean daily store turnover by the number of people in the community.[9] This method is most appropriate for a geographically isolated community, of small size, serviced by a single food store.

Assessing Nutritional Status: From a nutritional perspective, the role of the food and nutrition system is to provide the population with a safe, affordable, accessible and nutritious food supply. However, inequalities in the distribution and availability of foods, and hence nutrients, exist. These inequalities may be identified by assessing the nutritional status of those living in a particular community. The term 'assessment' is derived from the Latin ward assess are which means 'to sit by' or 'watch over'. In health care, the term nutritional status assessment describes "the process of collecting all pertinent information about the [nutritional] status of a person or group of persons"[12]. Since nutrition is one of the most important determinants of health, it follows that the assessment or "nutritional status should be one of the most important activities in monitoring the health of our nation"[13].

Biomedical Indicators: There is no single, reliable biomedical test that provides a direct measure of nutritional status. Instead, the assessment procedure involves collecting and interpreting data using a variety of methods. The biomedical data collected can be used to identify the presence of diseases with nutritional

components, such as hypertension and some cancers. The risk factors for such diseases also can be identified.[14]

Anthropometric Assessment: Anthropometry is "the process of measuring various dimensions of the body". The measures provide estimates of size, weight and proportions, particularly muscle and fat components of the body and are inexpensive and simple to obtain.[15] Accuracy depends on correct technique and reading of instruments, as well as accurate recording of the data. Owen and Frankle maniacs a number of anthropometric measurements used to assess the nutritional status of individuals. I will discuss briefly those measurements that are more commonly used.

Triceps skin fold (TSF) measurement: Skin fold measurements measure subcutaneous fast (underneath the skin) at various sites of the body. Since approximately half of the fat in the body is present as subcutaneous fat, the thickness of skin fold over the triceps muscle provides a good indication of fat, and energy stores.[16] The TSF measurement is taken at the midpoint of the non-dominant arm, using calipers. It may be interpreted by comparing the measurement with reference or percentile values, the latter providing adjustments for age.[17] Common errors made when obtaining a TSF measurement include measuring the wrong arm, incorrectly positioning the calipers, or taking the reading at the wrong time. The individual's state of hydration as well as the presence of oedema also may affect the accuracy of the reading.

Midarm muscle circumference (MAMC): This measurement indicates muscle or somatic protein stores. It is calculated using TSF and mid arm circumference (MAC) measurements. When obtaining the MAC measurement, care must be taken to ensure the tape is correctly positioned around the non-dominant arm, which should be loosely hanging by the side.

Waist to hip ratio (WHR): The WHR is used to measure abdominal obesity. It is calculated by dividing the abdominal circumference (halfway between the lowest lateral portion of the rib cage and iliac crest) by the hip circumference (at the level of the maximal protrusion of the gluteal muscles). Although cut off points for defining those persons at risk have yet to be identified, a WHR greater than 0.85 may be representative of excessive abdominal adiposity. Abdominal obesity (or android type) is seen more frequently in men while gluteofemoral (or gynoid type) is more common in women. After menopause, women tend to take on the male fat distribution pattern of abdominal obesity.[18]

Body mass index (BMI): This ratio provides a good indication of body fat in adults. It is calculated by dividing an individual weight (in kilograms) by their height squared (in meters). An index of between 25-30 is classifieds overweight, while obesity is identified if the BMI is greater than 30. As BMI increases beyond those values considered to be acceptable, health risks also increase. BMI fails to distinguish soft tissues and muscle bulk from fat.[14] Consequently, athletes frequently fall into the 'overweight' range but the excess weight is usually muscle. BMI also cannot distinguish between gluteo-femoral and abdominal obesity. As with other anthropometric measurements, the height and weight measurements used to calculate BMI are subject to errors. These errors may be due to the incorrect use of instruments, or the way the measurements are read and recorded. Day to day, or within day variations can influence monuments such as height by up to 2 centimeters.

Growth charts: Data from the National Centre for Health Statistics (NCHS) were accepted by the World Health Organization in 1978, and now ere the foundation for recognized growth chart development. Charts based on those data are used internationally, since there is little difference between the growth of children in developed societies and growth in those from privileged groups in developing countries.[18] Single points on a growth chart can rarely be interpreted accurately. Comments on the adequacy of a child's growth pattern can only be made from the plotting of multiple measurements taken over time. This establishes a percentile ranking, on the growth grid. Growth velocity, as well as acceleration or deceleration, can then be monitored which will reflect excessive or inadequate energy and/or nutrient intakes.

Biochemical Assessment: A number of biochemical tests are available for assessing nutritional status. These measure the concentration of nutrients circulating in biological fluids such its blood and urine, while haematological tests are concerned with the morphology and physiology of blood cells. Owen and Frankle lists a number of biochemical tests used in nutrition surveys. As discovered previously, improvements in the community's food intake and nutritional status only will occur if efforts are concentrated on making 'healthy choices - easy choices'. Knowledge of the food system is obtained by the collection of data, which then can be used as a guide for monitoring the system. In particular, it enables decisions to be made which enhance the food system's effectiveness and ultimately improve the population's nutritional status.

RESULTS

The mean age of respondents was 12.7±3.7 years. To the question as to how many days a week they have a certain meal or snack. One fifty-six reported missing at least one meal a day. Averagely the students consumed 4.2 meals per day. Most frequently missed meal was evening tea/snack (n=68), followed by midday snack (n=48). Few students reported missing lunch (n=12) or dinner (n=8) during the week (Table 1).

Table No.1: How many a day a week do you take the following meals?

Meal/day	0	1	2	3	4	5	6	7	Total
Breakfast	20	12	20	20	24	20	24	60	200
Midday	48	12	16	32	28	24	20	20	200
Lunch	12	12	20	24	36	28	44	24	200
Evening tea or snack	68	8	8	24	20	16	20	36	200
Dinner	8	0	4	0	28	60	52	48	200
Total	156	44	68	100	136	148	160	188	1000

Table No.2: Location where meal is consumed. (n=200)

Meal consumed	Home	School	Other (specify)
Breakfast	168	-	12
Midday	0	152	-
Lunch	52	80	56
Evening tea or snack	76	8	48
Dinner	116	-	76

Most students took meals at home except for midday snack, which was usually taken at school. The average number of times per week a student ate a meal outside the school or home was 2.6±1.2. There were six students who were on special diets. Four were on a low calorie diet and two were strict vegetarian. Four were self-prescribed, one was prescribed by a physician and one was prescribed by a yoga instructor (Table 2). Most children reported consuming most of the listed foods regularly. Two children were strict vegetarians. Chicken was the most frequently consumed meat followed by mutton and beef. Seventeen children reported non-consumption of fish. Milk was not taken out by two children, whereas majorly consumed milk on a daily basis. Fruits vegetables and pulses were no consumed too often (Table 3).

Table No.3: Type of foods consumed per week

Food/day	0		1		2		3		4		5		6		7	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Chicken	28	14.0	-	-	-	-	-	-	-	-	32	16.0	44	22.0	96	48.0
Beef	64	32.0	24	12.0	28	14.0	16	8.0	-	-	-	-	-	-	8	4.0
Mutton	28	14.0	-	-	4	2.0	12	6.0	16	16	28	14.0	36	18.0	64	32.0
Fish	68	34.0	68	34.0	32	16.0	8	4.0	12	12	4	2.0	8	4.0	-	-
Milk	8	4.0	-	-	-	-	-	-	8	8	24	12.0	56	28.0	104	52.0
Cheese	8	4.0	24	12.0	8	4.0	36	18.0	44	44	4	2.0	4	2.0	72	36.0
Eggs	32	16.0	-	-	-	-	4	2.0	8	8	4	2.0	48	24.0	104	52.0
Apples	8	4.0	4	2.0	8	4.0	20	10.0	24	24	12	6.0	52	26.0	92	46.0
Bananas	24	12.0	-	-	-	-	-	-	8	8	4	2.0	76	38.0	88	44.0
Watermelon	-	-	-	-	-	-	-	-	24	24	56	28.0	44	22.0	76	38.0
Potatoes	24	12.0	8	4.0	52	26.0	60	30.0	8	8	24	12.0	8	4.0	8	4.0
Cabbage	12	6.0	56	28.0	20	10.0	12	6.0	24	24	44	22.0	32	16.0	-	-
Cucumber	-	-	48	24.0	60	30.0	8	4.0	24	24	16	8.0	12	6.0	32	16.0
Salad leaf	-	-	48	24.0	60	30.0	8	4.0	24	24	8	4.0	8	4.0	44	22.0
Daal	-	-	44	22.0	32	16.0	48	24.0	24	24	28	14.0	12	6.0	12	6.0
Beans	-	-	60	30.0	76	38.0	48	24.0	4	4	4	2.0	8	4.0	-	-

DISCUSSION

Good nutrition is critical for development of healthy body and mind. Our study shows that even when food is adequately available it is the poor choice of food that can cause concern.[19] Children of high socio-economic class with access to all types of foods still do not consume adequate fruits and vegetables. They are likely to indulge in unhealthy junk food.[20]

A report by the US Department of Health and Human Services, examined similar variables in a much larger sample of US school children.[21] It recommends implementing, innovative strategies to reverse the rising trend of overweight in young children by promoting consumption of five or more servings of fruits and vegetables each day, increasing physical activity, increasing breastfeeding, and decreasing television viewing. Promote adequate dietary iron intake and screening of children at risk for iron deficiency. Overweight (high weight-for-length-BMl-for-age) in children and adolescents have reached epidemic proportions in recent years.[22] The prevalence of overweight in children in PedNSS from birth to age 5 is 13.1%. Overweight in children younger than 2 does not pose the same risk as it does in children aged 2 or older because little association has been found between their weight and increased risk for adult obesity.[23] Expert committees have recommended a two-level screening

for over weighting among children aged 2 years or older. The recommendations are to use BMl-for-age at or above the 95th percentile to define overweight and between the 85th and 95th percentile to define risk of overweight.[24]

CONCLUSION

Health education strategies can be effective implemented in a school setting. Health education through innovate means can ensure better outcomes. Students need to be encouraged to take healthy foods, by creating opportunities for better choices. School Canteens should also play its important part and provide nutritious food "Healthy choice-easy choices".

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

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Original Article

Dyslipidemia

To Determine the Frequency of Dyslipidemia in Primiparous Women, with Hypertension

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ABSTRACT

Objective: Objective of this study to determine the abnormalities of lipid profile in primiparous women with hypertension.

Study Design: Observational study.

Place and Duration of Study: This study was conducted at Gynae Department of MMC and of PUMHS Nawabshah from March 2012 to February 2014.

Materials and Methods: Total 100 primiparous women were chosen in this study after the diagnosis of the gestational hypertension. Cases from second trimester of gestational age were incorporated. Women with systolic blood pressure >130 mmHg, and d blood pressure >90 mm of Hg were considered as hypertensive. Every one of the women with known of fetal abnormalities, DM, abnormalities of the thyroid, ischemic coronary illness, renal failure, liver disease and previous history of lipid profile variations and hypertension before the pregnancy were rejected from the study. Blood tests of all the chose women's were taken in fasting and refer to hospital diagnostic laboratory for lipid profile. After the taking the reports information with respect to lipid profile and hypertension were recorded on the proforma.

Results: Total 100 ladies were incorporated, mean age was (mean+SD=28.5±4.2) years, gestational age was discovered (mean+SD=30.2 ± 3.1 weeks). Greater part of the women was found with overweight 68%. Dyslipidemia was found in 59% of the women, while 41% women were noted with ordinary lipid profile. As per the abnormalities of lipid profile, all total cholesterol found up in the 59% of the women, taking after by brought LDL was up in 42.0%, HDL was up in 20%, and TG was noted up in the 53.0% of the women. While in the 45% ladies HDL was noted decreased and 35% women were noted with normal HDL.

Conclusion: Variations in the lipid profile are the major reason for hypertension in primiparous women. Therefore it is very important that serum lipid profiles should be constantly observed all through the entire pregnancy period from ahead of diagnosis of hypertension to reduce the maternal morbidity and mortality in young women.

Key Words: Primiparous women, lipid profile abnormalities

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INTRODUCTION

Hypertensive issue stand for a most well-known therapeutic complication of pregnancy, influencing 6 to 8 percent of developments in the United States.[1] Pregnancy induced hypertension, characterized as high bold pressure following 20 weeks of pregnancy in the women with proteinuria and the edema without past history of high blood pressure is the significant reason for feto-maternal mortality and the morbidity. High blood pressure is available in 5% of whole pregnancies, in 10% of primiparous ladies and 20 –25% of ladies with past history of the chronic hypertension.[2] With expanding age, the risk of creating pregnancy induced hypertension raises.[3] Women with pregnancy induced hypertension will probably create overweight, dyslipidemia.[4] Hypertension during Pregnancy is one of the significant danger variables in now a days in services of the health, in light of the fact that it not only reason of mother mortality as well as decrease fetal growth for the duration of pregnancy.[5,6] High blood pressure is specifically connected with expanded stages of aggregate cholesterol (TC), triglycerides (TGs), low density lipoproteins (LDL) and low density lipoproteins (LDL) though; in the meantime, the stages of HDL are diminished. LDL-C stages top at mid 3rd trimester, most likely as a result of the hepatic impact of estradiol and the progesterone.[7] It has been recommended that the increment in plasma triglycerides and LDL-C stages for the duration of pregnancy may be utilized to distinguish women who will create atherogenic variations afterward in life.[8] Earlier research have demonstrated a decrease in cholesterol HDL up to ten years subsequent to the 1st pregnancy, free of weight, focal adiposity and chosen behavioral modifications.[9] Pregnancy induced hypertension be a factor of 15.6%

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maternal mortality.[10] Raised serum lipid fixations levels connected with seriousness of pregnancy induced hypertension.[9] Therefore the purpose behind this study to determine the dyslipidemia in primiparous women with gestational hypertension.

MATERIALS AND METHODS

This study was observational and was carried at Gynae Department of MMC and of PUMHS Nawabshah from March 2012 to February 2014. Total 100 primiparous ladies were chosen in this study after the diagnosis of the gestational hypertension. Women from second trimester of gestational age were incorporated. Women with systolic blood pressure >130 mmHg, and diastrolic blood pressure>90 mm of Hg were considered as hypertensive. Every women which known history of fetal abnormalities, DM, abnormalities of the thyroid, ischemic coronary illness, renal failure, liver disease and previous history of lipid profile variations and hypertension before the pregnancy were rejected from the study. Blood tests of all the chose women's were taken in fasting and refer to hospital diagnostic laboratory for lipid profile. After the taking the reports information with respect to lipid profile and hypertension were recorded on the proforma. All the data was analyzed on spss program version 17.0.

RESULTS

Total 100 women were included, mean age was (mean±SD=28.5±4.2) years, gestational age was (mean±SD=30.2 ± 3.1 weeks). Majority of women 61.0% were from rural areas. Table:1.

Majority of the women were found with overweight 68%, while 32% women were found with normal weight. Table:1.

Dyslipidemia was found in 59% of the women, while 41% women were noted with normal lipid profile. Figure 1.

Table No.1: Basic information of the patients n=100

Characteristics	Frequency/%
Mean age (mean±SD)	28.5±4.2 years
Gestational age (mean±SD)	30.2 ± 3.1 weeks
Blood pressure	
Systolic (mean+SD)	145.39 ± 7.68 mmhg
Diastolic (mean+SD)	97.80 ± 7.68 mmhg
BMI	
Over weight	68/68.0%
Under weight	32/32.0%
Residence	
Rural	61/61.0%
Urban	39/39.0%

According to the abnormal lipid profile, total cholesterol raised found in the 49% of the women, following by raised LDL was in 42.0%, raise HDL was in 20%, and raised TG was noted in the 53.0% of the women. While in the 45% women HDL was found decreased, and 35% women were noted with normal HDL Table:2.

Table No.2: Lipid profile of the patients n=100

Lipid Profile	Normal Frequency/%	Raised Frequency/%
TC	40/51.0%	60/60.0%
LDL	52/52.0%	42/42.0%
HDL	35/35.0%	20/20.0%
TG	53/53.0%	47/53.0%

Figure No.1: Frequency of dyslipidemia n=100

DISCUSSION

The abnormalities of lipids in plasma increase obviously along with pregnancy. Lipid stages are influenced due to changes in the maternal hormonal. Other components, for example, BMI, nourishment, pre-pregnancy concentration of the lipid and different pregnancy complications may perform important role in the metabolism of lipids and concentration in plasma. In this study women age was (mean±SD=28.5±4.2) years. Similarly Mankuta D et al[11] reported the mean age 30.4 years. Gestational age of the women was found (mean±SD=28.2 ± 3.1 weeks). Majority of the cases 61.0% were blongs rural areas. Sreekarthik KP et al[12] reported mean gestational age 39.16 ± 1.01 weeks. These findings are very as compare to gestational age of our study, this may because of majority of the cases in this study were observed during antenatal care time.

In the study of Akhavan et al[13] stated that association between hyperlipidemia and the gestational hypertension severity assessed and those cases having severe preeclampsia were found with significant difference of raised plasma TG, total ch, and raised LDL–C levels. In some studies mentioned that in starting of pregnancy serum lipid profile variations developed and create the risk of the PIH.[14,15] In several early published studies stated that raised abnormalities of the lipid profile had found in pregnant women with hypertension.[16-18] As well as in our series **d**yslipidemia was found in majority of the cases 59% of the women, while 41% women were noted with normal lipid

profile. Obesity or increased the pregnancy BMI is a authenticate, risk factor of the enhancement of endothelial dysfunction and preeclampsia, but this mechanism of this raised risk not finally understood.[20] Majority of the women was found with overweight 68%, while 32% women were found with normal weight.

Likewise patients with hyperlipidemia, particularly hypertriglyceridemia have a higher occurrence of and are inclined to grow more extreme instances of preeclampsia.[21] Most, in spite of the fact that not all studies have demonstrated a dyslipidemic example of expanded TG, cholesterol, LDL and diminished HDL levels in preeclampsia.[22] In our study according to the abnormal lipid profile, total cholesterol raised found in the 49% of the women, following by raised LDL was in 42.0%, raise HDL was in 20%,and raised TG was noted in the 53.0% women. While in the 45% women HDL was found decreased, and 35% women were noted with normal HDL As well **as** Anjum R et al[22] demonstrated that serum concentrations of TC, TGs, LDL and VLDL highly raised whereas, the HDL found was considerably reduced in pregnant women with hypertension.

CONCLUSION

Variations in the lipid profile are the major reason for hypertension in primiparous women. Therefore it is very important that serum lipid profiles should be constantly observed all through the entire pregnancy period from ahead of diagnosis of hypertension to reduce the maternal morbidity and mortality in young women. More research with big sample size is needed in primigravida women.

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

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Original Article

Effect of Aloe Vera Whole Leaf Extract on Blood Glucose, Hyperinsulinemia and Insulin Resistance in Streptozotocin Induced Type 2 Diabetic Rats

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ABSTRACT

Objective: To determine the effect of Aloe vera whole leaf extract and/or Rosiglitazone on plasma glucose, insulin and insulin resistance in type 2 diabetic Sprague-Dawley rats.

Study Design: Randomized control trail

Place and Duration of Study: This study was conducted at the Department of Physiology Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH) Islamabad from April 2009 to Oct 2010.

Materials and Methods: Type 2 DM was induced in 45 healthy Sprague –Dawley rats by feeding high fat diet for 2 weeks and injecting a low dose (35mg/kg) of streptozotocin intra peritoneally. Type 2 diabetic rats were randomly divided into three groups, each group having 15 rats and were labeled as diabetic group, Aloe vera group and rosiglitazone group. The diabetic group was injected normal saline, Aloe vera group was treated with Aloe vera whole leaf extract in dose of 300mg/kg body weight and rosiglitazone group was given 5mg/kg body weight of rosiglitazone I/P for 21 days.

Results: A significant reduction ($p<0.001$) in plasma glucose (62%), insulin (19%) and TG/HDL ratio (69%) was analyzed in Aloe Vera group as compared to diabetic control group.

Conclusion: The maximum impact in lowering plasma glucose, insulin and TG/HDL ratio was recorded in rosiglitazone group, followed by Aloe vera group. The results of present study provide a scientific basis of using Aloe vera whole leaf extract as antidiabetic in T2DM.

Key Words: T2DM. Aloe vera, Hyperinsulinemia, Insulin Resistance

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which is characterized by hyperglycemia due to the defects in secretion or action of insulin or both.[1] DM is increasing in alarming rate throughout the world especially in developing countries. Pakistan ranks sixth in the world's top ten countries with the highest number of diabetics[2]. Managing diabetes is difficult due to the number of side effects associated with drugs used for its treatment. Complementary and alternative medicine (CAM) for the treatment of diabetes mellitus is becoming popular.[3] The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs be further investigated.[4]

Aloe vera comes from a family called Aloaceae and related to the Liliaceae family. Among 360 known species only five have medicinal properties.[5]The Aloe vera plant has fleshy leaves which consist of gel, latex and outer green rind. Number of studies has been documented on gel and latex parts but with controversial reports.[6] A study based on the use of traditional phytotherapy for control and treatment of diabetes by rural inhabitants of district Attock showed that a large majority of people used the extract made from fresh leaves of Aloe vera, according to them this formula is very old and 100% effective.[7] Increasing incidence of diabetes mellitus in rural population, adverse effects of synthetic medicines and humble financial status of our people necessitates looking for indigenous and inexpensive botanical source of medicines with anti diabetic effects. Therefore present study was designed to analyze the effect of Aloe vera whole leaf extract on plasma glucose, insulin and insulin resistance in type 2 diabetic rats and to compare the results with known antidiabetic drug, rosiglitazone.

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MATERIALS AND METHODS

A whole leaf process was employed in making the Aloe juice. Leaves were cut into sections and were pulverized into a soup like structure by placing these in

a grinding unit. Cellulose was allowed to dissolve in a digestion liquid. Aloe emodin as well as aloin was removed by passage through activated charcoal column.[8]

Forty five healthy Sprague Dawley rats' about 90 days old, weighing between 220±50 grams were taken from National Institute of Health (NIH), Islamabad. Rats with deranged blood glucose levels and that which fail to become diabetic and insulin resistant after two weeks of high fat diet and injection of streptozotocin (35mg/kg) were excluded from the study

For induction of T2DM all animals were fed with high fat diet for 2 weeks after which a single intra-peritoneal injection of streptozotocin (available as 1 gram vial, Bioworld Pharmaceutical) in the dose of 35 mg/kg body weight was given.[9] For confirmation of T2DM fasting blood glucose levels along with total lipid profile were measured after 72 hours by tail vein sampling. The cut off value for hyperglycemia was of >11.11mmol/l. The development of insulin resistance was measured by using the surrogate marker of TG: HDL ratio. The cut off value of TG: HDL ratio >1.8 was used to establish insulin resistance[10]

After induction of T2DM, Sprague Dawley rats were randomly divided into three groups, diabetic control group, Aloe vera and rosiglitazone. Diabetic control group were admintered 0.1ml normal saline intraperitonealy (I/P) daily, Aloe vera group were given Aloe vera whole leaf extract in daily dose of 300 mg/kg body weight by gastric tubing and rosiglitazone group treated with 5mg/kg body weight of rosiglitazone I/P for next 21 days. After 21 days of treatment, overnight fasted rats were anesthetized and 5 ml of intra-cardiac blood was collected to analyze plasma glucose, insulin resistance (TG/HDL) and insulin levels.

Analysis of samples was done at Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi, Pakistan. Estimation of glucose was done by enzymatic colorimetric (TRINDER'S) method. Triglycerides (TG) and high density lipoprotein (HDL) were estimated simultaneously on automated chemistry analyzer (Vitalab Selectra E) An enzymatic colorimetric method GPO-PAP (Glycerol phosphate oxidase) was used for serum TG estimation. The direct method for quantifying HDL was done and their ratio was taken as marker for insulin resistance.[11,] Insulin measured by ELISA, based on the direct sandwich technique.

Data was entered into SPSS version 16.0. Mean and standard deviation was employed for all the values. Data within the groups were analyzed by using one-way analysis of variance (ANOVA) followed by Post Hoc (Tukey) test. The "p value" <0.05 was considered statistically significant.

RESULTS

At the end of the study the plasma glucose levels of diabetic control group was 20.15 ± 1.97 mmol/l with reduction in Aloe vera group upto 7.64 ± 0.71 mmol/l (62%), rosiglitazone group upto 6.54 ±0.64 mmol/l (68%) which revealed significant reduction ($p<0.001$) in the two treated groups. However, the reduction in plasma glucose level in rosiglitazone was maximum. The statistical difference among mean plasma glucose levels of the groups was found significant ($p<0.001$) by one way ANOVA. Post–Hoc (Tukey's) test was applied to calculate the statistical significance of the differences between the mean plasma glucose levels between the groups (table 1). The comparison revealed that mean plasma glucose levels significantly ($p<0.001$) decreased in Aloe vera and rosiglitazone group as compared to the diabetic group. However a greater reduction in rosiglitazone group was observed.

TG: HDL ratio in diabetic control group was 5.8 ± 1.40, in Aloevera group; 1.8 ± 0.20, in rosiglitazone group; 1.5 ± 0.30 as shown in table 1.

The statistical difference among mean TG: HDL ratio of the groups was found significant ($p<0.001$) by one way ANOVA. Post–Hoc (Tukey's) test was applied to calculate the statistical significance of the differences between the mean TG: HDL ratio between the groups (table 1). The comparison revealed that mean TG: HDL ratio significantly ($p<0.001$) decreased in Aloe vera and rosiglitazone as compared to the diabetic group as shown in table 1.

Table No.1: Comparison of plasma glucose, TG: HDL ratio and insulin levels in different groups by one way ANOVA

Variables	Diabetic control group	Aloevera group	Rosiglitazone group	p Value
Plasma glucose (mmol/l)	20.15 ± 1.97	7.64 ± 0.71	6.54 ± 0.64	<0.001
Triglyceride (mmol/l)	2.16 ± 0.14	1.08 ± 0.10	0.95 ± 0.13	<0.001
HDL (mmol/l)	0.39 ± 0.08	0.58 ± 0.06	0.65 ± 0.07	<0.001
TG:HDL ratio	5.8 ± 1.40	1.8 ± 0.20	1.50 ± 0.30	<0.001
Insulin (µU/ml)	20.63 ± 2.2	16.76 ±0.95	15.41 ± 1.06	<0.001

All values are presented as mean ±SD for 15 animals in each group

The TG and HDL levels of diabetic control rats were 2.16 ± 0.14 mmol/l and 0.39 ± 0.08 mmol/l respectively however TG decreased in Aloe vera group (1.08 ± 0.10 mmol/l) and rosiglitazone group (0.95 ± 0.13 mmol/l) as compare to the diabetic control group.. The serum HDL levels of diabetic control group was 0.39 ± 0.08 mmol/l, which increased in Aloe vera, and rosiglitazone group upto 0.58 ± 0.06 mmol/l, 0.65 ± 0.07 mmol/l respectively.

Statistical significance of difference between the mean level of lipid parameters were assessed by one way

ANOVA, which revealed significant difference ($p<0.001$) among the groups. Post–Hoc (Tukey's) test was applied to calculate the statistical significance of the differences between the mean TG levels between the groups (table 1). The comparison revealed that mean plasma TG levels significantly ($p<0.001$) decreased while HDL levels increased in Aloe vera and rosiglitazone group as compared to the diabetic group.

Plasma insulin levels in diabetic control (20.63 ±2.2 µIU/ml), which has been found decreased in Aloevera treated group (16.76 ± 0.59 µU/ml) and in rosiglitazone group (15.41 ± 1.06 µU/ml).

Table No.2: Statistical difference of plasma glucose, TG: HDL ratio and insulin levels between different groups using Post-Hoc (Tukey) test

Group comparison	Blood glucose (mmol/l)	Insulin (µU/ml)	Triglyceride (mmol/l)	HDL (mmol/l)	TG:HDL
Diabetic Vs Aloevera	<0.001	<0.001	<0.001	< 0.001	<0.001
Diabetic Vs rosiglitazone	<0.001	<0.001	<0.001	<0.001	<0.001
Aloevera Vs rosiglitazone	0.047	0.047	0.039	0.047	0.048

P value <0.005 is statistically significant

Table No.3: Percent reduction in blood glucose, TG:HDL and insulin levels in different treated groups in comparison to the diabatic control

Parameter	Control	Alovera	Rosiglitazone
Blood glucose mmol/l	20 .15	62 % ↓	68 % ↓
TG/HDL ratio	5.8	69 % ↓	74 % ↓
Insulin µU/l	20.63	19 % ↓	25 % ↓

The comparison of mean plasma insulin levels between all the groups assessed by one way ANOVA, revealed significant difference ($p<0.001$) amongst the groups.

Post–Hoc (Tukey) test was applied to calculate the statistical significance of differences between the mean plasma insulin levels between two groups, namely diabetic and Aloevera group, diabetic and rosiglitazone group, (table 1). The comparison revealed that mean plasma insulin level was significantly (<0.001) decreased in Aloevera supplemented group, rosiglitazone group as compared to diabetic control group (Table 2) Post-Hoc (Tukey) test was also applied between interventional groups. The comparison revealed that mean plasma insulin levels were significantly lowered in rosiglitazone group as compared to Aloe vera group.

DISCUSSION

We used the animal model of T2DM developed by Srinivasan, because it closely resembled the natural course and metabolic characteristics of the disease[9]. In previous studies most of the experimental models of T2DM were constructed with alloxan and streptozotocin to destroy only a portion of B cells which resulted in extreme insulin deficiency and overt hyperglycemia in rats and their characteristics were similar to type DM-1 than to DM-2.

Administration of high fat diet for 2 weeks followed by low dose of streptozotocin resulted in frank hyperglycemia, hyperinsulinemia and insulin resistance.These findings were consistent with the published data of different studies[12].

In our study TG/HDL ratio in all groups after inducing T2DM was more than 1.8, manifested the presence of insulin resistance. In a study by Srinivasan et al., (2005), TG/HDL ratio was not measured; however marked hyperinsulinemia (467.50 ± 32.43 pmol/l) in high fat fed rats was taken as the indicator of insulin resistance.

Aloe vera supplementation in the present study has resulted in statistically significant ($p<0.001$) reduction in plasma glucose levels when compared with diabetic control group. A study conducted by Noor et al, resulted in reducing fasting plasma glucose level in streptozotocin induced diabetic rats. They used the same dose and duration of treatment as in our study. However, by the end of the study, fasting blood glucose in their diabetic rats reduced by 41% while in our study blood glucose levels decreased by 62 % of the diabetic control rats[13] This could be due to the use of whole leaf extract rather only using the gel part and due better extraction of blood sugar-lowering active principles of whole leaf Aloevera extract.

Since it is difficult to quantify insulin resistance in daily practice, there are several methods to estimate it. Most commonly homeostasis model assessment for insulin resistance formula (HOMA- IR) is used[14]. However due to financial constraints we could not use HOMA- IR model. We used TG: HDL ratio to quantify it. In present study, there was a marked development of insulin resistance in the diabetic group as revealed by TG: HDL ratio of 5.8. The magnitude of insulin resistance was lowered in Aloevera group by 70%. This could be due to its glucose and lipid lowering property. The insulin sensitizing activity was attributed to the

presence of chromone lephenol and cycloartanol, a phytosterol in Aloevera extract manifesting the marked insulin sensitizing action of Aloevera. [15]

Kim studied the effect of Aloevera extract on diet induced obesity (DIO) mice. Aloevera was given in a dose of 100mg/kg for 8weeks that significantly lowered insulin resistance[16]. However, in their study they used HOMA- IR. The insulin resistance values of DIO group treated with 25, 50, 100 mg/kg Aloevera extract was 31.4%, 32.1% and 31.1% respectively, of that of the untreated DIO group. However our study results were more significant (70%) than Kim's study. This could be due to higher dose (300mg/kg) of Aloevera extract used in our study .

At the end of study the plasma insulin level in diabetic control group was (20.63 ± 2.2 µ U/ml) consistent with other studies.[17]Treating them with Aloevera extract resulted in significant decrease ($p<0.001$) in insulin level by 19%. This may be due to the fact that Aloevera extract increased the insulin sensitivity by decreasing plasma glucose and lipid levels, thus resulting in reduction in plasma insulin level.

A study conducted by Kim on C57BL/6J mice showed statistically significant ($p<0.05$) increased level of insulin (71%) after feeding them high fat diet for 12 weeks in comparison to regular diet fed mice.[16] A group of these mice with diet induced obesity (DIO) and hyperglycemia were treated with processed Aloevera gel (PAG), which resulted in 34% statistically significant decrease in insulin level ($p<0.05$) in comparison to the diabetic mice. The %age decrease in Kim's study was more profound than our study. This difference could be due to difference in type of model used in study.

Rosiglitazone is a known antidiabetic drug of thiazolidinediones family.[18] It increases insulin sensitivity and improves glycemic control. It also acts as a ligand for the gamma subtype of peroxisome proliferators activated receptor (PPARs- gamma), which is directly involved in the regulation of genes controlling glucose homeostasis and lipid metabolism.[19] In our study the plasma glucose levels are reduced by 68%, insulin 25%, TG 56%, HDL by 66%, TG:HDL ratio 74%, These findings of rosiglitazone group are similar to many clinical trials carried in the past[19].

CONCLUSION

In our study the treatments effects highlighted in percentage terms had recorded maximum impact in lowering blood glucose, insulin and insulin resistance in rosiglitazone treated group followed by Aloe vera. However, with prolong use, rosiglitazone is associated with weight gain myocardial infarction and heart failure making it an unlikely drug for T2DM.[20]. This opens the room to explore new strategy of treatment for T2DM, by looking for synergistic action of natural herb with synthetic drug (in half of effective dose). In addition to minimizing the side effects associated with synthetic drug, may help to lessen the financial burden associated with this disease especially in a country like Pakistan, where socio economic conditions of people are not strong enough to cope with chronic diseases like DM

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

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Original Article

Silymarin Therapy

Effect of Silymarin Therapy on Liver Aminotransferase in Non-alcoholic Fatty Liver Disease

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ABSTRACT

Objective: The present study evaluated the effects of silymarin on blood glucose, blood lipids and liver amino-transferase (AST & ALT) in Non-alcoholic fatty liver disease (NAFLD).

Study Design: Randomized Placebo Controlled (double blind) Trial

Place and Duration of Study: This study was carried out at Consultant Clinic Cant area Hyderabad and Department of Medicine, Isra University Hospital Hyderabad from April 2012 to August 2013.

Materials and Methods: A sample of 64 subjects (33 cases and 31 controls) was selected for evaluating effects of silymarin. Subject selection observed the inclusion and exclusion criteria. Subjects with aspartate transaminase (AST) and alanine transaminase (ALT) >1.2 of normal were included. NAFLD diagnosis was confirmed by ultra-sonography. NAFLD cases were given silymarin (140 mg x2 tablets) daily for duration of 3 months. Controls received placebo. AST & ALT were checked after three months. Data variables were analyzed by SPSS version 21.0.

Results: Mean ± SD of aspartate transaminase (AST) and alanine transaminase (ALT) before intervention were found as 73.2±9.7 vs. 69.3±17.6 IUL^{-1} ($p<0.021$) and 92.1±19.4 vs. 83±15.6 IUL^{-1} in cases and controls ($p < 0.0001$) respectively. After three months the AST and ALT were found reduced compared to baseline. AST and ALT were found as 39.3±7.5 IUL^{-1} and ALT to 39.3±10.9 IUL^{-1} 35.9±11.7 IUL^{-1} and 83±15.6 IUL^{-1} in cases and controls respectively. Blood lipids and blood glucose also showed statistically significant differences (p=0.0001).

Conclusion: Silymarin improves blood glucose, blood lipids and liver amino transferase in non-alcoholic fatty liver disease.

Key Words: Silymarin, NAFLD, Liver, Blood glucose, Blood lipids

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INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) is a metabolic disorder of characterized by abnormal fat deposition in liver parenchyma in the absence alcohol consumption. NAFLD clinically manifests as a metabolic disorder associated with obesity, systemic hypertension, hyperglycemia & diabetes mellitus and hyperlipidemia.[1,2] Estimated prevalence of NAFLD in industrialized countries is estimated as 45% in the general population.[3] NAFLD results in disordered liver metabolism and multiplies the risk of developing atherosclerosis manyfolds.[4] Many factors have been implicated in the etiology and risk of NAFLD. The factors include the obesity, insulin resistance, adipokine inter-play, oxidative stress and bacterial overgrowth syndrome.[5]

A change in diet, dietary habits, physical exercise, brisk walking and a total change in life style improves the NAFLD and guidelines recommend them for prevention.[6] Different drugs have also been recommended which modify and change the natural course of liver injury in NAFLD through various mechanisms. Many drug agents are now available and prescribed in clinical practice. Anti-oxidants agents,[7] metformin drug therapy,[8] receptor sensitizers,[7] Pioglitazone – a PPAR γ agonist,[9] and the ezetimibe[10,11] had been recommended and prescribed.

Silymarin is an herbal agent - a mixture of flavonolignans. Biochemical structure shows two diastereomers of "*silybin*". Silymarin exerts anti oxidant through a novel pathway of stimulation of nuclear transcription. Silymarin stimulates the nuclear polymerase, and mRNA formation. Silymarin inhibits toxins entry into hepatocytes by blockade at cell membrane levels. Silymarin protects against free radical mediated hepatocytes injury. It also inhibits the lipid peroxidation cascade initiated by free radical.

Previous studies had reported over expression of superoxide dismutase gene on the hepatocytes, this way it exerts anti oxidant activity. Silymarin increases stores of glutathione and anti oxidant enzyme – the glutathione peroxidase (GPX).[12]

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A search of Pakistani literature showed a limited experience of silymarin use in NAFLD, blood lipids and blood glucose in local population.[13-15] The financial prosperity has begotten obesity epidemic in urban areas. The obesity is a risk for metabolic disorders including NAFLD. The NAFLD may be the earliest manifestation of metabolic syndrome. In near future, the NAFLD may be a public health problem.

The present study evaluated the effects of silymarin on blood glucose, blood lipids and liver amino-transferase (AST & ALT) in Non-alcoholic fatty liver disease (NAFLD).

MATERIALS AND METHODS

The present randomized clinical placebo controlled (double blind) trial was conducted at the Consultant Clinic Cant area Hyderabad and Department of Medicine, Isra University Hospital Hyderabad from April 2012 to August 2013. Subjects complaining of upper gut symptoms were screened for the liver fatty infiltrations.

A sample of 64 subjects (33 cases and 31 controls) was selected for evaluating effects of silymarin. Subject selection observed the inclusion and exclusion criteria. Merits and demerits of study were explained to study subjects. Volunteers who singed informed consent were selected. Subjects were informed that they can withdraw at any time. Volunteer subjects who satisfied the inclusion criteria for NAFLD were studied.

Subjects with aspartate transaminase (AST) and alanine transaminase (ALT) >1.2 of normal for last six months were included. NAFLD diagnosis was confirmed by ultra-sonography.[13, 15] Subjects suffering viral hepatitis, history of alcohol use and hepatotoxic drug intake were excluded from study protocol. History of autoimmune disorders, diabetes mellitus, hypolipidemic drug intake, and diabetes mellitus was taken for exclusion.

Height, weight and body mass index (BMI) were noted. Subjects who satisfied the included criteria were block randomized into 2 groups – the cases and controls. NAFLD cases were given silymarin (140 mg x2 tablets) daily for duration of 3 months. Controls received placebo- identical appearing tablets. AST & ALT were checked after three months.

Participants were guided to take low fat low energy diet and regular physical exercise. Blood samples were collected from ante-cubital veins after three months therapy. Ethical issues were strictly observed. Data was noted in pre-structured questionnaire. Data variables were analyzed by SPSS version 21.0. (IBS-Corporation USA). Student's t-test and Chi square testing was used for numerical and categorical data respectively. Data was analyzed at CI of 95% interval ($p \leq 0.05$).

RESULTS

Age (mean± SD) in cases and controls was 49±9.7 and 48±8.9 years respectively ($p=0.07$). Male predominated over female and majority belonged to rural area. The baseline demographic data of study population is shown in table 1. BMI, blood glucose and blood lipids was observed between cases and controls ($p > 0.05$).

Mean ± SD of aspartate transaminase (AST) and alanine transaminase (ALT) before intervention were found as 73.2±9.7 vs. 69.3±17.6 IUL^{-1} ($p<0.021$) and 92.1±19.4 vs.83±15.6 IUL^{-1} in cases and controls ($p < 0.0001$) respectively. After three months the AST and ALT were found reduced compared to baseline. AST and ALT were found as 39.3±7.5 IUL^{-1} and ALT to 39.3±10.9 IUL^{-1} 35.9±11.7 IUL^{-1} and 83±15.6 IUL^{-1} in cases and controls respectively. Blood lipids and blood glucose also showed statistically significant differences ($p=0.0001$).

Table No. I: Baseline characteristics of cases and control subjects

	Cases (n=33)	Controls (n=31)	p-value
Age	49.0±9.70 years	48±8.9 years	0.071
Male	21 (63.6%)	21(67.7%)	0.091
Female	12 (36.3%)	10 (30.3%)	0.081
Rural population	22 (66.6%)	19 (61.2%)	0.072
Urban population	11 (33.3%)	12 (38.7%)	0.092
Weight (kg)	88.0±19.90	83.0± 21.50	0.063
BMI (kgm^{-2})	29.90±5.80	28.70±6.80	0.081
Postprandial blood glucose (mg/dl)	163.0±21.50	154.0±28.60	0.082
Triglycerides (mg/dl)	192.90±44.70	182.90±41.50	0.063
Total cholesterol (mg/dl)	199.10±23.80	198.30±21.40	0.08
HDLc (mg/dl)	37.10±8.10	36.90±9.50	0.092
LDLc (mg/dl)	95.30±17.50	97.20±15.30	0.063
VLDL(mg/dl)	41.0 ± 9.40	43.0± 14.60	0.074

Table No.2: Liver amino-transferase enzyme levels in cases and controls

	Cases		Controls		p-value
	Before intervention	After intervention	Before intervention	After intervention	
AST (IU/L)	73.20±9.70	39.30±7.50	69.30±17.60	35.90±11.70	0.02
ALT (IU/L)	92.10±19.40	39.30±10.90	83.0±15.60	51.20±19.10	0.001

DISCUSSION

Hepatoprotective mechanism of silymarin is now an established fact. Various underlying mechanisms have been proposed against oxidants in animal studies. Silymarin scavenges free radicals; therefore liver is an important site to be protected as free radicals are frequently formed there. Silymarin protects the phospholipids of cell membrane against free radical injury. Silymarin maintains cell membrane fluidity, and maintains cell membrane functions.[16]

Silymarin protects at sub cellular level through gene transcription. Silymarin facilitates gene transcription, mRNA formation and ribosomal translation. Thus silymarin produces new proteins which protect against toxic agents. Newly synthesized proteins may act as anti oxidant enzymes to neutralize free radicals and free radical mediated peroxidation of cell membrane phospholipids.[17]

Silymarin alleviates inflammation and exerts anti fibrogenic effects through inhibition of cytokine functioning.[18-22]

Silymarin maintains cell membrane fluidity and permeability, thereby helps to maintain mitochondria functions and energy production. Enhanced mitochondria functioning increases cellular capability against xenobiotc induced liver injury.[23]

Silymarin is reported to interfere directly with binding of toxins to cell membrane of hepatocytes. Thus the toxins mediated injury is minimized and cell functioning remains normal. Silymarin spares membrane transport proteins for normal cell functioning.[24]

A previous clinical study has reported effects of silymarin against drug induced liver injury, alcohol toxicity, and viral hepatitis induced liver injuries.[18]

The present study reports that the silymarin improves liver functioning as evaluated by the improvement of hepatocytes enzyme levels. Silymarin also improves blood glucose and blood lipid levels. Our findings are in parallel to previous studies[25,26]

Another previous study has also reported similar observations for silymarin.[27] A previous study reported that the silymarin reduces liver amino-transferase enzymes in NAFLD. Other liver functioning tests were also improved as reported by Hashemi.[28] The findings of above study are highly in keeping to our present study. Pro inflammatory cytokines are increased in NAFLD and they adversely affect the fat disposal in liver and promote fat deposition with hepatocytes. Lipid deposition sets up a vicious cycle which in turn increases the cytokine secretion.[28-29] As regards blood lipids, present study reports an improvement in the blood lipids which is an important finding and is in confirmation to above studies. Previous studies reported the liver amino-transferase was correlated positively with the pro inflammatory markers such as the C-reactive proteins. Hence, in present study if liver amino-transferase were reduced it means it might be due to a reduction in pro inflammatory cytokines. Our finding is of clinical importance and is consistent to previous studies.[30-33] Seemingly, it may be concluded that the Silymarin inhibits inflammatory cytokine release and thereby exerts hepatoprotective effects. Inhibition of cytokines improves liver fat deposition and facilitates fat disposal into circulation. Other underlying mechanisms may be working and need further elucidation. The limitations of present study are that we were not able to perform liver biopsy due to human ethical issues. The present study reports that the administration of silymarin improves liver parenchyma functioning in patients with NAFLD..

CONCLUSION

The present study reports that the silymarin improves blood glucose, blood lipids and liver amino transferase in non-alcoholic fatty liver disease. Liver amino-transferase are enzymes of hepatocytes cytoplasm compartment, hence present study concludes silymarin improves liver functioning at cellular levels which needs further elaboration.

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

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Original Article

Suicidal Hanging

Prevalence of Suicidal Hanging in males of 3rd Decade of life

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ABSTRACT

Objective: This study was carried out to find the manner and gender variation in various age groups of asphyxial deaths in Lahore.

Study Design: Observational study.

Place and Duration of Study: This study was conducted at the Department of Forensic Medicine King Edward Medical University, Lahore, during January 2006 to December 2008.

Materials and Methods: A total of 2979 autopsies had been carried out. After detailed scrutiny 220 cases of asphyxial deaths were selected for this study. The post-mortem reports, police papers and hospital notes were studied. The parameters selected in those asphyxial deaths were age, sex, means of constriction, level of application of force and fracture of hyoid bone.

Results: Amongst this total number of autopsies in 220 cases, the cause of death was asphyxia (7.39%). Hanging surpassed amongst all asphyxial deaths 104 which was 47.27%, ligature strangulation was next in number 64(29.09%) and then the throttling 52(23.64%)

Out of these 104 cases of hanging72 were males and males dominated as per M/F ratio shown as 2.25:1.0. The 3rd decade age group i.e.; 21-30 years had higher incidence of males than females. The homicidal manner was126 (57.27%), suicidal 68(30.91%) and in un-determined it was 26(11.82%).

Conclusion: Hanging remains the commonest method of suicide in males especially in age group of 21-30.Strangulation with ligature and manual throttling are the other methods used in homicidal deaths. The post-mortem findings showed damage to the structures above the thyroid cartilage which favors death due to hanging and throttling. And the trauma to the tissues below thyroid cartilage is consistent with ligature strangulation. The fracture of Hyoid bone is commonly seen in ligature strangulation.

Key Words: Mechanical Asphyxia, Hanging, Strangulation, Manual Throttling, Hyoid Bone.

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INTRODUCTION

Neck is a conduit of very important vital structures, Carotid, Vertebral and Jugular blood vessels, Spinal Cord and Cranial nerves, Trachea and Esophagus. So these vital structures are likely to be damaged in all sorts of trauma including mechanical interference at the level of neck. This will result in asphyxia and it is the commonest method adopted in homicidal asphyxial deaths by ligature or manual compression.

Hanging is defined as a form of asphyxial death in which the weight of the body acts as the mechanical force of interference in the process of respiration[1]. This compression can also be achieved by other means as direct trauma on neck by blow, neck being compressed in arms lock, accidental fall on the neck and entrapment in ropes[2].

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The resultant outcome of mechanical asphyxia depends upon the amount of compressive force applied and the physiological changes manifested by the involved structure, individually or in total. The result also depends upon the means used for constriction and the magnitude of force being used.

To compress jugular vein weight of 2 kg is required, it will block the return of blood flow to the heart and the pathological findings will be elicited as cyanosis, congestion and petechiae. Carotids arteries require 3.5 kg and will result in cerebral ischemia. Compression of carotid bodies by trauma will cause stimulation of Vagus nerve, which will cause sudden cardiac arrest. The air flow can be blocked either by direct force or indirectly by pushing the base of tongue against the posterior pharyngeal wall. As the trachea is a hard cartilaginous structure and it is not possible to occlude it easily, but a weight of 15 kg can achieve this. Direct trauma by any means on the larynx will cause fractures of hyoid and thyroid bones[2, 3, 4, 5, 6].

The resultant asphyxia so achieved will appear as non-specific asphyxial finding and can be seen as the pathological entities. The anoxia caused by falling oxygen level in blood will result in the form of damage to the endothelium which will result in capillary dilatation, increased permeability and pooling of blood. These pathological damages can be manifestedas cyanosis, congestion, petechial hemorrhages, oedema and fluidity of blood. The stasis of blood will cause further reduction in circulating volume of blood and further anoxia.

MATERIALS AND METHODS

This observational study was conducted at the Department of Forensic Medicine King Edward Medical University, Lahore, during January 2006 to December 2008. A total of 2979 autopsies had been carried out. After detailed scrutiny 220 cases of asphyxial deaths were selected for this study. The post-mortem reports, police papers and hospital notes were studied. The parameters selected in those asphyxial deaths were age, sex, means of constriction, level of application of force and fracture of hyoid bone.

Inclusion Criteria: The case in which the cause of death was hanging by ligature and constricting force was the weight of the body.

Exclusion Criteria: The other deaths which occurred due to any other form of trauma to the neck other than hanging were not included in this study.

RESULTS

During the study period of 2006-2008 the total number of autopsies was 2979 and out of these 220 (7.38%) were asphyxia deaths. (Table No. 1)

Table No. 1 Kinds of Weapons Used in All 2979.0 Autopsy Cases

	Total	%age
Blunt	403.0	13.52%
Sharp Edged	256.0	8.5%
Fire-arms	1285.0	43.13%
Poisoning	74.0	2.48%
All Types of Burns	50.0	1.68%
Mechanical Asphyxial Deaths	220.0	7.38%
Electricity	19.0	0.64%
Drowning	17.0	0.57%
Blasts	65.0	2.18%
Natural Causes	347.0	11.65%
Cause Un-Determined	213.0	7.15%
Total	2979.0	100.00%

Obstruction at the Level of Neck: In this study we included three types of neck compression. There were total of 220 cases of neck compression. Out of these 220, hanging was found in 104 cases (47.27%) and 64 were of ligature strangulation (29.09%).(Table No. 2) (Figure 1).

Table No.2 Types of Obstruction at the Level of Neck (220 cases of Asphyxial Deaths)

Types of Mechanical Asphyxia	Number of Cases	Percentage
Hanging Cases	104.0	47.27%
Ligature Strangulation Cases	64.0	29.09%
Throttling Cases	52.0	23.64%
Total Number of Cases	220.0	100.00%

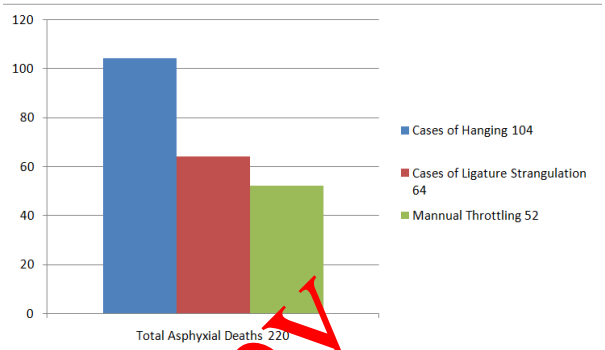

Figure No.1: Types of Obstruction at the Level of Neck (220 cases)

Age and Sex Distribution: The third decade of life between 21-30 years (35.91%) was the most prevalent age group. The next higher age was 31-40 years (25.91%) and the ages between 11-20 years was next in the sequence (17.27%). Out of these 144 (65.45%) were male in all 220 asphyxial deaths, and 76 were females (34.55%). (Figure No. 2)

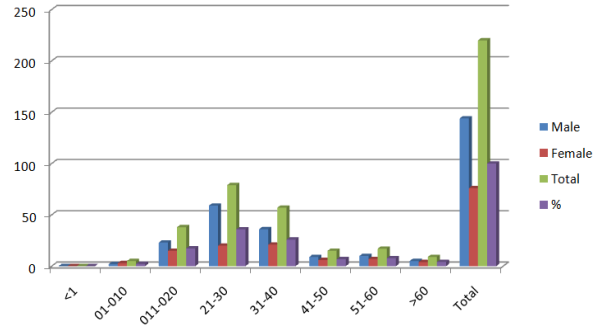

Figure No. 2: Age and Sex Distribution in 220 cases

Hanging had a higher incidence in both sexes in 3rddecade of life. Whereas in strangulation & throttling were high in 4th and 5th decade respectively. (Table No. 4) In first decade of life there was no case of hanging. The males cases were more in number than females (2.25:1) in hanging, as compared to strangulation (2.05:1) and throttling (1.26:1) (Table No. 3).

In all deaths male hanging showed highest incidence in 3rd decade and females in 2nd decade of age group. In ligature strangulation males were more in number in 3rd decade and females in 4th decade. Males, in throttling, were more in number in 3rd decade of age

group. While the females showed higher number in 4th decade. (Table No. 3)

The Manner of Death in all Cases of Hanging (104)

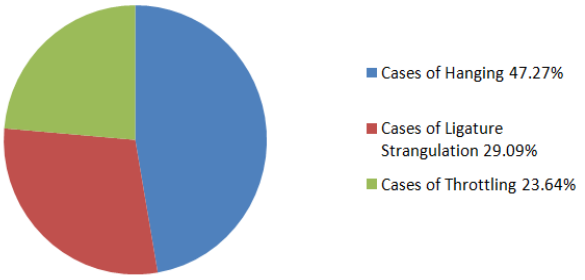

Figure No: 3: Manner of Death in all Cases of Hanging

Manner of Death: By manner of death we mean that the cause of death is natural or un-natural. Only the homicidal and suicidal deaths were included in this study as un-natural deaths. Those deaths in which the cause of death remained undetermined were not included. There was no case of accidental asphyxia.

57.27% (126) were cases of homicide, 30.90% (68) were suicide, and 11.82% (26) were cases in which the manner remained un-determined (Table 4).

In homicidal deaths M/F ratio was 2.15:1.0, in suicidal cases it was 2.77:1.0 and 1.6:1.0 in un-ascertained deaths (Table No. 4).

Manner of Death in Hanging: Out of all asphyxial deaths, 104 cases were that of hanging and amongst them 68 cases were suicidal showing an incidence of 68.50%.And the male to female ratio was 2.78:1.0. The predominant number was of males and particularly in 3rddecade while the females showed a rise in 2nd decade.

Homicidal hanging was 9.62% (10 cases). The highest incidence was seen in 3rd decade in both sexes. Male had higher incidence than females in all decades with M/F ratio of 1.5:1.

The un-determined hanging was seen in 26 cases (25%). The highest incidence was seen both males and females in 2nd decade. M/F ratio was 1.6:1. (Fig. No. 3) (Table No. 5)

Table No. 4: Sex& Age Variation in All Asphyxial Deaths (220 Cases)

Age In Years	Total Number of All Types of Asphyxial Deaths	Hanging Cases (104 Cases) Male/ Female Ratio 2.25:1		Cases of Ligature Strangulation Male/ Female Ratio 2.05:1		Cases of Throttling (52 Cases) Male/Female Ratio 1.26:1	
		Males	**Females**	**Males**	**Females**	**Males**	**Females**
<1 yrs.	-	-	-	-	-	-	-
1-10 yrs.	-	-	-	1.0	2.0	1.0	1.0
11-20 yrs.	38.0	14.0	10.0	6.0	3.0	3.0	2.0
21-30 yrs.	79.0	28.0	9.0	17.0	6.0	14.0	5.0
31-40 yrs.	57.0	19.0	4.0	12.0	7.0	5.0	10.0
41-50 yrs.	15.0	4.0	4.0	2.0	1.0	3.0	1.0
51-60 yrs.	17.0	5.0	4.0	3.0	1.0	2.0	2.0
>60 yrs.	9.0	2.0	1.0	2.0	1.0	1.0	2.0
Total No. of Cases	220.0	72.0	32.0	43.0	21.0	29.0	23.0

Table No. 5: The Manner of Death in All Deaths in this Study (Total Asphyxial Deaths=220)

Age In Years	No. of Cases	Homicidal Cases Male/Female Ratio 2.15:.01			Suicidal Cases: Male/ Female Ratio 2.77:1.0			Un-Determined Cases: Male/Female Ratio 1.6:1.0		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
<1 Yrs.	-	-	-	-	-	-	-	-	-	-
1-10 Yrs.	5.0	2.0	3.0	5.0	-	-	-	-	-	-
11-20Yrs.	38.0	8.0	6.0	14.0	5.0	9.0	14.0	7.0	3.0	10.0
21-30 Yrs.	79.0	36.0	11.0	47.0	20.0	6.0	26.0	4.0	2.0	6.0
31-40 yrs.	57.0	20.0	14.0	34.0	16.0	2.0	18.0	3.0	2.0	5.0
41-50 Yrs.	15.0	8.0	2.0	10.0	4.0	1.0	5.0	-	-	-
51-60	17.0	6.0	3.0	9.0	5.0	-	5.0	-	3.0	3.0
>60	9.0	6.0	1.0	7.0	-	-	-	2.0	-	2.0
Total	220.0	86.0	40.0	126.0 (57.27%)	50.0	18.0	68.0 (30.91%)	16.0	10.0	26.0 (11.82%)

Table No. 6: The Manner of Death in All Cases of Hanging (104)

Age In Years	Total No. of Cases	Cases of Homicidal Hanging 10 (9.62%) Male/Female Ratio 1.5:1.0		Cases of Suicidal Hanging 68 (65.38%) Male/Female Ratio 2.78:1.0		Un-Determined Cases 26 (25.0%) Male/Female Ratio 1.6:1.0	
		Male	Female	Male	Female	Male	Female
<1 Yrs.	-	-	-	-	-	-	-
1-10 Yrs.	-	-	-	-	-	-	-
11-20 Yrs.	24.0	2.0	1.0	5.0	6.0	7	3.0
21-30 Yrs.	37.0	4.0	2.0	20.0	5.0	4	2.0
31-40 Yrs.	23.0	-	-	16.0	2.0	3	2.0
41-50 Yrs.	8.0	-	-	5.0	1.0	0	3.0
51-60Yrs.	9.0	-	-	5.0	1.0	0	3.0
>60 Yrs.	3.0	-	1.0	-	-	2.0	-
Total Cases	104.0	6.0	4.0	50.0	18	16.0	10.0

DISCUSSION

Incidence of Death: Out of 2979 autopsies which were conducted during this study period, 220 cases were that of asphyxial deaths having an incidence of 7.39%. This incidence is greater than studied by 1.6%[7], 1.75%[8], and 1.88%[9] of all deaths of interference at the level of neck. While in other studies carried out it was 2.94%[10] of those all types of deaths and 24.53% of all asphyxial deaths. In another study it was 5%[11] of all types of deaths and 82% was of hanging in total of asphyxial deaths. Other studies when were compared they showed 1.17%[12], 12.4%[13] and 5.5% of all un-natural deaths but the incidence of our study was lower than 15.7%[14].

Types of Compression of Neck: In this study the number cases of hanging was 104, which was 47.27%. The cases of ligature strangulation were 64 and were 20.09%. The cases of manual throttling were 52 (23.64%). The findings in this study are comparable with those of (cases of hanging 57%,cases of strangulation 21%, and cases of throttling 18%) [9], (cases of hanging 61.17%, cases of ligature strangulation 21.19% and cases of throttling 17.64%) [8], (cases of hanging and ligature strangulation 80.7% and cases of throttling 19.3%) [7], (cases of hanging/cases of ligature strangulation 85% and cases of throttling 6%)[15], (cases of ligature strangulation 12.4%) [13], (cases of ligature strangulation 19.23%,cases of throttling 46.15%) [10], (cases of hanging 41.8%, cases of ligature strangulation 2.9% and manual throttling 2.3%) [14], (cases of hanging 69%) [11].

Distribution of Sex and Age: The age group 21-30 i.e.:3rd decade of life shows the highest incidence in all asphyxial i.e., hanging, ligature strangulation and throttling amongst all age groups. This incidence can be compared with the studies which have been carried out previously. They showed in one study57% [11]cases of hanging in all types of asphyxial deaths, and the age group with predominance was 3rd decade [12]. In another study average age was 41.9 years [14]. A study done by Bowen[16] showed greater incidence of hanging the age ranging from50-59 years. In another study Guarner & Hanzlick[17] found out the average age of 31 years the highest incidence in USA.

Male/Female Ratio: Male/female ratio in our study in hanging is 2.25:1.0, in ligature strangulation it is 2.05:1.0 and in manual throttling it is 1.26:1.0. So the incidence of males than females is clearly distinct in all three types of asphyxial deaths.

Hanging has shown that 69.23% were males. This is more than females, which is 30.76%. This incidence is comparable with that of Azmak[14]which showed 83.9% were male, and a ratio of male to female as 2.7:1.0in Bashir MZ[9] et al. The males were 73.07% and females 26.92% in this study.

The males were 58.9% and females 41.02% in ligature strangulation and throttling in the study of Bashir MZ[9]. The study of Azmak D[14] has shown 1.0:3.0 in cases of ligature strangulation and 1.0:2.0 in cases of manual throttling. Srivastava AK[10] had shown that in his study 30.77% were males and 69.23% were females, showing the incidence of females higher than the males.

Manner of Death: In our study the un-natural homicidal deaths have shown a higher incidence as compared to the study carried out by Bashir MZ[9]. In which the homicidal deaths were 45.05%. But our incidence is lower than that in the study of Demirci S[13] , which was85%.

The suicidal deaths in our study were 30.90%, which is lower than that in the study of Bashir MZ[9] which had shown 45.45% and the study of Azmak D[14] has shown it 47%. This is higher incidence than that shown by Demirci S[13] as 15%.

The incidence of suicidal hanging in our study was 65.38%, which shows at lower value that which was calculated by Bashir MZ[9] showing a percentage of 86.53%. 9.62% homicidal deaths have also shown a higher value than 3.84%[9]. Which is lower than the study of Bowen DA[16], showing 95%.

Accidental hanging was not reported in our study; whereas Bowen DA[16]had shown a percentage of 5% deaths of auto-erotic accidental asphyxia.

CONCLUSION

It is one of the commonest causes of deaths in our country. Suicidal hanging is the most preferred method of self- killing. Suicide occurs mostly in younger age groups especially in 3rd decade of life, and males showing higher incidence than females because of the responsibilities of living on their shoulders mainly.

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

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Original Article

TB Diagnostic Techniques

Comparison of Conventional TB Diagnostic Techniques with PCR IS6110 in Tertiary Care Chest Hospital Lahore Pakistan

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ABSTRACT

Objective: To compare the efficiency of conventional diagnostic techniques and Insertion Sequence (IS)6110 based PCR assay for M. tuberculosis in pulmonary and extra-pulmonary specimens from tertiary care chest hospital.

Study Design: Observational study.

Place and Duration of Study: This study was conducted at Gulab Devi Chest Hospital, Lahore from August to January 2013.

Materials and Methods: A total of 1599 (1417 pulmonary and 182 extra-pulmonary) non-duplicate clinical specimens, obtained over a period of six months, were tested by conventional techniques such as Ziehl-Neelsen staining (ZN), Lowenstein Jensen (LJ) medium and Fluorescent staining. MTB was extracted through DNAzol method. Insertion Sequence (IS) 6110 based PCR assay was used for M. tuberculosis from pulmonary and extra-pulmonary specimens. Of the 1599 specimens, 781 were suspect cases while 818 were MDR (follow up) cases. Mean age of TB patient was ±33 years. 18% of follow-ups and 20% of suspects were <20 year in age, 52% follow-ups and 36% suspects were about 20-40 years, and 30% follow-ups and 33% suspects were >40 years of age.

Results: It was seen that, among MDR cases (follow-ups) 68% were males and 32% were females. Similarly, among TB-suspects, 58% were males and 42% were females. Of total 168 suspected pulmonary samples ZN (48.2.7%), fluorescent microscopy (79.7%), LJ culture (52.9%) and PCR (91.6%) were positive for M. tuberculosis. In total 143 suspected extra-pulmonary samples, ZN (34.95%), fluorescent microscopy (45.5%), LJ culture (39.8%) and PCR (87.4%) were positive.

Conclusion: In contrast to conventional methods of TB diagnosis, PCR is more quick, sensitive, reliable and cost effective technique.

Key Words: MDRs, Bacteriocins, Lactobacilli, Antibiotic Resistance, MAR, Antibacterial activity

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INTRODUCTION

Tuberculosis (TB) a common and life threatening infectious disease and it is a burning issue for several years in health care setting. Pakistan stood on fourth position among TB burden countries.[1] It is globally prevalent chronic disease, caused by *Mycobacterium tuberculosis*, and is present devastatingly in the developing countries like Pakistan. But if accurately diagnosed and properly treated, this disease is quite curable. Accurate laboratory findings with accessibility is the need in the war against TB.[2] In 1993 global emergency was declared on tuberculosis by the World Health Organization. According to the latest estimates included in the "Global Tuberculosis Report" (2013) are that there were 8.6 million new TB cases in 2012 and 1.3 million TB deaths, showing subtle decline in overall ratio.[3] TB is slowly declining but MDR and XDR TB is becoming a serious challenge in the attempt to eliminate TB. In mycobacteriology, smear microscopy is still the most rapid easiest and cheapest procedure with specificity of over 99% (*Mycobacterium*spp). However, the sensitivity of microscopy is not up to the requirement since 25% to 50% smear microscopy of a respiratory specimen provides false-negative results.[4] Thus, where a positive smear test of a respiratory specimen helps in making a presumptive diagnosis of tuberculosis, a negative test does not rule out the disease.[5] However, the good laboratory practice requires the confirmation of any fluorescence microscopy smear-positive results by a ZN stain. Thus, the advantage of the rapid detection of acid-fast bacteria (AFB) by fluorescence microscopy is reduced. The recent re-emergence of tuberculosis has brought the shortcomings of the laboratory diagnosis of tuberculosis to light. In the last few years of the 20th century, the introduction of broth-based cultivation (BACTEC) and molecular biological methods(NAAT

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or PCR) gave birth to significant changes and improvements in the laboratory methods used.[6] However, the authenticity and accuracy of any new method can be assessed only by comparison with conventional techniques currently available in laboratories.[7]

The present study was conducted to compare the efficiency of conventional diagnostic techniques and Insertion Sequence (IS) 6110 based PCR assay for *M. tuberculosis* in pulmonary and extra-pulmonary specimens from tertiary care chest hospital.

MATERIALS AND METHODS

Gulab Devi Chest Hospital, Lahore is 1500 beds hospital giving tertiary health care to patients with all sorts of cardiac and pulmonary diseases, especially tuberculosis. One thousand five hundred and ninety nine samples were collected (based on clinical and radiological findings) during August to January 2013. Significant demographic data were analyzed. All important diagnostic techniques were performed to see the recent trends in TB diagnostics in collaboration with Citilab and Research Centre, Lahore. Sodium hydroxide (modified Petroff) method was practiced to process and decontaminate specimens for culture. Equal volume of 4% NaOH was added to the specimen (at least 2 ml, not more than 5 ml) contained in the centrifuge tube, vortexed and allowed to stand for 15 minutes at room temperature before filling the tube with phosphate buffer (0.067 Mol/liter, pH 6.8; Na_2 PHO_4 9.47g; KH2PO_4 9.07g). The specimens were then centrifuged at 3000g for 15 minutes and the supernatant discarded and deposits were resuspended in approximately 0.2 ml phosphate buffer. Ziehl–Neelsen (ZN) and fluorescence staining technique was performed (Auramine Fluro AFB Colour Medilines). Löwenstein–Jensen medium(Salt Solution (2.4g KH_2PO_4, 0.24g $MgSO_4$ $7H_2O$, 0.6g Magnesium citrate, 3.6g L-Asparagine, Sodium glutamate, Distilled water(600ml) Glycerol (ml) or pyruvate(g) 12 ml or 7.2 g);Egg homogenate1000 ml Malachite green (2%) 20 ml; pH about 6.8) labelled with the ID number and incubated at 37°C for 4 to 8 weeks for the isolation of Mycobacterium tuberculosis.

Extraction of the MTB DNA was done by using liquid samples directly. 130µl of sample was added in each labelled eppondorf followed by 300µl of DNAzol reagent. The mixture was mixed with pipette and waited for 5-10 minutes at room temperature. 200µl of chilled isopropanol in each mixture was added and vortexed for 1 minute. The mixtures were then centrifuged for 5 minutes and each sample was decanted on tissue paper. Then again 300µl dnazol in each sample was added, centrifuged for 5 minutes and decanted on tissue paper. 900µl 70% ethanol was added and centrifuge for five minutes. The pellets were dried at room temperature for 30-40 minutes. After that suspended themin 20µl of de-ionized water.

50µl reaction mixture was prepared which contained a buffer consisting of 50 mM KCL; 2mM MgCL2; 10 mMTris HCL (pH8.3); 200 µM dNTPs, 0.5 µM of each primer, 1.5 U of Taq polymerase (Perkin Elmer Cetus, Norwalk, CO), and 5 µl of extracted DNA from the pulmonary samples. The oligonucleotide primers (Loftstrand Laboratories, Gaithersburg, MD) used were TB1 (5'-GTG CGG ATG GTC GCA GAG AT -3'and TB2 (5'-CTC GAT GCC CTC ACG GTT CA -3'). These primers amplified a target fragment of 538 bp from the insertion-like M. tuberculosis sequence elementIS6110.27. In a thermocycler amplification was done with an initial cycle of denaturation (95ºC for 5 min), 40 cycles of amplification (95ºC for 1 min, 60ºC for 1 min, and 72ºC for1 min), and finally an extension cycle for 5 min. 5µl of the PCR product was loaded in 1.5% agarose gel in 1x Tris-Borate EDTA (TE) buffer and allowed for electrophoresis in a mini gel box for 30 minutes at 120 volts.

RESULTS

A total of 1599 TB cases were processed from August to January (2013) in tertiary care chest Hopital. Out of which 818 were the follow-up and 781 were the TB-suspect cases. On average 163 patients every month visit the laboratory for MDR-TB evaluation and 156 individuals come as TB suspects (Fig. 1). The six month record reveals the mean age of TB patient was 33 years. Three categories of the age of the patients were formed. 18% of follow-ups and 20% of suspects were <20 year in age, 52% follow-ups and 36% suspects were about 20-40 years, and 30% follow-ups and 33% suspects were >40 years of age. 11% of TB-suspects were not aware of their age. It is seen that, among MDR cases (follow-ups) 68% were males and 32% were females. Similarly, among TB-suspects, 58% were males and 42% were females (Table 1).

Table No.1: Tuberculosis prevalence in gender and age

TB Cases (1599)	Gender		Age (years)			
	Male	Female	<20	20-40	>40	Unknown
Follow-up (818)	32	68	18	52	30	-
Suspects (781)	42	58	20	36	33	11

Three hundred suspected cases (168 pulmonary and 143 extra-pulmonary) were processed for ZN, fluorescent microscopy, LJ-culture and PCR. Out of 168 pulmonary samples 147 were sputum and 21 were BAL. On the other hand extra-pulmonary samples include: lymph node 33, Pus 30, plueral fluid 48, urine 12, tissue 17, CSF 2, Pericardial fluid 1. PCR IS 6110wereperformed and positive samples showed a band on 538 bp. PCR results were more positive (91.6%) in pulmonary samples as compared to extra-

pulmonary samples (87.4%). Similarly, all other techniques showed high percentage of positivity in pulmonary suspected cases than exta-pulmonary suspected cases. Among the suspected pulmonary TB group of 168: ZN was positive in 97 (48.2%), Fluorescent microscopy in 129 (79.7%), LJ culture in 89 (52.9%) and PCR in 154 (91.6%). Whereas, in the 143 suspected extra-pulmonary TB group: ZN was positive in 39 (34.95%), Fluorescent microscopy in 50 (45.5%), LJ culture in 57 (39.8%) and PCR in 115(87.4%) [Table 2].

Table No.2: Comparison of ZN staining, Fluorescent Microscopy, LJ culture and IS 6110 PCR

Specimens of suspected tuberculosis		n=311	ZN staining		Fluorescent Microscopy		LJ Culture		PCR	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Pulmonary	Sputum	147	85	62	112	35	78	69	135	12
	BAL	21	12	9	17	4	11	10	19	2
	Positive (%)	168	97 (48.27%)		129 (79.7%)		89 (52.9%)		154 (91.6%)	
Extra-Pulmonary	Lymph Node	33	11	22	15	18	9	24	28	5
	Pus	30	11	19	14	16	9	21	21	9
	Pleural Fluid	48	16	32	20	28	22	26	41	7
	Urine	12	3	9	4	8	6	6	10	2
	Tissue	17	9	8	12	5	9	8	13	4
	CSF	2	-	2	-	2	1	1	1	1
	Pericardial Fluid	1	-	1	-	1	1		1	-
Total	Positive(%)	143	39 (34.95%)		50 (45.5%)		57 (39.8%)		115 (87.4%)	

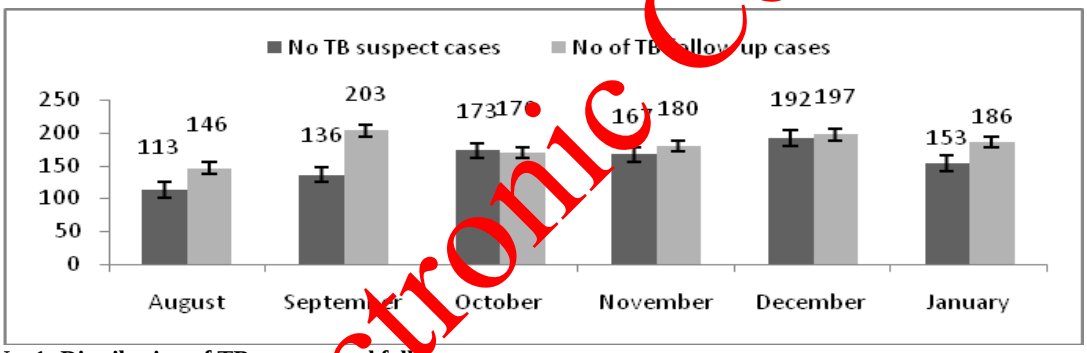

Figure No. 1: Distribution of TB suspect and follow-up cases

DISCUSSION

Many of the TB cases may be treated successfully with the appropriate chemotherapy, but diagnosis remains a huge hurdle to TB elimination. It is well-known fact that conventional methods to diagnose tuberculosis carry several limitations, which have encouraged the use of molecular techniques for AFB diagnosis.[8] Pulmonary and extra pulmonary TB types are different on the basis of the location of the infection which could be inside or outside lungs, respectively.[9] It is great success with PCR in diagnosis of MTB from gastric aspiration.[10] Pulmonary disease is believed to be more common, but extra-pulmonary remains quite severe because of unapparent and nonspecific outcome.[11] Auramine-rhodamine stains (FM) showed better sensitivity for extra-pumonary samples.

Three of the extra pulmonary samples: lymph node, urine and pleural fluid showed positive results for FM staining and so that for the culture, but they were negative for the ZN staining. Here, we found more smear-positive by FM staining. Additionally, in most of the cases ZN staining require two samples for reporting the results one at fasting condition and second after the breakfast or meal but FM is independent of this fact and yield even better results with only one sample. On the sensitive basis FM was observed to be a more cost-effective technique than ZN and applying FM on the sputum specimen.[12] By applying FM we can put on a larger number of patients on regimen which are positive for tuberculosis. This will lead to the better improvement outcomes in the treatment of the tuberculosis. Moreover with FM more patients can be diagnosing in lesser time and more importantly in an efficient way.[13] In case of MDR TB diagnosis, FM also has added advantage as in the progressing months of the treatment the sputum samples yield fewer bacilli which sometimes not detectable by the ZN staining. In general, tuberculosis are diagnosed by conventional methods which include sputum smear microscopy, chest radiographic findings and culture studies.[14] These methodologies prove unproductive due to low

Mycobacterium levels in the specimen or time consuming procedures.[15] Another important technique is nucleic acid amplification based assays are the most appropriate choice for the identification of MTB.[9] These molecular techniques have eliminated diagnostic limitations with better detection rates in smear negative samples with a high degree of sensitivity and specificity in both pulmonary and extra-pulmonary cases.[16] Direct detection of the *M. tuberculosis* from the sample using NAATs (Nucleic Acids Amplification Techniques) is one of the most promising and accurate way to evaluate the presence of the tubercle bacilli.[17] When compare with the culture, these techniques take an advantage of being rapid and free of labor.

CONCLUSION

Fluorescent microscopy is more sensitive than conventional microscopy when compared to culture as the gold standard. In contrast to conventional methods PCR is the most rapid, sensitive and reliable technique for pulmonary and extra-pulmonary tuberculsosis. Furthermore, MTB is still one of the worrisome issue being highly prevalent in this region.

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ACKNOWLEDGMENTS

List of all contributors who do not meet the criteria for Authorship, such as a person who provided purely technical help, writing assistance or department chair who provided only general support. Financial & Material support should be acknowledged.

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