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Editorial

Social Obligation of Medical Colleges and Teaching Medical Students Social Responsibility to Health

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World Bioethics Day 2022 was celebrated on 19th October 2022. The theme was 'Social Responsibility and Health'[1]. This is a reminder for medical schools to emphasize one of the important aspects of Social Responsibility and Health,' i.e. Social obligation of medical colleges and teaching health professions students' social compulsion to health". The medical schools have to develop socially trustworthy and accountable physicians/doctors who volunteer for activities that give contentment to the people and provide socially acceptable healthcare, thereby contributing towards the prosperity of communities[2].

We may ask two questions to ourselves; 1) Are our medical schools producing socially responsible physicians/doctors who engage themselves in activities contributing towards the bliss, well-being, and fortune of their communities? 2) "Are our medical education programs have state-of-the-art curriculums that produce a positive, constructive effect on the well-being of our communities?

We may uncover the answer to both. Our healthcare system must be person-centred, community-centred, focused on universal health coverage. Aiming for universal health coverage and person-centred and community-centred healthcare, we may have to measure our schools against at least three parameters:

1. Retention of doctors in socially deprived parts of the country,
2. Selection of career by the majority of students to become primary care physicians, a basic need of society, and
3. Practice prospects in multi-professional collaborative teams, primarily to manage highly prevalent communicable and non-communicable diseases in the community.

And the medical schools must be aware of healthcare system challenges and places themselves among the essential players to impact healthcare system strategies and plan through functional partnership with the main stakeholders[3].

Now we may focus on the social obligation of medical schools to produce graduates who can efficiently deal with priority health needs and challenges of people and society related to healthcare. The three distinctive modes for accomplishing such a social obligation are:[3]

1. Contributing to equity in healthcare,
2. Involving students in community-based activities, and
3. Engagement with possible employers of their graduates, in the public and private sector, with the anticipation that job prospects are opened in socially-deprived parts with conducive working environments and packages.

Charles B 2016[3] designed "The Social Obligation Scale" in Table I.

Table: The Social Obligations Scale

Scale	Responsibility ⇨	Responsive ⇨	Accountability
Social needs identified	Implicitly	Explicitly	Anticipatively
Institutional/ Educational Objectives	Defined by faculty	Inspired from data	Defined with Society
Educational Programs	Community-oriented	Community-based	Contextualized
Quality of graduates	Good Practitioners	Meeting Criteria of Professionalism	Health system change agent
Evaluation	Process	Outcome	Impact
Assessors	Internal	External	Health Partners

He elucidated the three distinctive categories in social obligation, from social responsibility to social responsiveness and social accountability, against six elements, i.e. social needs identified, institutional/ educational objectives, educational programs, quality of graduates, evaluation, and assessors). In the social responsibility category, good practitioners are being produced, but the medical school will decide the competencies suitable to meet the health needs of patients. Professionals are being made in the socially responsive category, meeting the criteria of professionalism to attain well-defined competencies based on the objective analysis of the healthcare needs of the people in a community. While in the social accountability category, health system change agents are being produced that create a better effect on the functioning and performance healthcare system, thereby on the health status of people, entailing a pursuit of novel practice modalities both for individual and population-based services under the same umbrella [3].

It is difficult to say how many medical schools of a particular country/state belong to one of the three

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mentioned categories as no research data is available. Still, Charles Boelen has his opinion regarding categories of medical school regarding the social obligation scale. He believed in 2016 that 90% of schools might be in the "social responsibility" category, 9% in the "social responsiveness" category and 1% in the "social accountability" category[3]. We perceive that Medical Colleges in South Asia including Pakistan variably falls in social responsibility" category with some compromises on the quality of graduate doctors.

Social accountability is the ability of doctors to respond positively to people's priority health needs and health system challenges to meet such requirements. Social accountability is a robust indicator in health professions education. The whole medical school needs to be dedicated to producing a quantifiable impact on community health, which is the need of the hour[4].

The curriculum must contain a good syllabus to teach social responsibility, social responsiveness and social accountability to the students through interactive teaching/learning and carefully designed assessment tools aligned with the teaching/learning methods and curricular objectives. The faculty must be identified and trained to teach and assess social responsibility, social responsiveness and social accountability component incorporated into the curriculum[2,3].

We must educate and train our students in medical schools with a well-designed curriculum that helps produce socially responsible, socially responsive and accountable physicians/doctors.

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Review Article

Application of Biosensors in Diagnosis of Human Parvoviruses

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ABSTRACT

Human parvovirus B19 is a viral pathogen that causes acute and usually self-limiting disease. Because the B19 virus predicates erythroid progenitor cells, it can cause a transient aplastic crisis in immunocompromised individuals. This infection has been associated with nonimmunologic fetal hydrops during pregnancy; also, B19 can persist for months in immunocompromised individuals. In B19 infection, viremia with a high titer is observed for approximately one week. After that, a specific immune response is critical to control the infection. Although molecular and serologic tests commonly diagnose the B19 virus, laboratory diagnostic tests have limitations. For the detection of human parvovirus B19, an inexpensive, effective, and rapid biosensor may be considered as an alternative.

KEYWORDS: Human Parvovirus B19, Biosensor, Molecular and Serological Diagnosis.

INTRODUCTION

Human parvovirus B19 is an unenveloped and small virus with a linear ssDNA (5 to 6 kb) encoding two capsid proteins (VP2 & VP1) and a single non-structural protein (NS1) required for viral replication[1]. The virus belongs to the Parvoviridae family and is a common human pathogen that can cause asymptomatic infection and various clinical symptoms such as erythema infectiosum (EI), fetal hydrops, transient aplastic crises after infection, and arthropathy in patients[2,3]. Human parvovirus B19 can replicate in erythroid progenitor cells, resulting in B19 infection and viremia with a broad spectrum of human parvovirus B19 titers lasting from a few days to several months[4,5].

Some clinical manifestations, such as arthropathy, anemia, and rash, also occur with other infections; therefore, a differential diagnosis is required to detect the presence of the B19 virus[6]. Many studies have shown that viral DNA or viral proteins can detect B19. Viral DNA is found in many healthy and dysfunctional tissues, suggesting that B19 infection persists in tissues throughout life[1]. On the other hand, the prevalence and transmission of the B19 virus are common worldwide in people of all ages, from children to adults. Transmission occurs via the respiratory tract, blood products with symptoms such as fever, malaise, headache, and myalgia, and also from mother to fetus with severe fetal anemia and fetal death[7-9]. Because B19 infection can spread and break out relatively quickly, control and prevention of the disease is an important issue. However, there are several laboratory diagnoses for B19, and early detection techniques are needed to control the spread of the virus, especially in children, pregnant women, and immunocompromised individuals. A biosensor is a device that can be used for disease detection and diagnosis, as well as environmental, medical, water, and food applications. Viral biosensors enable inexpensive, sensitive, and rapid diagnostic testing[10-12]. Therefore, in this review, a simple and reliable method for the detection of the B19 virus using a biosensor is presented.

Human parvovirus B19 detection methods and limitation

Antibodies, antigens, nucleic acid tests, DNA detection, and B19V cultures are used in laboratories to detect B19 infection (**Table I**)[13]. The most reliable test for acute B19 infection is antibody detection in serum, measuring IgM, which occurs a few days after clinical symptoms, and IgG, a marker of past infection[14]. B19-specific IgA and IgE antibodies have also been detected in human serum and sera but

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cannot be used to diagnose acute infection[15]. Due to the lack of antibody production, DNA detection is routinely used. At a very early stage, DNA detection is performed in the respiratory tract and blood by PCR and in cells and tissues by in situ hybridization[16]. Following the epidemic spread of real-time PCR in clinical laboratories, the novel high-resolution melting (HRM) analysis is being finalized as a rapid, cost-effective, and efficient laboratory method[17]. Antigen detection methods include monoclonal antibodies in EIAs, radioimmunoassays, and immunoblot assays, but they are not sensitive or reliable for detecting acute infections[18].

On the other hand, immunohistology can be a helpful antigen detection method for localizing viral particles in individual host cells in tissues by electron microscopy. Antibodies are used in immunohistochemistry (IHC), immunocytochemistry (ICC), and immunofluorescence (IF) to obtain visual information about the abundance of viral proteins[19]. Apart from these methods, technical developments must be advanced in both directions. First, point-of-care lab-on-a-chip devices are being developed to perform amplification and detection in a single device. Then, a novel array-based assay for B19 detection will be created. As a result, next-generation sequencing methods are being developed to screen for all pathogens present in a sample[20]. It should be noted that most of these laboratory diagnostic tests do not have high sensitivity at low B19 viral loads, and there is no cell culture system in the routine diagnostic laboratory to grow the virus.

Biosensors

Detecting biomolecules is essential in medical fields such as diagnostics and developing new drug molecules. Since Leland C. Clark's first stimulating study on biosensors in 1962, numerous studies have been approved[21]. Biosensors, used in various critical applications such as genetic engineering, sequencing, and disease diagnosis, are machines that identify gas molecules when screening chemical signals in biological cells. In addition, biosensors are potent tools for biohazard screening and fundamental research[22].

Biosensors for pathogen diagnosis are rapidly evolving. The specificity of a biosensor for target analytes determines its success. For analyte detection, a biosensor for viral infection diagnosis requires the effective immobilization of antibodies, aptamers, nucleic acids, or peptides on the surface of a transducer (**Figure I**)[23]. The transducer can convert biological signals into electrical or optical signals. In electrochemical biosensors, the analyte interacts with a sensing layer, resulting in an electrical pulse proportional to the concentration of the analyte. Biosensors are classified into different categories based on signal transduction[24]. Today, voltammetric, conductometric, calorimetric, optical, enzymatic, immunological, piezoelectric, DNA sensors, impedimetric, amperometric, and potentiometric sensors convert sensor data into a measurable signal based on this principle[21]. Incorporating nanoparticles in biosensors improves parameters such as validity, reliability, lower detection limit, retention time, sensitivity, stability, etc. Gold nanoparticles (GNPs) have recently been used in platforms to improve diagnostic sensitivity. GNPs have unique diagnostic properties such as stability, high biocompatibility, unique electronic properties, and fascinating electron transfer potential[25]. Today, there are numerous studies on biosensors for bacterial pathogens but few on biosensors for viral pathogens. Biosensors have been developed for Zika virus[26], swine and avian influenza[27], Ebola virus[28], dengue virus[29], and coronavirus-19[30]. For example, a magnetic bead-mediated surface plasmon resonance (SPR) biosensor platform for HIV-1 protease or viral load in blood has been reported, and a fibre-optic localized surface plasmon coupled fluorescence (LSPCF) biosensor for detection of hemagglutinin protein in influenza virus. A field effect transistor (FET) has also been developed for Ebola virus detection. In addition to these platforms, plasmonic photothermal (PPT) and localized surface plasmon resonance (LSPR) biosensors, in which sensor transduction occurs on a single gold nanoisland (AuNI) chip, are also helpful. Finally, paper-based multiplexed colourimetric (MPBC) sensors are the most popular point-of-care devices[24].

Biosensors for parvoviruses diagnosis

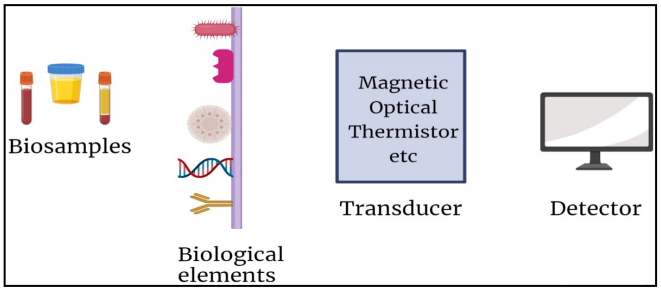
A biosensor is a portable analytical device detecting at least one biological or chemical substance[31]. Biosensors allow the detection of viral diseases in a sensitive, rapid, simple, and inexpensive manner[32]. Biosensors are distinguished according to the type of biological detection element or the type of physicochemical conversion. Depending on the type of transducer, biosensors are divided into optical, piezoelectric, electrochemical, and thermal biosensors[33].

The development of a B19 virus biosensor platform can be divided into three phases; 1) detection of various bioreceptors such as B19 DNA or proteins, human immunoglobulins, and human microRNA (miRNA); 2) hybridization detection methods such as electrochemical, piezoelectric, colourimetric, fluorescent, magnetic, and acoustic technologies, and 3) use of an immobilized bioreceptor such as a DNA probe, ligand, enzyme, antibody/antigen[34].

Table I: Human parvovirus B19 detection methods

		Technique	Time	Sample
Viral diagnosis	Antigen detection	* Counter immunoelectrophoresis * Immuno electronmicroscopy * Radioimmunoassay * Enzyme immunoassay * Blot immunoassay * Receptor-mediated hemagglutination	as soon as possible after the clinical presentation of the diseases	serum, bone marrow aspirates, cord blood samples, amniotic fluid samples and biopsy specimens of the placenta and fetal tissues
	Genome detection	* dot-blot hybridization assay * Microwell hybridization assay * in situ hybridization assay * amplification assays		
Serological diagnosis		* radioimmunoassay * enzyme immunoassay * Immunofluorescence	* IgM antibodies: the second week after viral infection to 4–6 months * IgG antibodies: persist for years	serum

Figure I: Various components of a biosensor for the B19 virus



DISCUSSION

Kim et al. used the quartz crystal microbalance (QCM) biosensor and ProLinkerTM B to rapidly diagnose parvovirus infection in dogs with 95.4% sensitivity and 98% specificity. They used ProLinkerTM B to bind antibodies to a quartz surface coated with gold in a regular pattern and in the correct orientation to attach to the antigen[35]. Mirasoli M et al.[36] developed a miniaturized multiplex biosensor for parvovirus B19 genotyping using a microfluidic oligonucleotide array and lensless chemiluminescence (CL). No cross-hybridizations between B19 genotypes were detected, and DNA-DNA hybridization reactions between sequences with different degrees of homology evaluated the assay's specificity. Another study developed a novel amperometric genosensor to rapidly detect parvovirus DNA from naturally infected dogs in faecal smears. Khatri R et al.[37] developed a biosensor that detects single-stranded genomic DNA (ss gDNA) isolated from a CPV vaccine strain in the 1.0-12.0 ng/l at 25°C for 10 min. Subsequently, the genobiosensor was used to detect CPV viral DNA in faecal swabs from naturally infected dogs. The detection limit of the sensor (LOD) was 1.0 ng/l of faecal viral DNA. In a study by Yamakawa AC 2023[38] AuNPs with antibody deposition were used to identify the presence of CPV-2 in stool samples. It has been demonstrated that AuNPs can be used with monoclonal and polyclonal antibodies, and combining both antibodies with LSPR can provide a reliable diagnosis compared to other molecular methods.

CONCLUSION

Biosensing technologies have been used as novel diagnostic tools for diagnosing viral pathogens. As susceptible instruments, biosensors provide results in a fraction of the time required by conventional methods. Because B19 diagnosis plays an essential role in infection control and public health interventions, developing a biosensor may be a crucial tool in detecting B19 infection; however, further research on parvoviruses is needed in this area.

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AUTHOR'S CONTRIBUTIONS

Hosseini F: Writing Original Draft
Zandi M: Conceptualization of the study
Behboudi E; Writing Original Draft
Salmanzadeh S; Writing Original Draft
Rasooli A: Investigation
Abbasi A: Investigation
Abbasi S: Writing original draft & Supervision
All authors reviewed and approved the final version of the manuscript.

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Original Article

Relationship of Serum Prostate Specific Antigen with Hirsutism in Women Having Polycystic Ovary Syndrome Belonging to the Province of Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

OBJECTIVE: To find the relationship of serum PSA with Hirsutism in PCOS females of Khyber Pakhtunkhwa.

METHODOLOGY: It was a cross-sectional/comparative study. This study was conducted in Khyber Teaching Hospital, Lady Reading Hospital, and Hayatabad Medical Complex from June to December 2021. A total of 172 study subjects were involved in this study. Serum PSA, testosterone and DHEAS levels of 86 newly diagnosed cases of PCOS (Group A) were compared with 86 healthy age-matched controls (Group B). These parameters were estimated using the ELISA method. Distribution of Hirsutism of the study subjects was also established with FGS. SPSS version 21 was used to analyze the data.

RESULTS: The results showed that serum PSA significantly increased (0.325±0.243 Vs 0.119±0.209, P <0.0001) in females with PCOS. Serum total testosterone (1.639±0.773 vs 0.739±0.965, P<0.0001) and DHEAS (3.397±1.243 Vs 2.035±1.203, P <0.0001) levels were also raised. A highly significant positive relationship was seen between PSA with Hirsutism through FGS (r 0-609, P 0.000), testosterone (r 0.352, P 0.000) and DHEAS levels (r 0.432, P 0.000)

CONCLUSION: This study observed significantly high PSA correlating positively with Hirsutism in females with PCOS.

KEYWORDS: Prostate Specific Antigen, Hirsutism, Polycystic Ovary Syndrome

INTRODUCTION

PCOS is a hormonal problem of childbearing age in females; roughly 5-6% of females are affected globally. The exact cause of PCOS is unknown; however, multiple factors can lead to this condition, such as family history, obesity, and insulin resistance[1]. According to the Rotterdam Consensus (2003-2004), PCOS is determined by the presence of at least two of the following features: i) Hyperandrogenism, clinical/ biochemical, ii) polycystic ovaries on ultrasound and iii) menstrual dysfunction including anovulation (no menses for more than three months) and oligomenorrhea (infrequent menses >35 days)[2]. Other causes of androgen excess, including Cushing's syndrome, androgen-secreting tumors, hyperprolactinemia and congenital adrenal hyperplasia, should be excluded. PCOS diagnosis is somewhat complicated for several reasons, such as menstrual irregularities in females, anovulatory cycles, especially in young girls, intricacy in explaining clinical features and biochemical indices of hyperandrogenic status and ambiguity concerning the importance of the ovarian cysts morphology[3, 4].

A prostate-specific antigen is a 33KD glycoprotein initially thought to be only secreted by a male prostate gland. Later studies showed that PSA is also produced in the female body by several different tissues like breast tissue, endometrial tissue, periurethral glands like skene glands and parotid glands[5]. PSA in normal females is <0.1ng/ml, which is markedly raised if there is a state of Hyperandrogenism like PCOS[6]. In this condition, androgen levels, such as testosterone, are high in females. Ovaries and adrenal glands produce androgens in PCOS due to faulty LH and FSH discharge because of defective gonadotropin-releasing hormone (GnRH) secretion[7]. LH causes theca cells of ovaries to produce more than normal androgens. Several studies show ovarian androgen synthesis in PCOS is more than in adrenal androgens. Hirsutism, acne, voice changes, hyperpigmentation and androgenic alopecia, characterize Hyperandrogenism. Hirsutism is the most disturbing psychosocial problem among females[8]. Hirsutism is the appearance of thick, course hair on unwanted body areas, in a pattern more common in males. Androgens have inconsistently diverse outcomes on hair follicles, varying according to body site[9].

Hirsutism is graded using the gold standard Ferriman Gallwey Scoring method[10]. The modified system

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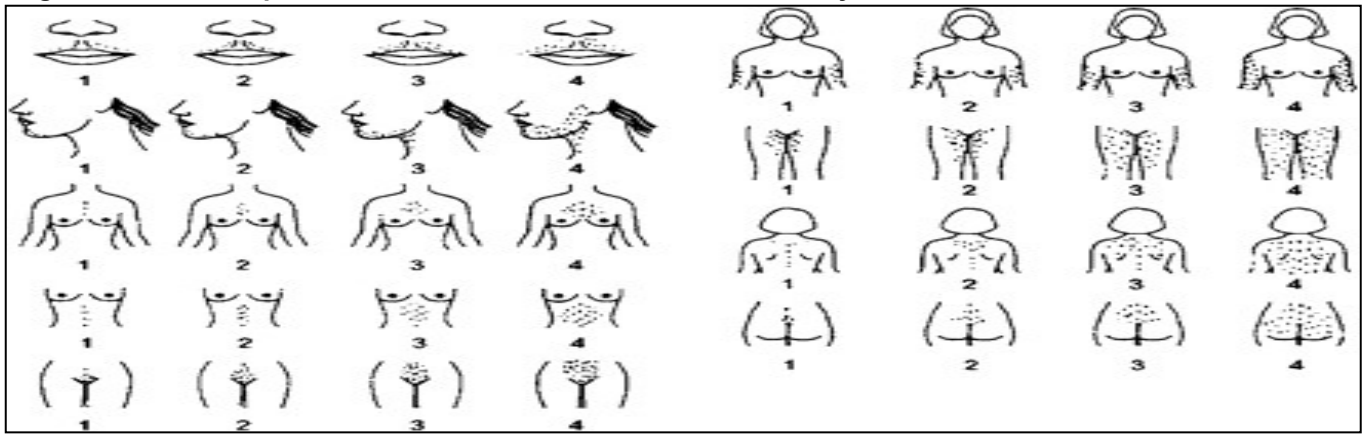
Figure I: Detailed representation of the modified Ferriman Gallwey Score



suggests examining nine body parts for hair growth. These include; i. upper lip, ii. chin iii. chest iv. upper abdomen v. lower abdomen vi. upper back vii. lower back viii. forearm and ix. Thigh[11]. Every part is given a maximum of 4 points and a minimum of zero. So, the maximum score is 36, and the minimum is zero. A score of 0-8 is considered normal, mild Hirsutism is 9 to 15, 16 to 25 is moderate Hirsutism, and severe Hirsutism is when the score is above 25[12].

However, Hirsutism may vary by ethnicity, and all other causes of Hirsutism, like hypertrichosis and idiopathic Hirsutism, should be excluded.

The study aims to evaluate the relationship between serum PSA levels with hirsutism status in PCOS females of Khyber Pakhtunkhwa.

METHODOLOGY

This cross-sectional, comparative study was conducted in the outpatient department (OPD) of Khyber Teaching Hospital, Lady Reading Hospital and Hayatabad Medical Complex, Peshawar, the three tertiary-level hospitals of Khyber Pakhtunkhwa from June 2021 to December 2021. One hundred seventy-two participants were involved in this study, separated into two groups; 86 cases (Group A) and 86 controls (Group B).

The cases group included newly diagnosed patients of PCOS, and the control group included healthy age-matched females with no major/chronic illnesses like diabetes mellitus, thyroid disease, and hypertension. Consent of all participants was taken. Data were taken and maintained on a well-designed questionnaire, including name, age, marital history, menstrual history, and obstetrical history. Clinical signs of Hyperandrogenism, including acne, pigmentation and Hirsutism, were checked. Hirsutism was scored according to the Ferriman Gallwey scoring system. Pelvic ultrasounds of all the study subjects were done. 5ml of blood was taken, and serum was collected by centrifuging at 3500 rounds per minute for 5 to 10 minutes in Eppendorf tubes, properly labelled and stored for later use at -20°C.

Biochemical analysis

The blood serum was tested for total PSA, testosterone and DHEAS levels. PSA was determined using the principle of sandwich enzyme-linked immunosorbent assay using a Calbiotech ELISA kit. Total testosterone was defined using Calbiotech ELISA kit no.TES5663 is based on the principle of solid phase competitive ELISA. The level of DHEAS was determined using Demeditec kit no.DEH3366, based on the principle of competitive ELISA.

Statistical analysis

We analyzed all data using SPSS and expressed the results as Means and Standard Deviation. The quantitative variables between the study groups were compared with the independent students' t-test. The relationship between PSA and other parameters was established with Pearson's correlation coefficient r.

RESULTS

Cases presented with a mean age of 25.24±4.395 years and mean BMI of 32.82±2.04 are shown in **Table I**. It can be seen that there was a significant difference in the mean BMI of cases and control with a p-value <0.0001.

Table I shows a statistically raised serum total PSA level in cases than in controls (0.325±0.243 Vs 0.119±0.209) with a p-value of 0.0001. Significantly higher levels of total testosterone (1.639±0.773 vs 0.739±0.965, p-value <0.0001) and DHEAS (3.397±1.243 Vs 2.035±1.203, p-value <0.0001) were observed in cases than controls. FGS shows that Hirsutism is statistically higher in cases than in control (p-value <0.0001).

Table II shows the relationship of serum PSA levels with other parameters. The association was established using Pearson's correlation coefficient r, and a P-value less than 0.05 was considered significant. A highly significant positive relationship is seen between PSA with testosterone (r 0.352, P 0.000) and DHEAS levels (r 0.432, P 0.000). A statistically significant and positive relationship was observed between PSA levels and FGS (Hirsutism) with r 0.609 and p 0.000.

Out of 86 cases, 70 subjects were obese, having BMI >30; 8 cases had borderline obesity with a BMI

between 25 & 30, and 8 cases were non-obese, having a BMI <25. While in the control group, 69 participants were non-obese, and 17 had borderline obesity.

Figure II shows the status of Hirsutism in the study groups. Out of 86 cases, 34 females had got mild Hirsutism (FG between 9 and 15), 33 females had moderate Hirsutism (FG between 16 and 25), 11 females had got severe Hirsutism (FG >25), and 8 cases did not have Hirsutism.

Table III shows the relationship of serum PSA with Hirsutism in cases according to the mild, moderate and severe groups. The PSA levels are significantly higher among the groups according to the severity of Hirsutism, further strengthening the positive association of PSA with Hirsutism in females with PCOS.

Table I: Comparison of clinical and biochemical characteristics between Group A and Group B

Variables	Group A (Cases) Mean ± SD	Group B (Control) Mean ± SD	P value
Age	25.24±4.395	25.860±4.229	NS
BMI	32.82±2.04	24.60±4.35	<0.0001
PSA	0.325±0.243	0.119±0.209	<0.0001
Testosterone	1.639±0.773	0.739±0.965	<0.0001
DHEAS	3.397±1.243	2.035±1.203	<0.0001
FGS	18.9±4.9	4.1±2.1	<0.0001

**P-value significant <0.05, NS not significant, PSA ng/ mL, Testosterone ng/mL, DHEAS µg/mL*

Table II: Correlation of PSA with Different Parameters in Study Groups

Parameters	r	p
Age	-0.093	0.143
Testosterone	0.352	0.000**
DHEAS	0.432	0.000**
FGS	0.609	0.000**

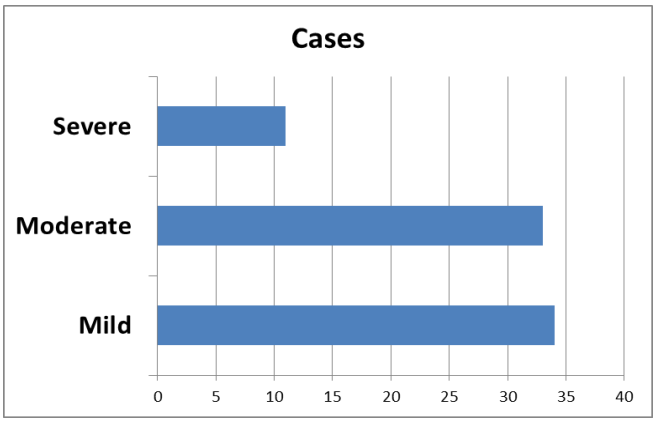
Table III: Relationship of serum PSA with a degree of Hirsutism in cases

PSA in Mild Hirsutism	PSA in Moderate Hirsutism	P-value
.104±.05	.203±.14	<0.0001
PSA in Moderate Hirsutism	PSA in Severe Hir-sutism	
.203±.14	.281±.17	<0.003

DISCUSSION

PCOS is associated with Hyperandrogenism causing Hirsutism, acne, obesity and menstrual irregularities[13]. The hyperandrogenic state in PCOS is associated with augmented production of luteinizing hormone by theca cells which causes increased DHEAS and testosterone levels in the body. Various studies have reported increased expression of the PSA gene in hyperandrogenic states (like PCOS), leading to increased serum PSA levels[14-16]. Female sources of circulating PSA are unclear; however, it may act as a reliable biomarker of the biological action of androgen in females[6,17]. Some studies have suggested the diagnostic role of PSA level in female colorectal and breast cancer[18,19]. Due to the lack of sufficient relevant research, the mechanism of PSA in the pathophysiology of PCOS remains to be elucidated to date.

Figure II: Description of Hirsutism in Cases



Vural B 2007[20] reported significantly raised serum PSA, testosterone and dehydroepiandrostenedione in PCOS cases. In a study conducted by Ibrahim WW 2016[21], a positive relationship between PSA with Hirsutism and DHEAS was observed. Similar results were observed in the present study. The results of Uknic K 2009[22], Rudnicka E 2016[23], and Tokmark A 2018[24] are consistent with the current research. Uknic K 2009[22] has recommended the diagnostic use of PSA in PCOS with high specificity, sensitivity and accuracy based on their outcomes. Similar results have been reported by Nagaraj S 2019[25] and Bhat K 2019[26].

The present study compared serum concentrations of PSA, testosterone and DHEAS and clinical evidence of Hirsutism in PCOS cases with healthy age-matched controls. This is a novel study in people of this area, which may show PSA as a more logical and sole biomarker of hyperandrogenic activity in PCOS females, reducing the health system's financial burden.

CONCLUSION

It is concluded that in PCOS, the level of androgens like DHEAS and total testosterone is raised, which in turn may cause the PSA levels to be increased than normal. Furthermore, Hyperandrogenism and raised PSA levels are also related to Hirsutism status in PCOS. Limitations of the study include its relatively small sample size. Thus, future large-group studies are recommended to investigate PSA's potential diagnostic and prognostic role in PCOS cases.

Ethical Permission: Khyber Medical College Peshawar, ERC letter No. 762/DME/KMC.

Conflict of Interest: No conflicts of interest.

Financial Disclosure / Grant Approval: No funding agency was involved in this research.

Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Khattak N: Design, acquisition, analysis, and interpretation of data
Durrani S: Design, acquisition, analysis, and interpretation of data
Ali S: Design, acquisition, analysis, and interpretation of data
Tariq K: Design, acquisition, analysis, and interpretation of data
Ur Rahman U: Design, acquisition, analysis, interpretation of data
Khan MA: Design, acquisition, analysis, interpretation of data

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Original Article

Frequency of Urinary Tract Infection, Microbial Patterns and Drug Resistance in Diabetic Patients in a Tertiary Care Unit

Salma Razzaque[1*], Ajeet Kumar[1], Asad Ahmed Khan[1], Masooma Abid[1], Syeda Seemin Raza[1], Ramsha Eraj[1]

ABSTRACT

OBJECTIVE: To determine the frequency of UTIs in people with diabetes and identify local bacterial flora and antibiotic resistance.

METHODOLOGY: An observational, analytical, cross-sectional study was conducted at the Medicine Ward of Jinnah Medical and Dental College Hospital (now Sohail Trust Hospital) from January-December 2020. A convenient sampling technique was applied, and the sample size was 132. Diabetic patients >18 years of age were included. Data was collected on a structured questionnaire. A urine sample was sent for culture sensitivity. Any growing flora and their sensitivity/resistance to antibiotics were recorded. All the data were analysed using SPSS version 21.

RESULTS: UTI was found in 31(23.5%) patients. Female gender, insulin therapy, and lower creatinine clearance were related to UTI, as shown by significant p values. Most common flora was E. Coli 26(84%), followed by Klebsiella 4(13%) and S. aureus 1(3%). E.Coli was 100% resistant to 3rd generation cephalosporins, 83.9% resistant to quinolones and 67.7% resistant to Penicillin (amoxicillin/clavulanic acid) and 43% resistant to aminoglycosides. Klebsiella was 100% resistant to Penicillin and quinolones and 85% to 3rd generation cephalosporin. E. coli and Klebsiella were 100% sensitive to carbapenems, nitrofurantoin and Fosfomycin, whereas S. Aureus was resistant to cloxacillin, clindamycin and clindamycin and quinolones, while sensitive to vancomycin., linezolid and aminoglycosides.

CONCLUSION: UTI was found in 23.5% of our diabetic patients; the most common organisms prevalent were E. Coli, Klebsiella and S. aureus, which was primarily resistant to Penicillins, Cephalosporins and quinolones while sensitive to nitrofurantoin, Fosfomycin and carbapenems.

KEYWORDS: Diabetes Miletus, Urinary Tract Infection, E. Coli, Antibiotic Resistance, Aminoglycosides, Quinolones.

INTRODUCTION

Diabetes Miletus is ranked as the fourth most cause of death worldwide[1]. The prevalence of diabetes in Pakistan is reported to be one in four adults (26.7%), the highest national prevalence in the world. In 2021, 33 million adults in Pakistan are living with diabetes – a 70% increase since 2019. Pakistan has the third highest number of people living with diabetes globally, after China (141 million) and India (74 million). In 2021, diabetes was responsible for 400,000 deaths in the country– the highest number in the Middle East and North Africa Region[2].

Diabetes is a multi-system disease affecting all significant organs, i.e. heart, brain, eyes and kidneys. It also increases the chances of certain infections like pneumonia, skin infections, foot ulcers and urinary tract infections. The incidence of repeated UTIs in people with diabetes is growing daily, especially in developing countries, because of a lack of diabetic control, poor hygiene and injudicious use of antibiotics. Thus, the high prevalence of diabetes is a significant burden on the health structure of the country[3].

Diabetes mellitus is a recognised risk factor for urinary tract infection (UTI). In Pakistan prevalence of UTI in people with diabetes ranges from 13-35% [3,4]. Multiple factors contribute to this high prevalence, ranging from poverty, lack of access to healthcare facilities, the self-prescription of antibiotics by the general population, and the over-prescription of antibiotics by healthcare workers without culture/sensitivity. All of this has contributed high prevalence of UTI in the diabetic population, which unfortunately leads to drug-resistant bacteria that were previously responsive to conventional antibiotics, especially beta-lactams and fluoroquinolones[5-7].

Data regarding UTI in diabetes has been studied in many countries. Most studies found gram-negative rods, Escherichia coli, proteus, Klebsiella, coagulase-negative staphylococci, enterococci, and pseudomonas, but there was marked variation in resistance patterns in different areas of the world. Data regarding drug sensitivity patterns is still limited, especially in the Asian population, with a high

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prevalence of diabetes. From the existing data, resistance to the conventionally used anti-biotics fluoroquinolones, penicillins, cephalosporin and aminoglycosides is constantly increasing, so there is a need to search for drug sensitivity patterns at the hospital level. It will help to find an alternative to these antibiotics[8-10].

Therefore, the rationale of this study was to help find local sensitivity patterns since, in clinics, it is not always possible to obtain a culture and sensitivity report for each case of UTI, especially the repeated one, because of financial constraints. Moreover, there is not enough information about the relationship between diabetic control (HbA1C), use of insulin and creatinine clearance on the occurrence of UTI. Our study aimed to fill these gaps in previous studies by identifying local bacterial and antibiotic resistance and assessing the effect of diabetic control, treatment and renal functions.

The objective of our study was to determine the frequency of Urinary tract infections in diabetic patients visiting the Medical ward and OPD of Sohail Trust Hospital and to assess the effect of age, gender, duration of diabetes, HbA1c, use of insulin/oral antidiabetics and creatinine clearance on the occurrence of UTI. We also identified the typical flora and their sensitivity and resistance to significant antibiotics, i.e. Penicillin, cephalosporin, Fluoroquinolones, Aminoglycosides, Carbapenems, Nitrofurantoin and Fosfomycin.

METHODOLOGY

It was a single-centred descriptive cross-sectional observational study conducted at the Department of Medicine, Sohail Trust Hospital Karachi (Jinnah Medical College Hospital) Korangi Karachi. The study was conducted from January - December 2020. The Convenient sampling technique was used for sample collection. All the patients of either gender admitted to the medicine ward or visiting medical OPD for treatment of diabetes, aged between 18-70 years, with a duration of diabetes mellitus for at least six months, were included. Diabetes mellitus was defined according to criteria of the American Diabetic Association as having Fasting blood glucose >126 on two occasions or HbA1C > 6.5 [11]. Patients with any structural abnormality of the urinary tract(Strictures, stones), using steroids or immunosuppressant drugs, antibiotics, and pregnancy are excluded since they are independent risk factors that can increase the prevalence of repeated and resistant urinary infections. The sample size was 132, calculated online using WHO sample size software, with a 95% confidence interval and a 5% chance of error[10]. Before starting the study ethically approved by the hospital URGC (Undergraduate Research Committee (Protocol I #: 00055/20). Researchers themselves collected data on a pre-designed questionnaire. Before the enrolment, participants were explained the details of the study and utilisation of data, the informed consent was taken from all the patients. The patients were asked simple yes/no statements or short answer questions in simple and understandable language. Qualitative variables were gender, presence of symptoms, previous H/O UTI, treatment (Insulin/Oral Antidiabetics), bacterial flora, and drug sensitivity, and quantitative variables were age, HbA1C, duration of diabetes, and creatinine clearance. After initial data collection, urine samples were sent to the hospital laboratory for D/R and culture sensitivity.

Samples were cultured on Blood agar and MacConkey agar. Antibiotic Susceptibility pattern was determined on Mueller-Hinton using Kirby –Bauer disc diffusion method. Urinary tract infection (UTI) is diagnosed with a positive urine culture with or without symptoms of UTI (dysuria, frequency and urine urgency). After collecting urine culture reports, any growing flora and their sensitivity/ resistance to all the effective antibiotics were recorded in the Performa. Data were analysed in SPSS version 21. Qualitative data are presented as frequencies/percentages, while quantitative data are presented as mean ± SD. The student T-test was applied to quantitative data, while the chi-square test was used to qualitative data. A P-value of < 0.05 is considered statistically significant.

RESULTS

UTI was found in 31(23.5%) diabetic patients; in comparison, 101(76.5%) patients had diabetes without UTI (**Figure I**). In comparing general characteristics between UTIs and non-UTI groups, we observed that females were more affected by UTI than males (p-value=0.02). Patients on insulin had a lower percentage of UTI than those on oral therapy (p value=0.045). HbA1C was also high in the UTI group (p-value = 0.042). Average Creatinine Clearance decreased in patients with UTI, as shown by significant p values (0.022). While the relationship between age, duration of diabetes and presence of symptoms was not found to be statistically significant (**Table I**)

In the culture/sensitivity report, the most common flora was E. Coli 26(84%), followed by Klebsiella 4(13%) and S. aureus 1(3%) (**Table II**).

E. coli was 65.3% resistant to Penicillin (amoxicillin/ clavulanic acid), 100% resistant to 3rd generation Cephalosporins, 84.6% to Quinolones and 42.3% to aminoglycosides. Carbapenems, Nitrofurantoin and Fosfomycin were 100% sensitive for E. coli. Klebsiella was 100% resistant to Penicillin and quinolones, 75% resistant to 3rd generation cephalosporin and 25% resistant to aminoglycosides while 100% sensitive to carbapenems, nitrofurantoin and Fosfomycin. (**Table III)**

S. Aureus isolated in only one sample was resistant to cloxacillin, clindamycin and quinolones while sensitive to vancomycin, linezolid and aminoglycosides. **(Table III)**

Figure I: Frequency of UTI

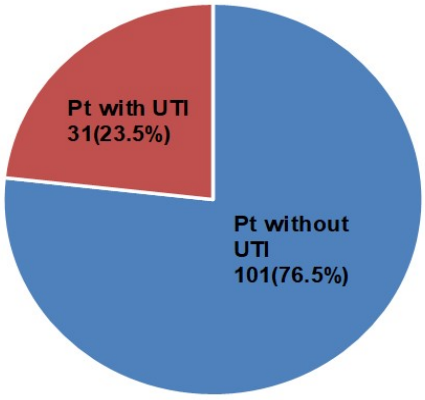

Table I: Demographic Characteristics of the Patients

Characteristics	Pt with UTI (31)	Pt without UTI (101)	P-value
Mean Age	56.74±12.85	52.60±12.43	0.110
Male Female	7 (22.6%) 24 (77.4%)	42 (41.6%) 59 (58.4%)	0.028
Mean duration of diabetes	10.71±6.07 year	9.40±6.16 year	0.301
Presence of symptoms (frequency, urgency)	12(38.7%)	32(31.7%)	0.303
On Insulin	14(45.2%)	63(62.4%)	0.045
HbA1c	9.28±1.88	7.63±2.08	0.042
Average Creatinine clearance	114.3±13.8	106.8±20.4	0.022

Table II: Frequency of Isolated Flora

Flora	N(%)
E. COLI	26(84%)
Klebsiella	4(13 %)
S. aureus	1(3%)

Table III: Frequency of Antibiotic Resistance

Flora	Antibiotic Groups	Resistance	Sensitive
E. COLI	Penicillin(Augmentin)	17(65.3%)	9 (34.6%)
	Cephalosporins (Ceftriaxone)	26 (100%)	0(0%)
	Quinolones	22 (84.6%)	4 (15.3%)
	Aminoglycosides	11(42.3%)	15(57.6%)
	Carbepenems (Imipenem)	0(0%)	26(100%)
	Nitrofurantoin	0 (0%)	26 (100%)
	Fosfomycin	0 (0%)	26 (100%)
Klebsiella	Penicillin(Augmentin)	4(100%)	0(0%)
	Cephalosporins (Cftriaxone)	3(75%)	1(25%)
	Quinolones	4(100%)	0(0%)
	aminoglycosides	1(25%)	3(75%)
	carbepenems (Imipenem)	0 (0%)	4 (100%)
	nitrofurantoin	0 (0%)	4 (100%)
	Fosfomycin	0 (0%)	4 (100%)
S. AUREUS	Penicillin (Cloxacillin)	1 (100%)	0 (0%)
	Clindamycin	1(100%)	0(0%)
	Quinolones	1(100%)	0(0%)
	Aminoglycosides	0 (0%)	1 (100%)
	Linezolid	0(0%)	1(100%)
	Vancomycin	0 (0%)	1 (100%)

DISCUSSION

Diabetes mellitus is a recognised risk factor for urinary tract infections. The incidence of repeated UTIs in people with diabetes is increasing daily, especially in developing countries, because of a lack of diabetic control, poor hygiene and injudicious use of antibiotics. In our study, we found the prevalence of UTI in people with diabetes in about 31(23.5%) patients; the other studies of the region have shown variable results ranging from 8-54%. Laway BA 2021[11] conducted a study in Kashmir and found 17% of patients had active UTIs. Ahmed S 2020[12] conducted a study in Peshawar and found 51% had culture-positive UTIs. Kumar R et al.[13] conducted a study in Sind, Pakistan, and found that 13% of the samples were culture-positive. This variation in the frequency of UTIs can be due to hygienic conditions, literacy, awareness in the community, poverty, access to healthcare facilities and diabetic control. That is why regional and hospital-based studies are essential sources to determine the local data about infection.

We also noted in our study that the occurrence of UTI was seen in females with low creatinine clearance and the use of Oral antidiabetic medications. Kande S 2021[14] conducted a study in 2021 in India and also observed that the incidence of UTI was more common in female diabetics and in patients with HbA1c of > 9%. Jha PK 2014[15] conducted a study in Nepal and observed a significant relationship between HbA1c and insulin with a low incidence of UTI.

Klinberg A et al.[16] and Raeispur M 2018[17] also observed that a high incidence of UTI was associated with elevated serum creatinine and HbA1c.

Our drug sensitivity data showed that E. coli, Klebsiella, and S. aureus were the most common organisms. We observed that both gram negatives were resistant to commonly used antibiotics like beta-lactam/lactamase (penicillins, Cephalosporins), fluoroquinolones and aminoglycosides, most of which are widely prescribed for treating UTI in general

practice. We observed that they were sensitive to Nitrofurantoin, Carbapenems and Fosfomycin. This changing trend shows the injudicious use of antibiotics, especially without drug sensitivity, leading to an alarming rate of resistance in gram-negative bacteria. The other studies in the subcontinent also show similar results. Forson AO et al.[18] conducted a study in 2020 that found that E.coli and Klebsiella were highly resistant to cefuroxime, ampicillin and gentamycin.

Nath T 2021[19] conducted a study in India that found high resistance in E.coli, Klebsiella and enterococcus to fluoroquinolones, aminoglycosides and co-amoxyclav.

Keshi L et al.[20] and Woldemariam HK et al.[5] also observed multi-drug resistance in gram-negative related urinary tract infections.

Our study observed that E.coli and Klebsiella were both sensitive to Carbapenems, nitrofurantoin and Fosfomycin, while staphylococcus was sensitive to vancomycin, linezolid and aminoglycoside. Nigussie D 2017[21] conducted a study in Turkey and observed that most gram-negative were resistant to aminoglycoside and ceftriaxone but sensitive to nitrofurantoin. A recent study conducted in India also showed high sensitivity of gram-negative infection for Carbapenems and nitrofurantoin while staphylococcus for vancomycin and linezolid[23].

Nongrum S et al.[23] and Yismaw G 2012[24] documented similar results in their studies supporting the same sensitivity pattern.

CONCLUSION

UTI was found in 23.5% % of our diabetic patients. The most common organisms prevalent were E. Coli, Klebsiella and S. aureus, which was primarily resistant to Penicillins, Cephalosporins and quinolones while sensitive to nitrofurantoin, Fosfomycin and carbapenem. This emerging resistance should be monitored frequently to observe any extension in resistance as we are recently experiencing extensive drug resistance cases of typhoid in Sindh. More studies with frequent monitoring in multiple centres are therefore recommended.

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AUTHOR'S CONTRIBUTION

Razzaque S: Major contribution in conceptualising the idea, study design, editing and supervision of the study process

Kumar A: Contribution in conceptualising the idea, study design, editing and supervision of the study process

Khan AA: Major contribution to the research proposal, data analysis, and manuscript writing.

Abid M: Major contribution to data collection and data analysis

Raza SS: Major contribution to patient selection, coordination and data collection.

Eraj R: Major contribution to data collection and writing the manuscript

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Original Article

Association of Dry Eye Disease and Diabetic Retinopathy with Glycated Hemoglobin at a Tertiary Care Unit of Karachi Pakistan

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ABSTRACT

OBJECTIVE: To investigate the association of dry eye disease and diabetic retinopathy (DR) with glycated haemoglobin at a tertiary care unit in Karachi, Pakistan.

METHODOLOGY: This cross-sectional observational study was conducted at Baqai Institute of Diabetology and Endocrinology, Baqai University Karachi, Pakistan, from July to December 2020. A total of 238 subjects having type 2, type 1, and gestational diabetes mellitus participated in this study. They went through a routine ophthalmic examination, breakup tear film time (BUT) test, Schirmer I test, staining fluorescein, and fundus photography performed to diagnose the DR. Baseline detail and biochemical parameters were recorded. Data analysis was done on statistical packages for the sciences social (SPSS) 20 version.

RESULTS: A total of 72 participants had dry eye disease (DED) conforming to a total prevalence of 30.25%; 11(4.6%) had severe dry eyes, 26(10.9%) had moderate dry eyes, 35(14.7%) had mild dry eyes whereas, 166(69.7%) participants had normal eyes. There was no significant association between gender, index mass body (BMI), smoking habits, history of family diabetes, and duration of Diabetes with DED. The frequency of diabetic retinopathy (DR) was registered as 23.5%; 29(24%) males and 27 (23.1%) females, respectively.

CONCLUSION: Overall, a 30.3% frequency of dry eye in diabetic individuals was observed. It should improve by having consistent follow-ups after three to six months, providing a distinct difference in the condition compared to the non-affected individuals.

KEYWORDS: Dry eyes, ocular surface disease, Dryness in the eyes, deficiency tear, lacrimal function unit, Schirmer's test, Diabetes.

INTRODUCTION

Worldwide, Diabetes is a rising trend, and it is projected by the Diabetes International Federation (IDF) that around 537 million folks are surviving with Diabetes, with predictions expected to upsurge above 643 million individuals by 2030[1]. In Pakistan, Diabetes is now known as a chronic disease due to the adaptation of Westernized diets and various changes in lifestyle and demographic features. According to the recent second National Pakistan Diabetes Survey (NDSP) 2016-2017, Diabetes has affected 26% of the country's population, which is alarming and a significant public health issue[2]. Diabetes, if not under reasonable control, can cause chronic complications that include retinopathy, nephropathy, neuropathy, and heart and vascular diseases.[3]

Blindness's major cause is DR in developed and underdeveloped countries[12]. The prevalence of diabetic retinopathy (DR) in Pakistan was 28.78% [4]. DR itself is a leading cause of blindness, and dry eye disease (DED) aggravates vision loss which usually may lead to poor quality of life[5]. DED has corneal complications such as superficial keratopathy punctuates, trophic ulceration, tear film hyperosmolarity, instability, neurosensory abnormalities, and persistent epithelial defects[6]. Hyperosmolarity and instability tear film are the procedures that cause dry eyes with Diabetes[7]. Tear film abnormality is a significant diabetic feature of the ocular disease surface due to low quality and tear functions that occur with the subnormal ocular surface[8].

Dry eyes are commonly observed in the age group between 60-70 years[9]. It's because aging results in acinar degeneration and nuclear abnormalities that entirely change the Lacrimal unit's function. It has also been reported that male gender, prolonged Diabetes, and elevated glycated haemoglobin (HbA1c) levels were positively linked to greater severity of DR[10]. A

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direct relationship was also found in an investigation between HbA1c levels and the prevalence of DED. The greater the HbA1c levels, the more likely to acquire dry eye conditions[6].

Early diagnosis of dry eyes has now become necessary for clinicians to determine the associated factors and to prevent visual acuity with early treatment. With the scarcity of data from this globe, we aim to assess the association of dry eye disease and diabetic retinopathy (DR) with glycated haemoglobin at a tertiary care unit in Karachi, Pakistan.

METHODOLOGY

This cross-sectional study was conducted at Baqai Institute of Diabetology and Endocrinology (BIDE), Medical Baqai University (BMU), Karachi – Pakistan. The study period was from July to December 2020; ethical approval was obtained from BIDE's Institutional Review Board (IRB). Each study subject was given a detailed methodology description, and written and verbal consent was obtained. Both males and females with known type 2 diabetes mellitus (DM), type I DM, and gestational DM, aged between 35-70 years, were included. Subjects with other types of DM and those taking other than Diabetes medications which can affect the production of tears were omitted from the study. Subjects with previous ocular surgery history, palsy Bell's person, Sjogren's syndrome, rheumatoid arthritis, or Parkinson's disease were also excluded.

Type II DM subjects were selected using a random sampling technique. Baseline details were obtained on a predesigned questionnaire. It includes diabetes duration, types of Diabetes, gender, age, smoking habit, alcohol habit, Diabetes family history, height, weight and blood pressure. Recent haemoglobin (Hb) A1c value and lipid profile levels were recorded from BIDE's hospital management system (HMS). A routine ophthalmic examination was done, which included a tear film breakup time (TBUT) test, Schirmer test, fluorescein staining and fundus photography for DR. Dry eyes were suspected based on a history of ocular discomforts such as soreness, gritty sensation, itchiness, redness, excessive watering of the eye, and blurred vision that improve after blinking. Subjects underwent lamp slit examination of surface ocular staining dye pattern with stain fluorescein. For the confirmation of the condition, a strip Schirmer test was made. The strip was placed at the middle junction and the third lateral of the lower eyelid, and the amount of wetting was measured after 5 minutes. The condition was graded on the scale: 0-5 mm; severe dry eye, 5-10 mm; moderately dry eye, 10-15 mm; mild dry eye, and ≥15 mm; normal tear function[11].

The glycemic index was targeted as HbA1c <7% (good glycemic control) and >7% (bad glycemic control)[12]. The subject was considered dyslipidemic if having a serum total cholesterol >200 mg/dl, serum LDL- cholesterol >130 mg/dl, serum HDL- cholesterol <40 mg/dl (for males) and <50 mg/dl (for females), and serum triglycerides >150 mg/dl [13]. Height was measured by directly measuring the length from the bottom of the feet to the highest point of the head. Weight was measured in kilograms by a weighing machine. BMI was calculated by dividing weight (kg)/ height $(m)^2$. BMI <23 kg/m^2 was considered normal, ≥23-24.9 kg/m^2 overweight and ≥25 kg/m2 obese per Asia Pacific Guideline[14]. Blood pressure was measured in a sitting position after 10 minutes of rest using a mercury sphygmomanometer. Hypertension was considered if the subject had blood pressure ≥ 140/90 mmHg[15].

Statistical analysis

Data was analyzed on Statistical Packages for Social Sciences (SPSS) version 20. Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were presented as numbers (percentages). The chi-square, ANOVA, and Kruskal-Wallis tests were applied where applicable to determine the association between variables.

RESULTS

A total of 238 individuals were assessed. **Table I**

Table I: Baseline characteristics of studied subjects

Variables		N (%) or Mean ± S.D
N		238
Gender	Male	121(50.8%)
	Female	117(49.2%)
Age (years)		50.52±11.85
Marital status	Single	13(5.5%)
	Married	214(90.7%)
	Widow	9(3.8%)
Body mass index (kg/m^2)		28.94±5.28
Systolic blood pressure (mm Hg)		124.15±18.51
Diastolic blood pressure (mmHg)		77.43±9.41
Type of Diabetes	Type 1	3(1.3%)
	Type 2	234(98.3%)
	Gestational	1(0.4%)
Smoking habit	No	169(81.3%)
	Ex-smoker	19(9.1%)
	Current smoker	20(9.6%)
Alcohol addiction	No	206(99%)
	Yes	2(1%)
Family history of Diabetes	No	51(24.5%)
	Yes	157(75.5%)
HbA1c (%)		9.13±1.99
Cholesterol (mg/dl)		161.28±44.76
Triglyceride(mg/dl)		191.24±126.53
High density lipoprotein(mg/dl)		32.75±8.24
Low-density lipoprotein (mg/dl)		112.15±39.87
Duration of DM	≤5 years	110(46.2%)
	5 to 10 years	57(23.9%)
	>10 years	71(29.8%)

shows the mean age of subjects (121 males, 117 females) was 50.5±11.85 years. The study subjects were primarily non-smokers, 169(81.3%), and married 214(90.7%). Cholesterol total, triglycerides, HDL, and LDL, were 161.28±44.76, 191.24±126.53, 32.75±8.24, and 112.15±39.87, respectively. Poor glycemic control was observed with mean HbA1c levels of 9.13±1.99. More than half of the subjects, 157(75.5%), had a family history of Diabetes, and 110 (46.2%) participants had Diabetes for less than five years.

Table II represents the assessment of tear meniscus height using Schirmer's strip. Out of 238 subjects, 11(4.6%) had severe dry eyes, 26(10.9%) had moderate dry eyes, 35(14.7%) had mild dry eyes whereas, 166(69.7%) individuals had normal eyes. The overall frequency of dry eye in diabetic individuals was found to be 72(30.25%).

Table II: Assessment of tear meniscus height using Schirmer's strip method

Schirmer's strip test (mm)	n (%)	Overall
N	**238**	
≤5	11(4.6%)	
6-10	26(10.9%)	72(30.25%)
11-15	35(14.7%)	
>15	166(69.7%)	

In males, the association was found to be 63.6% for severe, 53.8% for moderate, and 40% for mild dry eyes, whereas, in females, the association was 36.4%, 46.2%, and 60%, respectively. All the individuals lie in the obese range of ≥25 kg/m^2 for normal to severe dry eyes. Individuals with a positive history of Diabetes and a duration of fewer than five years suffered from moderate to severe dry eyes. There was no significant association between gender, body mass index (BMI), smoking habits, family history of Diabetes, and duration of Diabetes with tear breakup time (TBUT), p-value <0.05. Upon evaluating the relationship between biochemical parameters with TBUT, poor glycemic control was observed in severe (8.87±1.04), mild (9.45±2.31), and moderate dry eyes (9.84±1.33). No significant association was found between lipid profile, HbA1c, and TUBT, while a significant association was found in cholesterol levels among severe (170±53.39), mild (186.86±59.23), moderate (173.94±46.93), and normal eyes (152.83±38.58) p-value = 0.03 (**Table III**).

In participants who have Diabetes, the prevalence of diabetic retinopathy was found to be 23.5%. Among 56(23.5%) individuals having retinopathy, 29(24%) were males, whereas 27(23.1%) were females (**Table IV**). Furthermore, **Table IV** and **Table V** show the association of diabetic retinopathy with dry eye

Table III: Association of tear film breakup time (TBUT) with baseline and biochemical parameters

Variable		TBUT (mm)				P-value
		≤5	6-10	11-15	>15	
N		11	26	35	166	
Age (years)		47.91±12.58	48.54±11.75	51.43±11.24	50.81±11.99	0.868
Gender	Male	7(63.6%)	14(53.8%)	14(40%)	86(51.8%)	0.471
	Female	4(36.4%)	12(46.2%)	21(60%)	80(48.2%)	
Systolic blood pressure (mm Hg)		137.14±33.02	123.78±19.16	124.34±15.95	123.54±18.03	0.77
Diastolic blood pressure (mmHg)		77.14±11.13	78.87±9.16	77.16±8.8	77.28±9.57	0.895
Body mass index (kg/m^2)		30.05±3.95	29.42±4.79	28.2±4.19	28.97±5.65	0.772
Family history of Diabetes	No	2(28.6%)	5(21.7%)	10(31.2%)	34(23.3%)	0.786
	Yes	5(71.4%)	18(78.3%)	22(68.8%)	112(76.7%)	
Duration of Diabetes	≤5 years	6(54.5%)	12(46.2%)	9(25.7%)	83(50%)	0.114
	5-10 years	3(27.3%)	9(34.6%)	11(31.4%)	34(20.5%)	
	>10 years	2(18.2%)	5(19.2%)	15(42.9%)	49(29.5%)	
Type of Diabetes	Type 2	11(100%)	26(100%)	35(100%)	162(97.6%)	0.623
	Other	0(0%)	0(0%)	0(0%)	4(2.4%)	
HbA1c (%)		8.87±1.04	9.45±2.31	9.84±1.33	8.93±2.06	0.275
Cholesterol (mg/dl)		170±53.39	186.86±59.23	173.94±46.93	152.83±38.58	0.032
Triglyceride(mg/dl)		250.5±238.45	176.07±64.43	211.94±128.6	186.01±128.9	0.717
High-density lipoprotein (mg/dl)		31.75±6.13	35.14±7.44	36.18±7.54	31.53±8.45	0.122
Low-density lipoprotein (mg/dl)		98.25±40.94	123.67±53.73	121.05±35.46	108.64±38.08	0.432

Data presented as mean ± SD or n (%); P-value<0.05 considered statistically significant.

disease and gender. In contrast, no association significantly was found between diabetic retinopathy, dry eye disease, and gender was found.

Table IV: Association of Retinopathy with TBUT

Retinopathy	TBUT (mm)				P-value
	≤5	6-10	11-15	>15	
No#	11	26	35	166	
No	9 (81.8%)	21 (80.8%)	26 (74.3%)	126 (75.9%)	0.905
Yes	2 (18.2%)	5(19.2%)	9(25.7%)	40 (24.1%)	

Data presented as n (%); P-value<0.05 considered as statistically significant; NO# = numbers

Table V: Association of retinopathy with gender

Retinopathy	Male	Female	P-value	Overall
N	**121**	**117**	**-**	**238**
No	92(76%)	90(76.9%)	0.871	182(76.5%)
Yes	29(24%)	27(23.1%)		56(23.5%)

Data presented as n (%); P-value<0.05 considered as statistically significant

DISCUSSION

Overall, a 30.3% frequency of dry eye in diabetic individuals was observed, among which 4.6% had severe dry eyes, 10.9% had moderate dry eyes, and 14.7% had mild dry eyes. The prevalence of diabetic retinopathy was 23.5%, and participants were observed with poor glycemic control and bad triglyceride levels. The DED in diabetic patients varies in different populations. Fuerst N et al.[16] found a 47.8% dry eye prevalence higher than our study.

Dry eyes were more frequent in females than males, in line with a recent study that showed females were more prone to dry eye disease than males[17]. Females were more inclined to males, which may be due to the onset of menopause that causes decreased estrogen levels in females and leads to reduced tear film[9]. Misra SL et al.[18] reported that due to the lesser production of androgens, a type of protective hormone, there is an upsurge in the incidence of dry eye disease in women. However, other studies have negated the association of gender in diabetic individuals with dry eye disease[19]. This research revealed that reduced tear formation in some diabetic individuals is linked to autonomic nervous system dysfunction[20]. We found the component of total cholesterol were similar in levels among diabetic participants with DED and non-DED. Our data show no association between dry eye disease and lipid profile. In our study, the prevalence of diabetic retinopathy was 23%, and gender had no statistically significant influence on the condition. We observed most participants with less than five years of Diabetes, in contrast with ul Islam Q 2017 [21] studies which found participants with longer diabetes duration.

In this investigation, we observed participants with poor glycemic control; however, the association of HbA1c with dry eye was statistically insignificant, in contrast with the findings of other studies[22-26], which found a strong positive connection between HbA1c and dry eye. Manjula TR 2019[27] stated that DM management with dry eyes is statistically significant. A study from the Asian population also found a strong association between dry eyes with poor glycemic control[28].

CONCLUSION

Overall, a 30.3% frequency of dry eye in diabetic individuals was observed, among which 4.6% had severe dry eyes, 10.9% had moderate dry eyes, and 14.7% had mild dry eyes. It may improve by having consistent follow-ups after three to six months, providing a distinct difference in the condition compared to the non-affected individuals.

Limitations

This study has some limitations due to its relatively small sample size and the fact that it was conducted at only one clinical site. Moreover, menopausal history, which significantly affects the development of DED, was lacking in our study.

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AUTHOR CONTRIBUTIONS

Sultan S: Concept, design, edited and approved the manuscript

Khanzada MA: Interpretation of data, edited and approved the manuscript

Shakeel A: Interpretation of data, edited and approved the manuscript

Ahmed N: Literature search, interpretation of data, and wrote the manuscript

Fawwad A: Concept, design, Edit, and support the final manuscript

Basit A: Concept, design, Edit, and approve the final manuscript

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Original Article

Sonographic Ultrasound Evaluation of Mastalgia and to Determine the Relationship between Breast Duct Diameter and Severity of Mastalgia

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ABSTRACT

OBJECTIVE: To determine the breast ultrasound findings in patients with Mastalgia and the relationship between breast duct diameter and severity of Mastalgia.

METHODOLOGY: A prospective cross-sectional study carried out at Dow Institute of Radiology, Dow University of Health Sciences from June-December 2021. Female patients, irrespective of age, presenting with Mastalgia without any prominent finding on clinical examination were included. A fellowship-trained radiologist performed an ultrasound. Data analysis was performed on SPSS 22.0

RESULTS: Total 380 patients were included. The mean age of the patients was 36.3±11.5 years, and the mean VAS score was 3.2±1.2. A total of 234(61.6%) had mild pain, 139(36.6%) had moderate pain and 7 (1.8%) had severe pain. The mean duct diameter in patients with Mastalgia was 2.9±3.7 mm, with 220 (57.9%) patients having a diameter <2.0 mm and 160(42.1%) having a diameter >2.0 mm. Duct ectasia was found in 161(42.4%). Mastalgia was significantly higher in patients with duct ectasia (p-value <0.001) and in patients with fibrocystic change (p-value <0.001).

CONCLUSION: Duct ectasia, fibrocystic changes, infective/inflammatory changes, and axillary lymphadenopathy were sonographic features of Mastalgia. Mastalgia was significantly higher in patients with duct ectasia, duct diameter >2.0 mm and in patients with fibrocystic changes.

KEYWORDS: Mastalgia, Duct ectasia, fibrocystic changes, breast pain.

INTRODUCTION

Breast pain, often termed medically as Mastalgia, is a common condition among females. Women usually describe it as a dull ache. However, some females may have breast heaviness, numbness, tightness, or discomfort. Pain severity may be variable, ranging from mild to severe. Moreover, its nature may be intermittent or continuous. In extreme cases, it may hamper the quality of life[1,2]. Mastalgia may be cyclic or non-cyclic. Cyclic pain is usually due to hormonal variation during the menstrual cycle, often described as severe one to two weeks before the start of periods and declining on the day of bleeding, subsiding in the next few days. It improves during pregnancy or lactation or in menopause[3]. Non-cyclic pain is usually unrelated to the menstrual cycle and may be related to injury, surgery, infection or intrinsic breast diseases such as cysts or neoplasm[4].

According to a study, Mastalgia is present in 51.5% of the population[5]. Moreover, it was frequent in older patients and less active patients. 35% of the patients reported sleep disturbance due to Mastalgia.[5] Mastalgia has also been linked to ingestion of a high-fat diet, intake of high-caffeine drinks, smoking, and use of some antidepressants, antibiotics or hormonal treatment. However, the mechanism is not well understood[6].

Commonly used imaging modalities for breast pain evaluation include mammography and ultrasonography. Ultrasound employs sound waves, predominantly in females under 35 years and those with dense breasts[7]. Females with Mastalgia may have a normal ultrasound or fibrocystic disease, duct ectasia, mastitis and fibroadenoma in 32.3%, 8.8%, 0.6% and 6.1% of the cases respectively[8]. It is essential to exclude any serious underlying disease process in patients presenting with Mastalgia. According to Breast Imaging Reporting and Data System (BI-RADS)[9], a previous local study has focused on findings. However, no relationship between Mastalgia and its severity has been made with the breast duct diameter in our population. Moreover, a study reported a higher duct diameter in patients with painful breasts than in non-painful breasts[10]. Therefore, this study aimed to determine the breast ultrasound findings in patients with Mastalgia and the relationship between breast duct diameter and severity of Mastalgia.

METHODOLOGY

A prospective cross-sectional study was undertaken at Dow Institute of Radiology, Dow University of Health Sciences, Ojha campus, from June - December 2021.

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The sample size was calculated using an Openepi calculator, taking the frequency of duct ectasia in mastitis as 8.8% [8], the confidence interval (CI) as 95%, and the margin of error as 3%. The total sample size came out to be 342. Non-probability consecutive sampling technique was used. Female patients of either age presenting with Mastalgia without any prominent finding on clinical examination were included as described in the operational definition and prescribed ultrasound by their primary physician. Patients with a positive physical examination, including palpable breast mass, skin thickening, ulceration, nipple discharge, nipple retraction, known breast carcinoma, post-breast surgery, lactation, or already diagnosed with duct ectasia/follow-up patients. In included patients, Mastalgia was defined as breast pain for one day or more without any prominent finding on clinical examination. Its severity will be labelled on a visual analogue scale (VAS)[11]: VAS 1-3 was considered mild pain, VAS 4-6 was moderate pain, and VAS 7-10 was severe pain. Among sonographic findings of Mastalgia, duct ectasia was regarded as the abnormal widening of one or more breast ducts (tubular branching structures) to greater than 2 mm diameter, or 3 mm at the ampulla[12,13]. On ultrasound, fibrocystic change was defined as prominent fibro glandular tissue without any palpable mass, which may include cysts and areas of fibrosis.

Patients presenting with Mastalgia for ultrasound breast were initially to grade their pain on the visual analogue scale (VAS). The patient signed a written consent form before the procedure. Following pain grading, an ultrasound breast was performed by the principal investigator and also by a consultant radiologist with more than three years of experience post-fellowship in female imaging. Ultrasound was performed on Toshiba Aplio 300 ultrasound system machine. All four quadrants of the breast and retroareolar region were scanned systematically. Patients were again asked to pinpoint the exact location of the pain to re-look that area again for any abnormality. Patient demographics, pain score, severity, and ultrasound findings were recorded.

Statistical package for social sciences version 22.0 was used for data entry and analysis. Mean and standard deviation (SD) was calculated for quantitative variables such as age, weight, height, BMI, VAS score and duct diameter. Frequency and percentages were computed for qualitative variables such as pain severity (mild, moderate or severe), duration of pain, cyclical variation (cyclic or non-cyclic), marital status (unmarried or unmarried), menstrual status (normal cycle, premenopausal or menopause), co-morbidities (hypertension, diabetes, nil), drug history (hormone therapy, antidepressants antibiotics), occupation, and residence. Effect modifiers such as age, duct diameter, cyclical variation, marital status and menstrual status were stratified to see their effect on pain severity. Post-stratification, the chi-square test was applied, and a p-value of less than or equal to 0.05 will be taken as significant.

RESULTS

A total of 380 patients were included in this study. The mean age of the patients was 36.3±11.5 years. The

Table I: Baseline characteristics of the patients with Mastalgia (n=380)

	n	%
Age (years)	36.3±11.5*	
≤40	259	68.2
>40	121	31.8
Height (cm)	164.3±5.5*	
Weight (kg)	67.7±9.32*	
BMI (kg/m^2)	25.0±3.1*	
Duct diameter (mm)	2.9±3.7*	
≤2.0	220	57.9
>2.0	160	42.1
VAS score	3.2±1.2*	
Pain severity		
Mild	234	61.6
Moderate	139	36.6
Severe	7	1.8
Duration of pain (months)	3.4±1.4*	
Cyclic variation		
Cyclic	54	14.2
Non-cyclic	326	85.8
Marital status		
Married	273	71.8
Unmarried	107	28.2
Menstrual status		
Normal cycle	272	71.6
Peri-menopausal	41	10.8
Menopause	67	17.6
Diabetes Mellitus		
Present	42	11.1
Absent	338	88.9
Hypertension		
Present	66	17.4
Absent	314	88.9
Medication use for co-morbid conditions		
Present	57	15.0
Absent	323	85.0

**Mean±SD*

Table II: Ultrasound features in patients with Mastalgia (n=380)

	n	%
Duct ectasia		
Present	161	42.4
Absent	219	57.6
Fibrocystic changes		
Present	66	17.4
Absent	314	82.6
Infective/inflammatory changes		
Present	56	14.7
Absent	324	85.3
Axillary lymphadenopathy		
Present	12	3.2
Absent	368	96.8

mean duration of symptoms was 3.4±1.4 months. Mean height, weight and BMI were 164.3±5.5 cm, 67.7±9.32 kg and 25.0±3.1 kg/m^2, respectively. The mean VAS score was 3.2±1.2. A total of 234 (61.6%) had mild pain, 139 (36.6%) had moderate pain and 7 (1.8%) had severe pain. 326 (85.8%) had non-cyclic Mastalgia, and 54 (14.2%) had cyclic Mastalgia. Out of 380 patients, 273 (71.8%) were married. Multiple findings in mastalgia patients were found in 69 (18.2%), and a single result was found in 311 (81.8%). A total of 272 (71.6%) had normal menstrual cycles. The mean duct diameter in patients with Mastalgia was 2.9±3.7 mm, with 220 (57.9%) patients having a diameter <2.0 mm and 160 (42.1%) having a diameter >2.0 mm. The baseline characteristics of the patients are summarized (**Table I**).

Table III: Comparison of mastalgia severity with patient characteristics

	Mastalgia severity			Total	P value
	Mild	Moderate	Severe		
Age					
≤40 years	186 (48.9%)	70 (18.4%)	3 (0.8%)	259 (68.2%)	<0.001*
>40 years	48 (12.6%)	69 (18.2%)	4 (1.1%)	121 (31.8%)	
Cyclic variation					
Cyclic	44 (11.6%)	10 (2.6%)	0 (0.0%)	54 (14.2%)	0.004*
Non-cyclic	190 (50.0%)	129 (33.9%)	7 (1.8%)	326 (85.8%)	
Marital status					
Married	152 (40.0%)	114 (30.0%)	7 (1.8%)	273 (71.8%)	<0.001*
Unmarried	82 (21.6%)	25 (6.6%)	0 (0.0%)	107 (28.2%)	
Menstrual status					
Normal cycle	191 (50.3%)	78 (20.5%)	3 (0.8%)	272 (71.6%)	<0.001*
Peri-menopausal	12 (3.2%)	25 (6.6%)	4 (1.1%)	41 (10.8%)	
Menopause	31 (8.2%)	36 (9.5%)	0 (0.0%)	67 (17.6%)	

**Chi-square test applied*

Table IV: Comparison of mastalgia severity with duct diameter, duct ectasia and other ultrasound findings

	Mastalgia severity			Total	P-value
	Mild	Moderate	Severe		
Duct diameter					
≤ 2.0 mm	187 (49.2%)	33 (8.7%)	0 (0.0%)	220 (57.9%)	<0.001*
>2.0 mm	47 (12.4%)	106 (27.9%)	7 (1.8%)	160 (42.1%)	
Duct ectasia					
Present	47 (12.4%)	107 (28.2%)	7 (1.8%)	161 (42.4%)	<0.001*
Absent	187 (49.2%)	32 (8.4%)	0 (0.0%)	219 (57.6%)	
Fibrocystic changes					
Present	30 (7.9%)	32 (8.4%)	4 (1.1%)	66 (17.4%)	0.001*
Absent	204 (53.7%)	107 (28.2%)	3 (0.8%)	314 (82.6%)	
Infective / Inflammatory changes					
Present	14 (3.7%)	36 (9.5%)	6 (1.6%)	56 (14.7%)	<0.001*
Absent	220 (57.9%)	103 (27.1%)	1 (0.3%)	324 (85.3%)	
Axillary lymphadenopathy					
Present	2 (0.5%)	6 (1.6%)	4 (1.1%)	12 (3.2%)	<0.001*
Absent	232 (61.1%)	133 (35.0%)	3 (0.8%)	368 (96.8%)	

**Chi-square test applied*

Among ultrasound features of Mastalgia, duct ectasia was found in 161 (42.4%), fibrocystic changes were found in 66 (17.4%), infective/inflammatory changes in 56 (14.7%) and axillary lymphadenopathy in 12 (3.2%) (**Table II**).

A total of 65(19.0%) patients had isolated duct ectasia. 34(9.9%) patients had duct ectasia together with fibrocystic changes. 51(14.9%) patients had duct ectasia together with inflammation. A total of 11(3.2%) patients had duct ectasia and axillary lymphadenopathy.

Mastalgia was significantly higher in patients aged ≤40 years age (p-value <0.001), in patients with non-cyclic variation (p-value of 0.004), married patients (p-value <0.001) and patients with normal cycles (p-value <0.001) (**Table III**).

Mastalgia was significantly higher in patients with duct ectasia (p-value <0.001) and in patients with fibrocystic change (p-value <0.001) (**Table IV**).

DISCUSSION

Mastalgia, commonly referred to as breast pain, is also described by some females as aching, dull pain or by some females as discomfort, breast heaviness or tightness. It usually ranges from mild to severe and may affect the quality of life[1]. It may be related or unrelated to the menstrual cycle. Hormone-related changes may be responsible for cyclic mastalgia[3]. Whereas vascular, inflammatory, infective, or neoplastic may result in non-cyclic Mastalgia. Ultrasound and mammogram are the modalities to evaluate for mastalgia[7,14].

In our study population, most females experiencing Mastalgia were less than 40 years. Another study showed that Mastalgia was common in young patients[15]. Our study further showed that mild Mastalgia was more common than in the previous study[15], leading to moderate to severe Mastalgia in younger patients; this could be attributed to hormonal changes in younger age.

In our study, non-cyclic Mastalgia was more common than cyclic Mastalgia. Another study showed a higher prevalence of non-cyclic mastalgia[16,17]. However, another study showed a high prevalence of cyclic Mastalgia.[15] It can be theorized that hormonal changes could result in Mastalgia during the menstrual cycle. In contrast, other causes such as neoplasm, trauma or other non-specific causes can result in non-cyclic Mastalgia.

Mammary duct ectasia is one of the common benign breast diseases. It is an underestimated disease, and confusion about its overlap with plasma cell mastitis still exists presently[18]. Our study showed a high prevalence of duct ectasia in patients with Mastalgia, and a higher diameter was observed in patients with moderate and severe Mastalgia. Another study showed a significant association of Mastalgia with breast pain.[19] This previous study also showed nipple discharge as another presenting symptom in duct ectasia[19]. Another study showed a very low prevalence of breast duct ectasia in patients with mastalgia[20]; this could be attributed to the small sample size of that study.

Fibrocystic breast disease, now more commonly termed fibrocystic breasts, is a common benign breast disease. Our study also observed a high prevalence of fibrocystic disease in patients with Mastalgia. Another study showed that almost 50% of patients had fibrocystic breast disease with mastalgia[21]. Pain, discomfort and tenderness are common symptoms of this condition, and some women also experience nodularity. It can be assumed that Mastalgia may result in changes leading to fibrocystic breast development.

A high prevalence of infective/inflammatory changes was reported in our study in patients presenting with Mastalgia. However, another study reported a relatively lower prevalence of infective diseases such as mastitis and abscess in such patients[22]. This reported difference could be related to a difference in sample size. Our study had a high sample size, whereas a smaller sample size was present in the previous study.

Our study reported the presence of axillary lymphadenopathy in a few patients with Mastalgia. Isolated axillary lymphadenopathy in Mastalgia is usually rare. However, we believe that axillary lymphadenopathy may be present in cases of mastitis if ancillary findings in ultrasound breast are observed, such as mastitis, abscess or any neoplastic cause. Therefore, adequate history followed by a detailed physical examination of all four quadrants, regional lymph node areas, and axilla, such as supraclavicular and infraclavicular regions, should be adequately and thoroughly examined.

Our study was not without certain limitations. The first limitation was that it was a single-centre study, and another limitation was that its relationship with nipple discharge was not observed in our population. A previous study showed a significant correlation of duct ectasia with nipple discharge[19]. Another limitation was that the association with the use of lactation was not evaluated. Lactation has also demonstrated a strong association with duct ectasia and mastalgia[19]. Our study assessed the use of co-morbid medications; however, the association between oral contraceptive medications and mastalgia or duct ectasia was not evaluated.

Despite these limitations, we believe that the prospective nature of the study is its strength, and another strength is its large sample size. It is recommended that further multicentric studies incorporating various other symptoms such as lump and nipple discharge and other variables such as smoking should be carried out to obtain additional insights regarding mastalgia and duct ectasia.

CONCLUSION

Duct ectasia, fibrocystic changes, infective/ inflammatory changes and axillary lymphadenopathy

were sonographic features of Mastalgia. Mastalgia was significantly higher in patients with duct ectasia, duct diameter >2.0 mm and in patients with fibrocystic changes.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Aziz S: Concept, Final approval of the manuscript
Sattar A: Literature review
Walid A: Proofreading, literature review
Rahim A: Final draft of the manuscript
Naz N: Data interpretation, analysis
Ara H: Supervision and proofread of final manuscript draft

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Original Article

Effect of Dietary Counselling on Anthropometric Measurements of Liposuction Patients

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ABSTRACT

OBJECTIVE: To observe the effects of dietary counselling on various anthropometric parameters in patients after liposuction.

METHODOLOGY: This non-randomized educational intervention study was performed at a cosmetic surgery centre in Islamabad, Pakistan, from July to December 2017. Among 83 subjects, 43 were in the intervention group, and 40 were in the control group from both genders. Patients undergoing primary liposuction, abdominal liposuction, and abdominoplasty with liposuction were included in this study; patients on any weight loss diet or pills in the last six months were excluded. The intervention group was followed up three months after dietary counselling, and the control group was without dietary advice. Anthropometric measurements of both groups were done before liposuction, just after liposuction, and then three months after surgery, which included body weight, body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), and mid-upper arm circumference (MUAC). Data analysis was done with SPSS version 20.

RESULTS: Significant changes were found in all parameters except for MUAC in the control group. Independent-sample T-tests showed no statistically significant difference in any parameter at any time.

CONCLUSION: Liposuction resulted in a reduction in weight, BMI, WC, WHR, and WHtR in both groups, but dietary counselling could not show its effect on any parameter in our study. Further reduction of these parameters in the intervention group could occur if the intervention lasted longer.

KEYWORDS: Liposuction, BMI, WC, WHR, MUAC, dietary counselling.

INTRODUCTION

Liposuction is not an obesity treatment[1]. Liposuction is used for the removal of genetically disturbed or diet-resistant fat[1]. It has been observed that liposuction patients regain fat after a certain period of time[2] because these patients do not follow dietary management following surgery. Logically speaking, without control of dietary intake and physical activity, the regain of excessive body fat cannot be avoided. In the literature, indications and general liposuction complications were encountered, but the effect of dietary modifications following liposuction could not be found.

There is a gap in the literature about the effect of dietary intervention following liposuction. The present study was conducted to observe the impact of dietary intervention on various anthropometric measurements of post-liposuction patients.

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METHODOLOGY

The study was of the educational intervention category. It was carried out at a cosmetic surgery centre in Islamabad, Pakistan, from July to December 2017, with purposive sampling done. Permission was obtained from the clinic administration to carry out the study. Eighty-three candidates participated in this study, and all the participants signed informed consent.

Patients undergoing liposuction and abdominoplasty were followed for three months post-operatively. Eighty-three subjects from both genders were enrolled in the study (43 subjects in the intervention group and 40 in the control group). Subjects undergoing primary liposuction, abdominal liposuction, and abdominoplasty with liposuction were included in this study. Patients on any weight loss diet or pills during the last six months were excluded. The intervention group was followed after counselling for diet plans, but the control group was observed without any dietary counselling. Anthropometric measurements of both groups were done before liposuction, immediately after liposuction, and then three months after surgery.

BMI is the most commonly used measurement for obesity, but BMI is associated with total body fat and does not reveal body fat distribution. Central obesity, characterized by excessive abdominal fat, has been associated with a higher mortality risk. Other

anthropometric measurements have been formulated to explain the extent of central obesity, like waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR).[3] Anthropometric measurements included body weight (Wt.), body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), and mid-upper arm circumference (MUAC). SPSS version 20 was used for data analysis.

RESULTS

The study was comprised of 83 participants. Fifty two percent of participants were in the intervention group, and 48% were in the control group. Twenty three percent of participants in both groups were males, and 77% were females (**Table I**). Two-thirds of the study participants were younger than 40, and the mean age of the whole sample was about 37±7.9 years. The youngest subject in the sample was 18 years old, and the oldest subject was 58 years old.

Table I: Characteristics of study participants

	Categories	Frequency	Percentage(%)
Groups	Intervention	43	51.8
	Control	40	48.2
Gender	Male	19	22.9
	Female	64	77.1
Age	<40 years	55	66.3
	≥40 years	28	33.7

The mean weight of the intervention group (males) was about 79 kg before surgery, about 78 kg immediately after liposuction, and about 72kg after three months of dietary counselling (**Table II**). The differences between all these readings were statistically significant (**Table II**). The mean BMI of the intervention group was about 31 kg/m² before surgery, 30.8 kg/m² immediately after liposuction, and about 28 kg/m² after three months of dietary management (**Table II**). The differences between all these observations were also statistically significant. Similarly, the differences between WC, WHR and WHtR observations were also statistically significant (**Table II**). However, the mean MUAC of the intervention group was about 34 cm before surgery, remained the same right after surgery, and then decreased to 32 cm after three months of dietary advice. Hence, the differences between MUAC pre- and post-liposuction were not different, but immediately after liposuction and after three months of dietary counselling were statistically significant (**Table II**). Similarly, the difference between MUAC before surgery and after three months of dietary counselling was substantial.

The mean weight of the control group (males) was about 76.5 kg before surgery, 75.5 kg immediately after surgery, and about 70 kg three months after surgery. The mean BMI of the control group was approximately 31.4 kg/m² before surgery, about 31 kg/m² immediately after surgery, and 30.6 kg/m² three months after surgery (**Table II**). Differences between values of both these parameters at different points in

Table II: Comparison of means (Male participants)

Measures	Groups	Before liposuction	After liposuction	Three months after liposuction	p-value	P-value: Post-liposuction and three months after liposuction	P-value: Pre-liposuction and three months after liposuction	Intervention group Vs control group (Before liposuction):	Intervention group Vs control group (After lipo-suction):	Intervention group Vs control group (Three months after liposuction):
Weight (kg)	Intervention n=10	79.25±9.28	78.57±8.84	72.03±8.34	<0.05	<0.001	<0.001	<0.005	<0.005	<0.005
	Control n=9	76.44±10.33	75.65±10.05	70.24±9.67	<0.005	<0.001	<0.001			
BMI*	Intervention n=10	31.07±3.01	30.81±2.74	28.20±2.31	<0.05	<0.001	<0.001	--	--	--
	Control n=9	31.39±5.75	31.01±5.58	30.67±5.70	<0.005	<0.001	<0.001			
WC† (cm)	Intervention n=10	114.54±12.41	111.60±11.64	106.46±12.46	<0.005	<0.01	<0.005	--	--	--
	Control n=9	107.01±6.63	104.68±6.46	100.24±6.11	<0.001	<0.001	<0.001			
WHR‡	Intervention n=10	1.01±0.11	0.99±0.11	0.95±0.12	<0.005	<0.05	<0.005	--	--	--
	Control n=9	0.94±0.01	0.92±0.01	0.89±0.02	<0.001	<0.001	<0.001			
WHtR§	Intervention n=10	0.67±0.07	0.65±0.07	0.62±0.07	<0.001	<0.05	<0.005	--	--	--
	Control n=9	0.63±0.04	0.62±0.04	0.59±0.04	<0.005	<0.005	<0.001			
MUAC‖ (cm)	Intervention n=10	33.85±1.59	33.85±1.59	32.88±2.7	--	<0.05	<0.05	--	--	--
	Control n=9	35.72±4.02	35.72±4.02	35.72±4.02	--	--	--			

**Body mass index, †Waist circumference, ‡Waist-to-hip ratio, §Waist-to-height ratio, ‖Mid-upper arm circumference, Bold=statistically significant values*

Table III: Comparison of means (Female participants)

Measures:	Groups	Before liposuction	After liposuction	Three months after liposuction	p-value: Pre - and post-liposuction	P-value: Post-liposuction and three months after liposuction	P-value: Pre-liposuction and three months after liposuction	Intervention group vs control group (Before liposuction)	Intervention group Vs control group (After liposuction)	Intervention group Vs control group (Three months after liposuction)
Weight (kg)	Intervention n=33	85.39±16.35	84.39±16.26	79.05±16.38	<0.001	<0.001	<0.001	--	--	--
	Control group n=31	78.90±13.68	78.05±13.43	76.63±13.62	<0.001	<0.001	<0.001			
BMI*	Intervention n=33	31.73±4.29	31.25±4.41	29.28±4.39	<0.001	<0.001	<0.001	--	--	--
	Control n=31	30.65±4.46	30.40±4.39	29.81±4.40	<0.001	<0.001	<0.001			
WC†(cm)	Intervention n=33	101.62±9.50	98.44±8.90	93.16±8.22	<0.001	<0.001	<0.001	--	--	--
	Control n=31	99.61±6.33	96.31±6.23	92.33±6.09	<0.001	<0.001	<0.001			
WHR‡	Intervention n=33	0.93±0.07	0.90±0.07	0.87±0.05	<0.001	<0.001	<0.001	--	--	--
	Control n=31	0.94±0.02	0.91±0.02	0.88±0.02	<0.001	<0.001	<0.001			
WHtR§	Intervention n=33	0.63±0.06	0.61±0.05	0.58±0.05	<0.001	<0.001	<0.001	--	--	--
	Control n=31	0.62±0.04	0.60±0.04	0.57±0.03	<0.001	<0.001	<0.001			
MUACǁ (cm)	Intervention n=33	35.23±2.92	34.58±5.13	34.71±2.98	--	--	<0.001	--	--	--
	Control n=31	33.90±2.19	33.90±2.19	33.90±2.19	--	--	--			

**Body mass index, †Waist circumference, ‡Waist-to-hip ratio, §Waist-to-height ratio, ǁMid-upper arm circumference,*
Bold=statistically significant values

time were statistically significant. Similarly, differences in WC, WHR, and WHtR values of the control group at different points in time were also statistically significant (**Table II**). But the mean value of MUAC of the control group before, right after, and three months after surgery had almost no differences (**Table II**).

All these parameters were also compared between interventional and control groups at these three points, i.e. before liposuction, immediately after surgery, and three months after surgery. A statistically significant difference was found only in body weight because there was already a statistically significant difference between mean body weight between the intervention and control group before liposuction (**Table II**). No statistically significant difference was found between any parameter at any point in time.

All parameters were also compared between interventional and control groups at these three points in time in females (**Table III**). Results were similar to those in males, except for body weight (**Table III**).

DISCUSSION

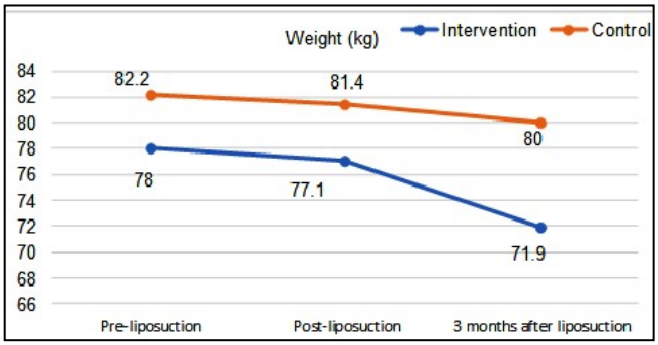
In our study, about 77% of study participants were females, and about 66% of participants were below 40 years of age (**Table I**). These findings aligned with the literature[1,4], where primarily young females opted for liposuction.

Many studies showed that liposuction could cause a decline in body weight and BMI, as well as WC and WHR, in obese patients[1,3,5,6]. Some studies showed the effects of specific alternative procedures to liposuction, like cryolipolysis and lipocavitation, along with dietary intervention on these parameters of interest. Significant effects of the dietary intervention were found on body weight, BMI, and WC, with or without these procedures, after three months[7,8]. No such study was done in liposuction patients in the past. Our study also found a significant change in all anthropometric measures in the control group after liposuction, except for MUAC. Both groups expected weight and BMI to change right after the liposuction procedure, as a large amount of fat was removed. WC, WHR, and WHtR were also expected to decrease as most fat removed during liposuction was from the waist area. But MUAC was not expected to drop immediately after surgery. Weight loss requires a negative energy balance where energy intake is lower than energy expenditure.[9] Our study observed three months of dietary restriction in the intervention group. In the intervention group, all anthropometric measures significantly decreased right after liposuction, except for MUAC, but after three months of dietary intervention, all anthropometric measurements dropped, along with MUAC. A decrease in MUAC possibly occurred due to an overall effect of dietary intervention on the body.

Although our study showed that liposuction reduced weight, BMI, WC, WHR, and WHtR in both groups, no statistically significant difference could be found between both groups during different points in time, but it is evident from **Figure I** that the weight decline

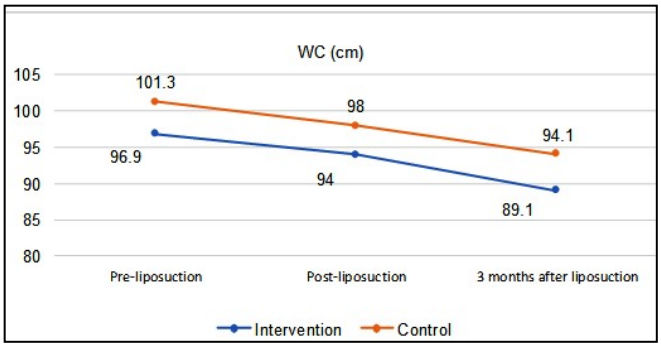
was similar pre- and post-liposuction in both groups, but the decline was steeper in the intervention group as compared to the control group (**Figure I**). However, the decrease in WC was similar in both groups (**Figure II**). Literature showed that dietary intervention alone for at least six months could result in statistically significant weight, BMI, WC, WHR, and WHtR changes[10]. As **Figure I** shows, the difference in the decline in weight between these two groups could have widened further. It could have become statistically significant if the intervention had lasted for longer. The effects of dietary intervention possibly could not reflect significantly in our study because the intervention lasted only three months.

Figure I: Comparison of a trend of weight



reduction between groups:

Figure II: Comparison of a trend of waist



circumference (WC) reduction between groups

Logically speaking, the effects of liposuction cannot persist for long without lifestyle changes. There is always a risk of weight regaining after liposuction. It happens because the fat condition, related to the concurrent obesity status, becomes a reference point where the metabolism becomes focused[2]. It means that the energy balance regulates the metabolism to keep the fat content at a level when obesity develops. This results in increased food intake and decreased energy expenditure. Hence, preventing weight regain means going against these biological mechanisms[2]. Results from short- and long-term outcomes after liposuction showed that patients have an initial fat mass loss lasting up to three months, and then body fat gradually restores, usually in one year[6,11]. As our study lasted for only three months, and the control group was showing a decline in all anthropometric measures, probably the patients were still in the phase of losing fat mass rather than regain of fat.

It should be emphasized that liposuction is a cosmetic procedure[12]. It can be utilized as a feasible method for aesthetic purposes and improving insulin resistance with appropriate diet and exercise[13,14]. Although liposuction should be used only as an adjunct to bariatric surgery in massively obese patients, many patients are only interested in changing their appearance rather than their behaviours. It should always be emphasized that the effects of the procedure are reversed if the lifestyle is not changed. Patients who want to benefit from liposuction in the long term should try to improve their appearance through diet and exercise[1].

CONCLUSION

Liposuction definitely reduced weight, BMI, WC, WHR, and WHtR in both groups, but dietary counselling could significantly affect any of these parameters. Dietary counselling could have probably resulted in a further reduction of these parameters compared to the control group if the diet restriction was prolonged for an adequate period, like six months or more.

Ethical Permission: Allama Iqbal Open University, Islamabad, IRB letter No. AIOU-6-2017-IRB-A-006.

Conflict of Interest: No conflicts of interest, as stated by authors.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Khan AJ: Conception, drafting, final approval
Mohmand H: Conception, drafting, final approval
Bushra S: Data collection, revision, final approval
Sajid S: Data collection, revision of the manuscript, final approval
Jehan T: Analysis, revision of the manuscript, final approval
Kashif S: Analysis, revision of the manuscript, final approval

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Original Article

Clinico Demographic Profile and Outcome of neonates born to Mothers with COVID-19 Infection at a Tertiary Care Hospital

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ABSTRACT

OBJECTIVE: To determine the Clinico-demographic profile and outcome of neonates born to mothers with COVID-19 infection at a Tertiary Care Hospital.

METHODOLOGY: This cross-sectional study was conducted at Pediatrics Department, Ziauddin Hospital Karachi, from March 2020 to August 2021. The sample size was 31, non-probability purposive sampling technique was used. Neonates of COVID positive mothers who consented to Covid-19 testing were included. Neonates of Covid positive mothers who refused to participate were excluded. Diagnosis of coronavirus infection was based on Novel Corona Virus-2019 (nCoV-19) qualitative PCR test through nasopharyngeal swab. Clinico-demographic profile and outcome of neonates data were recorded on self -developed proforma. Standard protocols were followed per National Command and Operation Center guidelines. Data were analyzed by SPSS version 20. Frequencies and percentages were calculated, and the chi-square test was applied as a significance test with a P-value of <0.05.

RESULTS: First virology test was positive for 6 (19.4%) neonates, and the second was positive for only 1 (3.2%) neonate; 17 (54.8%) neonates who were negative in the first virology test and stayed less than three days in the hospital refused to undergo a second test by their parents. After 14 days of follow-up, 29 (93.5%) neonates became asymptomatic, while 2 (6.5%) had symptoms of neonatal respiratory distress and required admission to the neonatal intensive care unit.

CONCLUSION: Vertical transmission of covid-19 can occur if the mother acquires infection in the last trimester. However, these infected neonates' morbidity and mortality rate is negligible; therefore, breastfeeding and rooming should be encouraged.

KEYWORDS: COVID-19; neonates; Coronavirus; outcome; vertical transmission; respiratory distress; neonatal intensive care unit; Polymerase chain reaction; mortality

INTRODUCTION

The Corona Virus infection originated in Wuhan, China, and became a pandemic. The World Health Organization (WHO) officially named the novel coronavirus disease Corona Virus Disease 19 (COVID -19). Corona (SARS-Cov2) is an enveloped RNA virus that causes respiratory, gastrointestinal, hepatic, and neurological manifestations in varying degrees[1]. The time lapse between exposure and the appearance of symptoms of COVID-19 infection is two weeks but usually, patients present in the first week. Symptoms such as high body temperature, dry cough, headache and muscle aches are universally present. There is little understanding of the clinical spectrum of this disease in neonates and children. Initial research data from China showed that severe acute respiratory syndrome coronavirus (COVID-19) infection could harm pregnant women. However, newborns did not show clinical manifestations and viral testing results were negative[2]. Though most studies suggest that vertical transmission of this infection is relatively weak, there are few case reports of neonates with early positive testing offering in-utero transmission of the infection[3].

Despite a significant increase in both the incidence and prevalence of Coronavirus infection, there is limited data on the in-utero transfer of the virus from the mothers to infants during pregnancy, at the time of delivery or soon after. Feto-maternal virus transfer is possible as its genetic material has been detected in body fluids. Still, due to a high rate of false positive results, the mere detection of specific IgM antibodies on testing is insufficient evidence of infection[4]. Studies have also reported that lactation does not pose a risk of infection in babies of mothers with COVID-19 disease; therefore, breastfeeding is recommended. Research literature reports that infection in late pregnancy can lead to premature labor, neonatal respiratory difficulty, thrombocytopenia and low oxygen saturation in neonates and also increases the risk of unfavourable perinatal occurrences[5] These neonates may develop breathing problems, cyanosis, increased work of breathing, and rapid heartbeat,

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temperature instability, decreased oral intake, drowsiness and gut issues such as emesis, loose stools and increased abdominal girth. A few can also experience acute respiratory distress syndrome (ARDS)[6,7].

Babies born at appropriate gestational age to mothers who test positive primarily do not exhibit signs or symptoms[8]. Maternal vaccination in the antenatal period effectively reduces the risk of severe infection and pregnancy-related complications, especially in those with coexisting chronic diseases.[9]Nasopharyngeal swabs are the most common way of sampling, but its "positive detection rate" is less than 50%, so it needs to be repeated. Most studies have not suggested that infection can be transferred in utero from mothers to neonates. However, it is essential to analyze additional cases to determine if this remains true[10,11]. Therefore, this study is being conducted considering the existing shortage of research data, especially in the local context on the clinical presentation and outcome in babies with maternal infection. Moreover, testing neonates born to infected mothers helps better understand the disease pattern and aids in making informed decisions about instituting protective plans for better postnatal care.

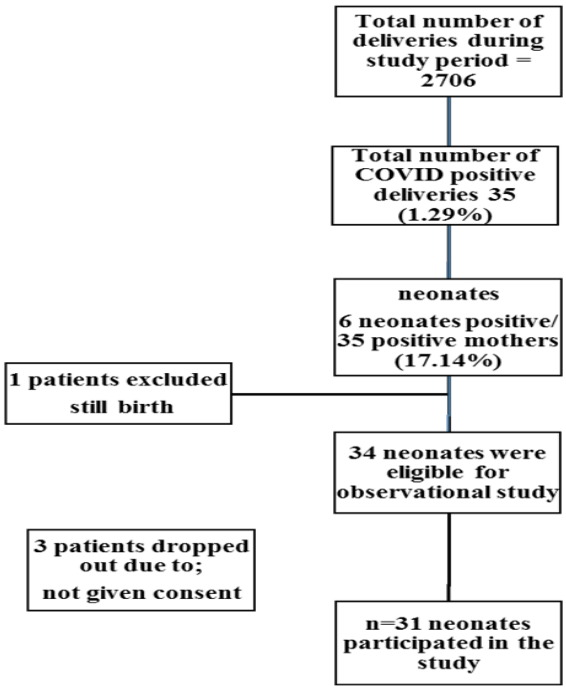
METHODOLOGY

This prospective cross-sectional study was conducted at the Pediatric Unit of Ziauddin University Hospital Karachi from March 2020 to August 2021. The sample size was calculated following the results of Anand P et al.[12]. By keeping a frequency of 2.3%, and our total estimated sample size was n= 31 at a 95% confidence interval and a 5% margin of error. For recruiting participants, a non-probability purposive sampling technique was used.

Newborns of COVID positive mothers of either gender and whose parents consented to conduct COVID-19 PCR tests in neonates were included. Newborns whose parents were unwilling to participate in the study were excluded.

Data was collected on a pre-designed proforma from parents/caregivers of neonates meeting the inclusion criteria and born to COVID-19-positive mothers admitted for delivery at Ziauddin Hospital. Diagnosis of coronavirus infection in the mother and baby was based on Novel Corona Virus-2019 (nCoV-19) Qualitative polymerase chain reaction PCR (COVID-19) test through the nasopharyngeal swab. Antigen Detection Rapid Test (Ag-RDT) through nasopharyngeal swabs was considered for diagnosis for emergency surgeries. Informed and written consent was taken from the patient's parents/ guardian. After taking detailed history and examination, a duty pediatric team member recorded the mother's and neonate's demographic and clinical features on the pre-designed proforma. Neonatal clinicians attended deliveries with all due precautions for Infection-control as per the hospital infection control policy. In babies with a potential requirement for resuscitation, measures were taken according to the hospital infection control policy.

Figure I: Algorithm for recruitment of study participants



Infants born at term to Covid-positive mothers were kept with mothers if the neonates were asymptomatic. A post-discharge telephone follow-up was performed by one of the pediatric residents. The newborns were bathed within 4 hours after Birth to remove the virus potentially present on skin surfaces. The clinical staff ensured the use of Droplet and Contact precautions until the newborn virology status was known to be negative by COVID-19 PCR testing. Hospitalization of neonates was done if they were symptomatic or their guardians wanted a hospitalized quarantine. Symptomatic and preterm neonates born to Covid -19 positive mothers were admitted to specific isolation areas physically separate from other newborns whose mothers were COVID Negative.

Maternal infection status was assessed according to WHO COVID severity staging. Information about the mode of delivery and if the pregnancy was booked or not was recorded on the proforma. If the newborn showed breathing problems and chest symptoms, these were recorded along with the duration of the hospital stay and outcome.

Neonate gestational age assessment was done by last menstrual period (LMP), first-trimester ultrasound, or Ballard examination (in case LMP and ultrasound were unavailable). Intrauterine growth status at Birth was assessed by birth weight and neonatal anthropometric charts. Mode of delivery, APGAR score, the need for ICU, signs and symptoms, duration of stay and outcome was recorded. The Workup of the healthy neonate was only a COVID PCR test after 24 hours of Birth. Subsequent testing

was done as indicated (e.g. if the baby became unwell after a negative maternal result or as recommended by the clinician). For neonates who were positive at initial PCR testing, follow-up testing was done at 48-72 hours. For Sick neonates, laboratory tests were performed on admission, including a complete blood picture, acute phase reactant (CRP/ Procalcitonin), and blood culture to investigate for co-infection and X-ray/CT scan chest. All blood reports, X-ray/CT scans, and chest scan findings were discussed and noted. Management was provided, including supportive management, oxygen therapy, Non-invasive ventilation, mechanical ventilation, nutritional support, and maintaining fluids and electrolyte balance. Specific management, including antibiotic, antiviral and immunomodulatory treatment, was given per WHO and National covid-19 guidelines. The outcome and prognosis of the neonates were assessed based on the duration of clinical and radiological improvement of disease, length of NICU stay and COVID-19 negative report. Data was recorded on predefined Proforma.

Data Analysis: It was done by the statistical software package SPSS version 20.0. The data was calculated in frequencies and percentages, and the chi-square test was applied with a P-value of <0.05, considered statistically significant.

RESULTS

A total of 31 neonates were included in the study. Among the newborns, 20 (64.5%) were males, and 11 (35.5%) were females. The mean gestational age of the neonates was 35.97±2.8 weeks. There was no significant difference among the demographic variables covid-19 positive and negative neonates. **Table I** shows the demographic and laboratory data of neonates. There was no significant difference (p=0.237) in Appearance, Pulse, Grimace, Activity and Respiration (APGAR) scores of neonates who were covid-19 positive and those who tested negative. Most neonates, i.e. 45.2%, were born by Emergency caesarian surgery, whereas 16.1% were born through spontaneous vaginal delivery and 38.7% via elective caesarian section.

Table I: Demographic and laboratory parameters of neonates born to covid-19 positive mothers

	Mean	SD
Gestational age (weeks)	35.97	±2.81
Birth weight (kg)	2.71	± .69
APGAR score at 1 minute	7.53	±1.46
APGAR score at 5 minutes	8.83	± .95
Hb (g/dl)	14.64	±3.33
TLC (/mm^3)	16.96	±6.40
Platelet count (/ mcL)	242.64	±128.39
CRP (mg/dl)	5.95	±13.18

CRP = C-reactive protein; Hb = Hemoglobin; TLC= Total Leukocyte count
APGAR= Appearance, Pulse, Grimace, Activity, Respiration

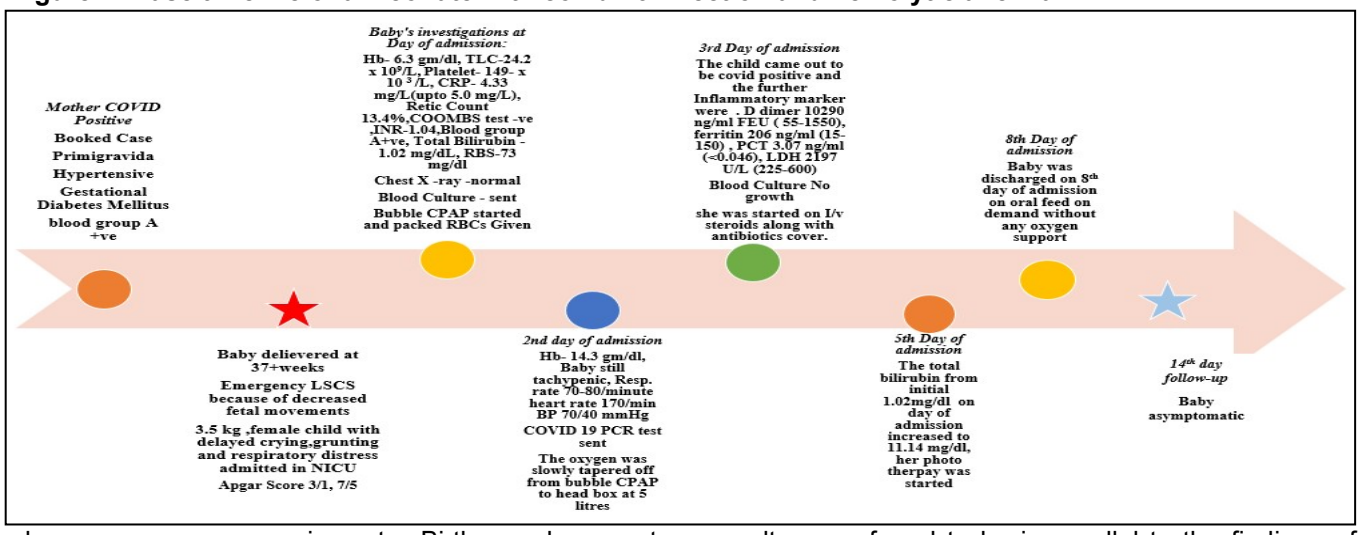
After delivery, out of 31 neonates born to COVID-19-positive mothers, 22 (71%) neonates required admission to NICU for various reasons. **Table II** shows the causes of NICU admission, symptoms of newborns at delivery and outcome in the study population. Out of 22 (71%) neonates who were admitted to NICU, 8 (36.3%) were admitted for less than three days, 7 (31.8%) were present in NICU for 4 -7 days, and 7 (31.8%) required observation in NICU for more than seven days. The first PCR test was positive for 6 (19.4%) neonates, and the second virology test was positive for only 1 (3.2%) neonate. Furthermore,17(54.8%) neonates who were PCR negative in the first virology test and had a hospital stay of fewer than three days, their caregivers refused to send the neonates' second PCR test. At 14 days of follow-up, 29 (93.5%) neonates were asymptomatic. However, 2 (6.5%) had symptoms of respiratory distress. Three neonates born on the 28th, 29th and 33 weeks of gestation expired. They all were COVID PCR negative, done after 24 and 48 hours of delivery per the methodology's protocol. The mortality rate was, therefore =9.7%. Two newborns remained admitted for > 14 days, and one expired within 48 hours of delivery due to severe hyaline membrane disease. After delivery, 13 (41.9%) neonates were exclusively breastfed, 3 (9.67%) were kept on mixed feeding practices (Mother feed and formula milk), and 15 (48.3%) neonates received formula milk. Furthermore, it is also worth mentioning that one of the term neonates born during the study period had

Table II: Symptoms of neonates at birth, the reason for NICU admission and the outcome

Symptoms at the Time of Birth		
	Yes	No
Fever	7 (22.6)	24 (77.4%)
Respiratory Distress	20 (64.6%)	11 (35%)
Cyanosis	0	31 (100%)
Asymptomatic	13 (42%)	18 (58%)
Reason for NICU admission		
Preterm	11 (35%)	20 (64.5%)
Low Birth weight	10 (32.3%)	21 (67.7%
Congenital pneumonia	7 (22.6)	24 (77.4%)
Observation	4 (13%)	27 (87%)
Neonatal sepsis	7 (22.6)	24 (77.4%)
Respiratory Distress	10 (32.3%)	21 (67.7%
Outcome of Neonates		

Discharged early(<7days)	Expired	Discharged late (>7 days)	Shifted with Mothers	LAMA
15 (48.4%)	3 (9.7%)	7(22.6%)	5(16.1%)	1 (3.2%)

Figure II: Case timeline of a neonate with covid-19 infection and hemolytic anemia



shown severe anemia at Birth; subsequent investigations revealed hemolytic anemia secondary to COVID infection. **Figure II** shows the case timeline of the neonates.

DISCUSSION

Due to the risk of development of congenital Covid-19 disease in newborns, data regarding the vertical transmission of this infection needs to be elaborated. The current study evaluated the outcome in neonates of 31 pregnant covid-19 positive mothers. Most literature does not support that Covid-19 can be transmitted vertically from diseased mothers to their neonates. In one study, the vertical transmission of covid-19 was assessed, and the findings revealed no risk of vertical transmission, and the viral markers in the placenta were not detected[13]. However, it was later refuted by Seymen CM 2021[14] that the virus can enter the placenta and cause severe pathological changes. Contrary to these findings, 6 (19.4%) neonates were Covid-19 positive in our study.

In a systemic review, it was concluded that there are chances that if the disease occurs in the 3rd trimester of pregnancy, a small number of neonates at Birth may get infected. Furthermore, it was mentioned in the data regarding mothers who got an infection in the first trimester that the outcome of those pregnancies did not show any potential risk for consequent fetal morbidity and mortality[15]. These findings are similar to those of our study. However, we found that one baby who was COVID-19 PCR positive developed severe anemia with positive inflammatory markers strongly suggestive of vertical transmission of infection. The correlation of vertical transmission was further strengthened by negative bacterial culture. However, to confirm vertical transmission, we need to check the COVID variants at the genomic level to identify the similarities in the genome of the mother's and neonates' viral samples, which was a limitation of our study. Regarding the second serological analysis of Covid-19 in three COVID positive neonates, our results were found to be in parallel to the findings of Martínez-Perez O et al., 2020 who reported that when evaluated for the second time after 48 hours, the neonates were covid-19 negative despite being positive in the first PCR. The reason for a negative PCR test in these neonates has not been identified yet.

In our study, there was no relation between the mode of delivery of COVID-19-negative and covid-19 positive mothers. However, neonates born to mothers who underwent Emergency lower segment caesarian section required NICU admission[16]. The most common reasons for NICU admission were preterm delivery, respiratory distress and low birth weight. The same results were documented by Anand P et al.[17]. It is established in the research literature that neonates born to Covid-19 mothers with late disease onset, i.e. in the late third trimester, may not develop symptoms of the disease. It is also highlighted that the neonates should not be kept separately from the mothers, and rooming in should be encouraged with standard precautions[18]. In our study, there was a positive outcome, i.e. out of 31 neonates, 27 (87.1%) were discharged, three expired, and one left against medical advice. Our findings are consistent with those of other studies documented in the research literature[19,20]. Our study found that one covid-19 positive neonate had hemolytic anaemia. To the best of our search, we could not find a similar case elsewhere in the neonatal age group in the online literature. However, similar findings were reported by Kosmeri C et al. in older children secondary to covid-19 infection[21].

CONCLUSION

The possibility of vertical transmission of covid-19 cannot be ruled out, primarily if the mother acquires infection in the last trimester. Even with positive PCR, most neonates had good outcomes, with fewer or no disease-specific symptoms. However, morbidity and mortality seemed to be directly related to prematurity

and low birth weight. Furthermore, it is recommended to encourage breastfeeding in neonates born to COVID-positive mothers with standard precautions.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Zafar F: Study design, literature review, data collection and drafting of the manuscript
Ehsan S: Contributed to the idea, data collection and critical manuscript review.
Khan L: Contributed to data collection and literature review
Fawad B: Analyzed data and contributed to data interpretation

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Original Article

Sero-prevalence and Perception of Brucellosis among High-Risk Groups, A Cross-sectional Study

Mohamed Osman Elamin Bushara[1]

ABSTRACT

OBJECTIVE: To determine the seroprevalence of Brucellosis and assess the disease's perception among high-risk groups.

METHODOLOGY: This descriptive cross-sectional, community-based study was conducted among 125 high-risk group individuals vulnerable to infection with Brucellosis and compared to 50 individuals of non-risk groups. Obtained sera were tested for the presence of antibodies to Brucella using the Rose Bengal plate test and standard tube agglutination test. A questionnaire was designed to assess the risk behavior and previous knowledge about the disease.

RESULTS: The males were 78%, and the females were 22%. The seropositivity of Brucellosis was 3.2% among people at high risk and nil for the non-risk groups. The disease was prevalent in dairy farmers, which was more prevalent in dairy farmers (2.4%) and slaughterhouse workers (0.8%). All infected persons were males; the disease is most common in the age group 15-25 years.

CONCLUSION: There was a statistically significant correlation between education level and the prevalence of Brucellosis. The two laboratory methods used to diagnose Brucellosis gave the same results. Routine checkups and education of at-risk individuals are recommended to help control the disease.

KEYWORDS: Brucellosis, High-risk groups, Perception, Sero-prevalence, Sudan, Weil Flix Test Method, Widal Method

INTRODUCTION

Brucellosis is an infectious disease previously known as Rock, Cyprus, Undulated, Gibraltar, Malta, and Mediterranean[1]. Bacteria of the genus Brucella are major zoonotic pathogens responsible for considerable human morbidity in areas where they are endemic in livestock. Either direct contact with infected animals or their infected products infect humans[2]. People working in jobs requiring frequent contact with animals or meat, such as slaughterhouse workers, farmers, veterinarians, and dairy workers, are at high risk[1]. Brucellosis has been documented worldwide over several years in various wildlife. Recently Brucellosis has been reported in a wide variety of marine mammals. A significant consideration regarding Brucellosis in wildlife is distinguishing between spillover infection from domestic animal management and environmental factors. Brucella organisms are shed in milk, urine, and vaginal discharges, contaminating the environment. The infection occurs through ingesting the non-boiled milk of infected animals, contact with vaginal discharge, urine, stools, and the blood of infected animals, breached skin and the mucous membrane of the conjunctiva, and inhalation[3].

In this study, we aimed to determine the seroprevalence of Brucellosis among the people in the high-risk group in Atbra, River Nile State, Sudan, and to assess the knowledge of the study population about the disease and to compare the two laboratory methods that are commonly used in the diagnosis of Brucellosis (Rose Bengal Plate (RBP) method and Standard tube agglutination (STAT) Method).

METHODOLOGY

This descriptive cross-sectional community-based study was conducted among people with a high risk for acquiring Brucellosis compared to non-risk groups. The study was conducted at the veterinarian laboratory research and special lab in Atbara City, Sudan. The investigation for Brucellosis was conducted by two methods Rose Bengal Plate (RBP) method and the Standard tube agglutination (STAT) Method. The Study area was Atbara City, located in River Nile State in Sudan about 310 km north of Khartoum and lies on the junction of River Nile and Atbara River with a total population of 134568. The participants were recruited from the security forces' agricultural project, the slaughterhouse, the veterinary hospital, Um-Altuor, and the Atbara market, Sudan. The study was conducted in June 2011. The sample size of 175 individuals, including 125 from at-risk groups and 50 recruited from non-risk groups. The high-risk group was as follows: veterinarian, dairy worker, butcher, farmer worker, and slaughterhouse.

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We selected 25 participants per group (A total of 125 participants). The control group was recruited from Nile Valley University Employees and housewives in the same area. The number of the control group was 50 individuals. During the study period, the samples were taken by simple random technique. We included a healthy population who agreed to participate in the study and excluded the very elder population and anyone who refused to participate. The data were collected by a pre-tested questionnaire.

Methods used for detection of Brucellosis:

Method 1 Rose Bengal Plate (RBP): Acid antigen stained with Rose Bengal plate being susceptible and rapid for human diagnosis, the test is simple to spot agglutination and buffered to low PH 3.65 + 0.05. (O.I.E).

Principle: The test is based on antigen-antibody reaction. If the antibody in the serum corresponding to the antigen, the reaction appears as visual agglutination. Agglutination means a positive result; if there is no agglutination, it means a negative result. The Procedure was done according to the manufacturer's instructions.

Blood sampling: we took 3 ml of venous blood drawn from each participant in a dry glass tube by standard technique and biosafety. We prepared the serum by centrifugation of the blood. After that, we took the reagents from the refrigerator and stood till the reagents became room temperature. Then we took 25µl of serum volume by automatic pipette into the white plastic plate and gently shacked the reagents, and an equal volume of antigen was placed in the serum spot, immediately, after that mix suspension using a glass plastic rod for each test to produce an oval or circular zone. The mixer was rotated gently for 4 minutes. After 4 minutes, the agglutination was read; any visible reaction was considered positive. Comparing with positive control in the patch.

	T	C
R	25 µl	25 µl
S	25 µl	-
C	-	25 µl

R: Rose Bengal reagent, S: Serum, C: Positive control.

Method 2 Standard tube agglutination method (STAT): These stained bacteria antigen suspensions were killed and stained to enhance the reading of the agglutination reaction. There were two bottles, one containing Brucella abortus, and the other was Brucella melitenisis and control rabbit sera. Principle: The Ag/Abs reaction was based and gave agglutination. We required: 8 small plastic test tubes for each sample, dispensing pipette 0.005 ml – 1 ml, 0.85% normal saline, and a water bath. Procedure: we placed eight test tubes in a rack and labeled 1 – 7 and control, we put 1.9 ml of 0.85 ml of normal saline into the first tube (tube 1) and 1 ml saline in the remaining 7 tubes. Then, we added 0.1 ml of undiluted serum into the first tube and mixed them well. Then, we transferred 1 ml from the first tube into the second tube and mixed it well. This process, as double serum dilutions were continued, and 1 ml was discarded from the seventh tube. The eighth tube was the control and contained 1 ml of saline only. The tubes were shacked well, and we added one drop of the undiluted antigen suspension to all the tubes, including the control, and mixed them well. The tubes were incubated in a water bath at 37˚C for 24 hours. After incubation, we carefully removed the rack, and agglutination was observed. The titer was taken as the last tube showing agglutination and then considered a positive result. While if no agglutination occurred, then it was considered a negative result. We used negative and positive control to ensure the reagent worked well. This procedure used for two antigen Brucella melitenisis and Brucella abortus.

Statistical analysis

We enter data, clean, and analyze using the statistical package for social sciences (SPSS) Version 26 (IBM Corp., Armonk, NY, USA). In the descriptive analysis, we analyzed the socio-demographic variables and presented them as frequencies, and in inferential statistics, Chi-square and t-tests were applied. We considered statistically significant for all two-sided p-values was ≤ 0.05 and 95% confidence interval (CI).

RESULTS

In this study, we identified that headache and joint pain were the most common symptoms of Brucellosis among the patients 48 (27.4%) and 42 (24%), respectively, while the lymph node was the least sign 3 (1.7%) (**Table I**). The most common age group of positive results was 15-25 years in 2 patients. (**Table II**). Most of the participants were males, 137 (78%), and the female were 38 (22%) (**Table III**). We identified that the most educational level was university level 57 (32.6%). The study reported that there was a significant correlation between education level and the prevalence of Brucellosis (**Table IV**).

Table I: The frequency distribution of symptoms and signs of Brucellosis among the participants

Symptoms and signs of Brucellosis	Yes Frequency (%)	No Frequency (%)
Headache	48 (27.4%)	127 (72.6%)
Joint pain	42 (24%)	133 (76%)
Neck pain	33 (18.9%)	142 (81.1%)
Testes pain	38 (21.7%)	131 (74.9%)
Fever	29 (16.6%)	146 (83.4%)
Liver and spleen	5 (2.9%)	170 (97.1%)
Lymph node	3 (1.7%)	172 (98.3%)

Table II: The association between the Rose Bengal method and the age group of the participants

Rose Bengal		Age group						Total
		15-25	26-35	36-45	46-55	56-65	66-75	
Positive	Count	2	1	0	0	1	0	4
	% within Rose Bengal	50.%	25.%	0.%	0.%	25.%	0.%	100.%
Negative	Count	35	54	45	25	7	5	171
	% within Rose Bengal	21.%	32.%	26.%	15.%	4.%	3.%	100.%
Total	Count	37	55	45	25	8	5	175
	% within Rose Bengal	21.%	31.%	26.%	14.%	5.%	3.%	100.%

P-value < 0.05

Table III: the association between the Weil Flex reaction method and the sex of the participants

Well Flexreaction Method		Sex		Total
		Male	Female	
Positive	Count	5	0	5
	% within well Flex reaction	100.%	0.%	100.%
Negative	Count	132	38	170
	% within well Flex reaction	78.%	22.%	100.%
Total	Count	137	38	175
	% within well Flex reaction	78.%	22.%	100.%

P-value < 0.05

Table IV: Relationship between education level and mode of transmission

Educational level		Transmission				Total
		Raw milk	Raw meat	Both	I don't know	
Illiterate	Count	2	1	5	23	31
	% within education	7.%	3.%	16.%	74.%	100.%
Basic	Count	6	0	3	24	33
	% within education	18.%	0.%	9.%	73.%	100.%
Secondary	Count	15	3	14	22	54
	% within education	28.%	6.%	26.%	41.%	100.%
University	Count	20	3	22	12	57
	% within education	35.%	5.%	39.%	21.%	100.%
Total	Count	43	7	44	81	175
	% within education	25.%	4.%	25.%	46.%	100.%

DISCUSSION

This study was conducted in northern Sudan to determine the seroprevalence and perception of Brucellosis among risk groups. One hundred and seventy-five individuals were selected randomly. One Hundred and twenty-five individuals from at-risk groups and 50 from control groups. The Sero-prevalence of Brucellosis among the study group was 2.3%. This result is less than the fact result because the standard tube agglutination method gives a false negative in chronic Brucellosis. So, it must be confirmed by other common techniques such as the 2ME method or the (PCR) method. This result is lower than that reported by Ismail A 2007 (11.4%) [4], similar to the study by Mohammed N et al. (3.3%) [5] in Bangladesh.

The study revealed the highest prevalence of Brucellosis among dairy workers and slaughterhouse workers, and this is similar to that reported by Ismail A 2007[4], and Muhammad N et al.[5]. The study reported that there was a significant correlation between educational level and the prevalence of Brucellosis and these findings disagreed with Cetinkaya Z 2005[6] in western Anatolia. The study documented a

statistically significant association between the prevalence of the disease and previous knowledge of transmission of the disease, knowledge of Brucellosis, and causative agent. However, the study showed no difference between the two methods used in the detection of Brucellosis, as they gave the same results. This fact is in agreement with Muhammad N et al.[5].

CONCLUSION

From our findings and relevant previous studies, it is clear that there are some significant reasons leading to this increase in the prevalence of Brucellosis, such as educational status, prior knowledge, and being at occupational exposure, such as dairy and slaughterhouse workers. These risk factors should be addressed to control and prevent the disease. The health authorities need to highlight the health education of the population, especially those in contact with animals, to raise their awareness about Zoonotic diseases, the method of transmission from animal to human, and how to treat the residents of animal and their products. The role of animal health and veterinary health care can be strengthened by early discovering and excluding the infected animals from the herd. Additionally, the health authority should start treating infected animals and humans promptly, following up with the livestock, and advising the animal owners about the methods to be used in diagnosis and treatment. Furthermore, the population needs to be educated about avoiding drinking unpasteurized milk and eating raw meat, as this is a social habit present in specific communities. Moreover, the vaccination of animals is crucial to eradicating the disease.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Bushara MOE: All research was done by the principal author

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Original Article

Effectiveness of Mulligan's Movement with Mobilization and Muscle Energy Technique on Pain, Functional Status, and Depression in Students with Sacro Iliac Joint Dysfunction

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ABSTRACT

OBJECTIVE: The study aims to identify the effectiveness of Mulligan's movement with mobilization and Muscle energy techniques on pain and functional disability in students with Sacro Iliac joint dysfunction.

METHODOLOGY: This randomized control trial was conducted on college students with sacroiliac joint dysfunction recruited from KMCH Institute of paramedical sciences, Coimbatore, Tamil Nadu, India, from August 2019 to July 2020. Group A included 16 subjects and Group B with 15 subjects. The subjects who fall into the inclusion criteria were included, and subjects who are willing to participate in the study were included in this study. Neurological signs, Radiating pain below the knee, recent surgery & fracture around the hip, and hypermobile joint were excluded. Group A received Mulligan's mobilization and core stability exercise; Group B received muscle energy technique with core stability exercise. Outcome Measures: Pain and functional disability were measured by the modified Oswestry disability index; Kinesiophobia was by the Tampa scale.

RESULTS: The level of significance was 0.05. The mean values of the post-test show marked improvement between the groups, with a p-value of 2.69 for functional disability and a p-value of 0.45 for pain. Significant progress was observed in participants who received Mulligan's movement with mobilization. Statistical calculation was done with the help of SPSS version 18.

CONCLUSION: Mulligan mobilization is more effective than the muscle energy technique in managing sacroiliac joint dysfunction.

KEYWORDS: Sacro Iliac joint dysfunction, Mulligan's movement with mobilization, Muscle Energy technique, modified Oswestry Disability Index, Tampa scale for kinesiophobia

INTRODUCTION

Musculoskeletal disorders are common in all groups, with the prevalence in India being 21.3%. Sacroiliac joint dysfunction has been linked to low back pain for many years[1]. 15% to 30% proportion of lower back pain cases are thought to be related to the sacroiliac joint[2]. The mechanics of the spine and pelvis can theoretically be altered by excessive or restricted mobility at the SIJ, leading to pain[3].

Mennell introduced the term "joint dysfunction" for arthrokinematics dysfunction in the absence of pathological alterations in the joints, including capsules and ligaments[5], and linked muscular discomfort and muscle spasms to challenges with typical arthrokinematics mobilization in joint capsules, which restrict joint movement when participants tried to move joints exhibiting symptoms of joint dysfunction[6].

"Sacroiliac dysfunction syndrome" refers to sacroiliac joint abnormalities where a biomechanical issue would be present but no obvious lesion[5]. The pain pattern from the sacroiliac joint is usually below the L5 region (the most specific area is the Fortin area), radiating posteriorly towards the toe, and occasionally mimicking sciatica[3,4]. SIJDF sufferers described pain made worse by bending, sitting, driving, standing, or walking. These actions can also make the pain go away. Although the disease can be unilateral, it often favors the right side when it is bilateral [6].

Mobilization can provide a mechanical effect, such as stretching or rupture of constricted tissues[7], as well as a neurophysiological result to alleviate muscle discomfort and guarding. According to a study, the gate control theory put forth by Melzack and Wall can account for the physiological consequences of joint mobilization[8], which aims to increase the joint Range of motion and reduce discomfort. By blocking the pain

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signal conveyed by thin filaments, a sluggish stimulus in conduction velocity, while proprioceptive neurons of thick filaments are stimulated, the vicious cycle of pain and spasm can be halted[6,7].

Stabilization and management of sacroiliac joint problems are achieved through manual therapy. Research has established the efficacy of Mulligan's manual treatment technique at peripheral joints, known as mobilization with movement (MWM)[8]. The effectiveness of mobilization with actions in managing diseases and joint dysfunction has been shown[7].

The joint is repositioned during mobilization with movement to correct instability, enabling the joint to track normally. Subsequent research to date also reveals that the processes underlying the effect of MWM are based on mechanical dysfunction, necessitating the repair of positional faults[9,10]. A typical conservative method for treating various degenerative disorders of the spine, especially lumbopelvic pain (LPP), is the muscle energy technique (MET)[11].

The muscular energy technique is considered moderate for restricting movement in the spine and extremities[11]. With the muscular energy technique, the patient must actively contract their muscles confidently while the practitioner administers a counterforce that prevents movement. The Muscle energy technique has long been preferred for treating pelvic asymmetry and muscle imbalances in the lumbopelvic region[12]. Unfortunately, fewer studies have investigated the effectiveness of the Muscle energy technique. The study aimed to determine the effect of MWM and Muscle energy techniques on selected variables in students with SI joint dysfunction.

METHODOLOGY

A randomized controlled trial was conducted on college students with sacroiliac joint dysfunction recruited from KMCH Institute of paramedical sciences, Coimbatore, Tamil Nadu, India, from August 2019 to July 2020. A simple random technique was used to select the samples. Group A included 16 subjects and Group B with 15 subjects. Subjects who met the criteria were recruited in this study: A Minimum of 3 out of 6 positive sacroiliac pain provocation tests: Fortin's finger test. Gaenslen's Test, FABER Test, Thigh Trust Test, joint compression test, and distraction test. The age group of 18-26 years, both gender, subjects with clinical or subclinical sacroiliac joint dysfunction, and subjects willing to participate in the study. The subjects with neurological signs, diagnosed with other than SIJD, recent fractures & surgeries around the hip, hypermobile joint, and malignancy, were excluded.

Thirty-one eligible subjects were selected from the population and aligned into two unequal groups. All the participants were obtained with consent forms, and eligible participants were randomly assigned by lottery method into Mulligan's movement with a mobilization group with sixteen subjects and fifteen to the Muscle energy technique group by purposive sampling technique. Subjects were requested not to receive any other treatments or exercises for treatment (4 weeks). The participants in group A were administered Mulligan's movement with mobilization[9,10]. The participants were instructed to lie prone, and the therapist's hands were positioned to perform the techniques.

Position of the therapist for an anterior innominate fault: Standing directly in front of the patient's pelvis on the side of non-affected SIJ. Stabilizing hand: palms down on the sacrum, fingers pointing caudally so that the ulnar border is directly next to the SIJ on the same side. On the side of the affected sacroiliac joint, the fingers of the hand used for mobility are around the anterior part of the ASIS. The mobilizing force applied to the anterior aspect of the ASIS. **For a posterior innominate fault:** Mobilizing hand: The lowermost hand was used as a moving hand, and its thenar eminence was positioned just medial to the prominent portion of the posterior iliac crest; thus, the fingers pointed outward, the same side's heels were employed to rotate or glide laterally toward the innominate about the sacrum. The second hand's palm can either stabilize the rest of the pelvis or support the mobile hand and aid in the execution of the lateral glide. Three sets of this approach, each with ten repetitions, were administered for 12 sessions on alternate days. The pain is a result of the mobilization being performed in functional positions[10]. The exercise was done while standing and walking for participants who experienced sacroiliac joint pain when walking, which is thought to be caused by an anterior or posterior innominate defect.

Self-Mobilization: At the end of the treatment session, self-mobilization was taught to the patient for anterior innominate: The patient was in all fours position with a towel under the ipsilateral knee and asked to sit on their feet with the hands relatively fixed on the couch, which produces the postero-lateral glide. For posterior innominate, the towel was placed on the contralateral knee and asked to sit on his feet, providing an anteromedial glide.

The participants in group B were given the Muscle energy technique[11,12]. The participants were told to lie prone. The hands are set up to extend while lying passively. Muscle energy technique (MET) exercises include post-isometric relaxation techniques for spinal stabilizers like the erector spine and hamstrings, anterior stabilizers like the Iliopsoas muscle, which stabilizes the spine anteriorly and regulates the lumbar pelvic rhythm, and lateral stabilizers like the quadrates lumborum muscle[11]. It was administered thrice for 12 sessions, each position held for 7–10 seconds. The limitation barrier was then identified, and the subjects were instructed to do a 20–30% isometric contraction, maintain it for 7–10 seconds, and then relax for 2–3 seconds. Instructions for proper breathing were given. Then, three times per session,

the limb was moved past the restriction barrier on an exhalation and kept there for 10 to 30 seconds[12,13].

The outcome measures are Pain, Functional status & Depression in students with SI joint dysfunction. Pain and disability were assessed using a modified Oswestry disability index[14,15,] and Depression was assessed using the Tampa scale for kinesiophobia[16]. These were evaluated before and after the intervention. An independent t-test analyzed the obtained data. Both an independent and paired t-test were used to analyze the data.

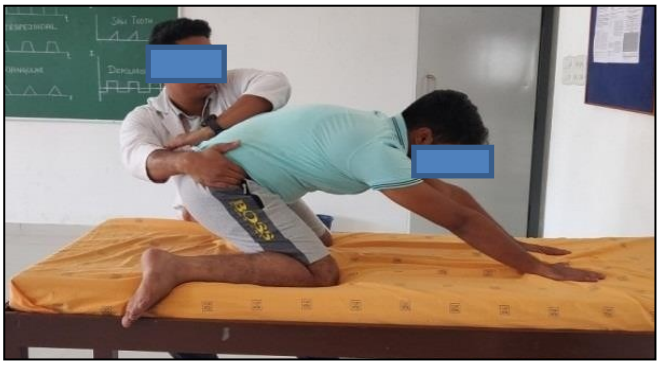
RESULTS

The results of this study were that Mulligan's movement with mobilization along with core stabilization had a significant effect in improving pain, functional status, and Depression when compared to the muscle energy technique with core stability exercises.

Independent t-test reveals no significant difference in the pre-test mean of Group A and Group B. Regarding the post-test, there was a significant difference in the mean value of Group A & Group B. Group A had more improvement than Group B. **(Table I)**

The post-test mean value revealed an improvement in both groups. Still, the more significant improvement was observed in group A, which received Mulligan's movement with mobilization, where the values of the modified Oswestry disability index were found to be 12.87% from 24.75%, and group B which showed an improvement level from 26% to 17.06% and the Tampa scale score for Kinesiophobia showed a difference of 30.25 from 39.37 in group A and group B showed no percentage difference of 30.26% from 30.25% were found following the treatment. **(Table II)**

Picture II: Mulligan mobilization for anterior innominate dysfunction in the quadruped position



Picture III: Mulligan mobilization for anterior innominate dysfunction in sit-to-stand position

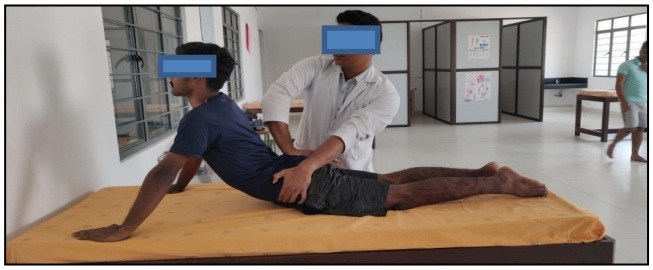
Table I: Mean difference in disability index (Group A and Group B)

Variable		SD for Mean value		Table 't' value	Level of significance
		Group A	Group B		
Modified Oswestry disability index	Pre-test	24.75±12.26	26.01±11.93	2.043	P>0.05 insignificant
	Post-test	12.87±5.56	17.06±7.92	2.043	P<0.05 significant

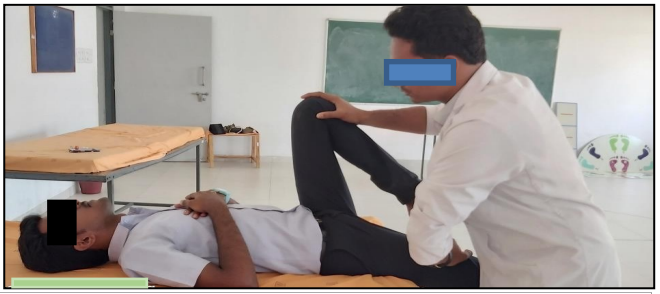
Table II: Mean difference IN PAIN (Group A and Group B)

Variable		SD for Mean value		Table 't' value	Level of significance
		Group A	Group B		
Tampa scale for kinesiophobia	Pre-test	39.37±4.84	38.93±6.94	2.043	P>0.05 insignificant
	Post test	30.25±3.70	30.26±5.36	2.043	P<0.05 significant

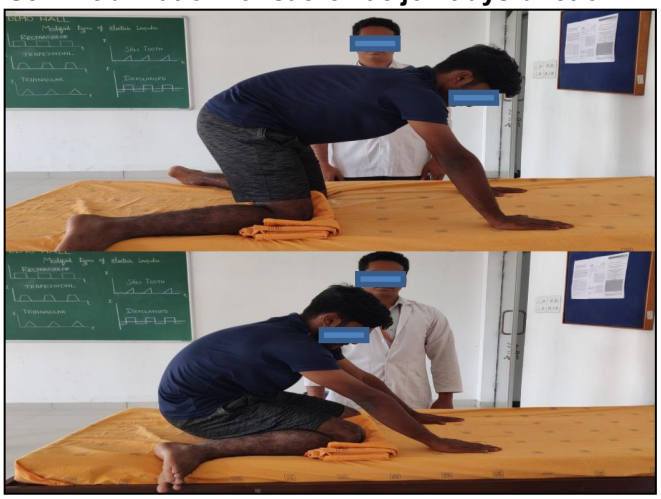
Picture I: Mulligan mobilization for anterior innominate dysfunction in lying



Picture V: Muscle energy technique for the



Picture IV:
Self-mobilization for sacroiliac joint dysfunction



Picture VI:
Muscle energy technique for hamstring muscle

Picture VII:
Muscle energy technique for erector spine muscle

DISCUSSION

Ishak NA 2017[16] speculate that muscle imbalance or ligament sprain may cause discomfort in the sacroiliac joint and related structures. The pain pattern is where the sacroiliac joint's pattern of discomfort is more frequently felt[18]. The study aimed to determine the effectiveness of MWM, muscle energy technique, and core stability exercises for students with sacroiliac joint dysfunction.

Picture VIII: Muscle energy technique for quadratus lumborum muscle

Sacroiliac joint dysfunction and stabilization have often been treated using manual therapy. Research offers a lot of evidence on Mulligan's mobilization[11]. Brian Mulligan claims that when accurate mobilization is used to treat sacroiliac joint pain, the pain usually goes away[18]. However, persistent corrective mobilization restores the pain-free function, and repeated applications result in long-lasting gains[20].

Based on Chaitow's description of the neurophysiology of the muscle energy technique, which shows a subsequent decrease in the tone of the agonist's muscle following isometric contraction, it is possible to extrapolate that the technique reduces pain[11]. Lewis supports this observation that the increased muscle tension of the involved muscles, which produces discomfort and dysfunction, is reduced by restoring the full length[19] of the muscle also because of the Golgi tendon organ responses to overstretching of the muscles by preventing further contraction[20].

Paired t-tests and unpaired t-tests were assigned to find the difference between the group and between the group, respectively, and the recorded values were examined and explained. There was a statistically significant difference within both groups and also independent- test showed a significant difference within both groups on the selected variables like MODI and TSKP.

Mulligan's movement with mobilization had a significant effect on improving pain, functional status, and depression[21]. In the table, pre and post-test values of the MODI and TSKP showed reduced pain, improved functional status, and Depression following four weeks of treatment[22]. The improvement is significant with $p<0.05$.

LIMITATIONS

The limitations of this study include: short-term follow-up only for four weeks, the limited age difference between 18-26 years, all types of sacroiliac joint dysfunction were taken, the amount of resistance applied by the patient during muscle energy technique was not quantified, and additionally, students were not permitted to change their regular schedules, which would have made their suffering worse while receiving treatment.

CONCLUSION

MET and core stability exercises and Mulligan's mobilization were equally beneficial in treating participants with SIJDF. However, Mulligan's mobilization group found a significant improvement concerning reducing pain intensity, functional ability, and Depression.

Ethical permission: Saveetha Medical College ERC letter No. 001/05/2019/IEC/SMCH.

Conflict of Interest: No conflicts of interest, as stated by authors.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR CONTRIBUTIONS

Sivakumar S: Concept, Resources, Data Collection and Processing analysis, Manuscript writing
Kamalakannam M: Supervision of research
Kalpana AP: Analysis, manuscript correction
Prakash J: Design materials, manuscript corrections
Gowtham R: Materials, Data collection and processing

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Original Article

Association of Upper Trapezius Pain with Perceived Exertion and Depression in Workers of the Food Industry

Saba Irfan[1], Wajeeha Mahmood[1], Tahir Mahmood[2*], Hafiz Zohaib Shahid[1]

ABSTRACT

OBJECTIVE: To determine the association of upper trapezius pain with perceived exertion and depression among food industry workers.

METHODOLOGY: It was an analytical cross-sectional study carried out from November 2021 to March 2022 after Ethical approval. One hundred food worker samples in Lahore were selected by a non-probability convenient sampling technique. The participants were male and female in the age group of 25-40, with 8-12 hours of duty time/day. The outcome tools were the Visual Analogue Scale, Borg Exertion Scale and Depression Inventory Scale. All the data was entered in SPSS V.25 for analysis of data. Quantitative variables are presented using Mean± SD (Standard deviation) and categorical variables with frequencies, while the association was determined using the Chi-square test.

RESULTS: In the current study, there were 76% male and 24% female participants with a mean age of 30.53±5.30 years. Among the participants, 32% had moderate pain, 24% had severe pain, 21% had strenuous physical activity exertion, and 19% had light physical activity exertion, But 21% had mild depression, and 9% had severe depression. There was a significant association between upper trapezius pain rating with physical activity exertion level at P= 0.007 and depression at P= 0.000

CONCLUSION: This study concluded that there was a significant association between upper trapezius pain, the level of Physical Exertion and Depression. The upper trapezius pain intensity increases the level of depression and physical exertion.

KEYWORDS: Association, Depression, Food Workers, Perceived Exertion, Pain, Upper Trapezius.

INTRODUCTION

Musculoskeletal disorders (MSDS) are problems within the tendon, ligaments and muscles. At the same time, intervertebral discs and vascular and nervous systems don't directly result from acute or instant events but affect an area unit put in step by step and inveterately. The primary factor in developing MSDS of the shoulder and neck is tiredness due to overuse of muscles[1]. Cooks and restaurant staff is at high menace for occupational musculoskeletal disorders (WMSDs) because of increased stress on the body related to getting ready materials and cooking. A high rate of WMSDs has become prevalent among food industry workers (FSWs) due to muscle overuse. Myofascial trigger points (MTrPs) are considered a common leading source of musculoskeletal pain and discomfort. These are the hyperirritable nodule and present with tenderness in the tight band of muscles, producing a massive contribution to the development of discomfort and leading to motor pathology. Among the upper quadrant, the upper trapezius (UT) is the most reported issue; most suffer from the development of MTrPs[2]. Occupational musculoskeletal disorders are the major reported causes of rigorous long-run pain and physical incapacity, mainly owing to adverse geographic point factors and awkward postures that may influence all body parts[3]. It is a fact that work-related neck disorders (WRNDs) are usually caused by poor positioning of head and neck muscles during long working hours[4]. E Wood E 2018[5] stated that systematic disorders were the leading reason for injury and lost time at work. Food service staff are exposed to a range of stressors together with repetitive manual labor, lifting, and forceful movements in addition to awkward postures and prolonged standing. In a recent study, chefs or cooks reported a 7% prevalence rate. Depression is related to underprivileged health behaviors and communal challenges.

Consequently, it's obligatory to order and build up anticipatory approaches for each disorder[6]. In the construction of workstations and work activities, the safety of the person performing the tasks should be kept in mind. Perception of effort has been deliberated for a spread of physical exertions, typically for exercise and rehabilitation programs[7]. The perceived rate of exertion (RPE) is a simple scale to assess muscle fatigue during daily activities. This scale is also called Borg rate of perceived exertion (BCR-10), and the Rate of perceived Discomfort (RPD) is obtained

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with a modified version. Many of the authors had stated that there is an association of muscle fatigue measures, including myoelectric indicators, time or endurance and rate of perceived exertion is moderate to high[8]. Mobbing has emerged inside the work and is more and more spreading. It affects the employees expressively (aggression, reduced resistance to worry, irritability, nervousness) and physically (fatigue, weakness, chronic fatigue syndrome and pains in several body elements). In contrast, severe bullying will even end in depression and suicide cases[9]. Glock CH 2019[10] stated that RPE scores of the arm, shoulder, and trunk were overstated because of the continual physical efforts of the staff over time; therefore, prolonged and repetitive packaging tasks were the main reason for subjective discomfort.

Upper trapezius muscle pain is a growing public health problem in food workers and other people that required to be addressed. To the researcher's knowledge, there is insufficient data on this topic in Pakistan. The current study aimed to determine an association of upper trapezius pain with perceived exertion and depression among food industry workers. This study will help to assess the association between upper trapezius pain and its associated factors among food industry workers. Results will help develop preventive measures in posture, limiting heavyweight, overuse of upper trapezius muscles and reducing working hours among them. They can get rid of the pain as well as depression in future, and it may also improve their work efficiency.

METHODOLOGY

It was an analytical cross-sectional study carried out from November 2021 to March 2022 after Ethical approval. One hundred food worker samples in Lahore were selected by a non-probability convenient sampling technique. The data was collected from different restaurants in Lahore, including Cakes & Bakes, United Restaurant, McDonald's and Big Man Pizza House. Male and female food workers aged 25-40 years, with work duration longer than eight months, unilateral and non-traumatic shoulder pain, tenderness of upper trapezius muscle more than twice over the past week and having 8-12 duty hours were included[11]. The sample size was calculated using $n= Z_{1-\alpha/2}\ \rho\ (1-\rho)/\ d^2$. where $Z_{1-\alpha/2=}$ is the standard normal variate (at 5% type I error ($p<.05$), it is 1.96, and at 1% error ($p<0.01$) considered significant below 0.05, hence 1.96 is used in the formula. P= expected proportion in population based on previous studies—and d= Absolute error or precision. We calculated Z= 1. 96, P=0.54, 1-p= 0.46 and D= 0.007. Using 54% proportion of the condition sample size is n=100.[2] While the participants with any preceding diagnosis of shoulder instability, shoulder fracture, any of the systematic illness, i.e. arthritic swelling, systemic lupus erythematous, shoulder surgery and systematic diseases, i.e. Epigastritis were excluded.

The outcome was measured using Borg-Perceived Exertion Scale, Depression Inventory and Visual Analogue Scale. The Borg rating scale is based on the assessment of sensation in a person that is experienced during any physical performance, including respiratory and heart rate and level of fatigue and sweating. Physical exertion is based on rating it from 6-20, providing an estimated heart rate depending upon the performed physical activity. On scale value of 6 indicates "no exertion at all", 12 to 14 "moderate exertion", and 20 "exertion at all" stop activity[12]. Major depressive inventory was used, which has 10 to 12 questions, and each question has six options, including "all the time" (5) to "no time" (0). A score of 4 or 5 on two of three scale items indicates "mild", and a score of 4 or 5 on two of 3 items indicates "moderate". In comparison, a score of 4 or 5 in all three items indicates "severe", and no of items reduced to nine indicates "major" [13]. The visual analogue scale was used for pain, with 0 to 10 scoring in which 0 shows no pain, 1-3 mild pain, 4-6 moderate pain and 7-10 severe pain[14].

Participants were assured that their personal information would not be disclosed following the ethical principles of human subjects. Participants in the study were informed, and the participants signed a consent form. SPSS version 25 was used for the analysis of data. Mean, and standard deviations were calculated for quantitative variables and frequency/ percentages for categorical variables, and the Chi-square test was used to determine the association between the outcomes.

RESULTS

In the current study, there were 76% male and 24% female participants with a mean age of 30.5±5.3 years. The participants' mean weight was 69±13.9 kg, and the mean height was 5.57±.38 feet. The Mean BMI of the participants was found to be 23.9±4.37.

Among the participants, 32(32%) had moderate pain, and 24(24%) were suffering from severe pain and, 21 (21%) were having hard physical activity exertion level, 19% had a light physical activity exertion level. While 48 (48%) had no depression, 21(21%) had mild depression, and 9(9%) had severe depression. **(Table I)**

Table I: Demographics

Category	Frequency (%)
Male	76(76%)
Female	24(24%)
Age Mean	30.53±5.30
Height(ft) Mean	5.57±.38
Weight(kg) Mean	69.03±13.91
Body Mass Index Mean	23.90±4.37

Ft= feet, Kg=Kilograms

The mean pain score was 3.90±2.81, Mean Depression Score was 20.43±9.18, and the Mean physical exertion score was 4.63±1.91. P value 0.007 indicates a significant association between Physical activity Exertion level and upper trapezius Pain Rating. **(Table II)**

Table II: Frequency Distribution of Pain, Exertion and Depression

Outcome	Category	Frequency (%age)
Pain Rating	No Pain	15 (15%)
	Mild Pain	29 (29%)
	Moderate Pain	32 (32%)
	Severe Pain	24 (24%)
Physical activity exertion level	No Exertion	7 (7%)
	Extremely light	8(8%)
	Very Light	13 (13%)
	Light	19 (19%)
	Somewhat Hard	16(16%)
	Hard (heavy)	21 (21%)
	Very Hard	10 (10%)
	Extremely Hard	5 (5%)
	Maximal Exertion	1 (1%)
Depression Level	No Depression	48 (48%)
	Mild Depression	21 (21%)
	Moderate Depression	22 (22%)
	Severe Depression	9(9%)

A P-value of .000 indicates that there is a significant association between upper trapezius pain with the level of Depression Level. The P-value indicates a significant positive association between pain score and depression score. Increasing pain increases the level of depression score among workers. **(Table III & Table IV)**

DISCUSSION

This study aimed to find an association between upper trapezius pain with perceived physical exertion and depression among workers in the food industry. Cooks and restaurant employees are at high risk for Work-Related Musculoskeletal Disorders (WMSDs) because of the increased stress on the body related to getting ready materials and cooking. This analytical study was conducted on a sample of 100 food workers, both male and female participants. There was a significant association of upper trapezius pain rating with physical activity exertion level and depression level among the participants. The current study found 56% moderate to severe depression among the participants due to upper trapezius tightness, and it was associated with exertion and depression among industry workers. Williams N 2017[12] anticipated factors related to biomechanical factors, stress level, and shoulder pain prevalence among workers. It was found that shoulder ache was directly related to biomechanical factors and the stress level perceived among the food industry workers. The perceived stress level was linked to the psychological demand of each population under study. Still, the social support of workers was correlated negatively to the level of perceived stress solely within the pharmaceutical producing website[20]. In the current

Table III: Association of Pain and Physical Exertion Level

Pain Rating	Physical activity Exertion level									Total	P-value
	No Exertion	Extremely light	Very Light	Light	Somewhat Hard	Hard (heavy)	Very Hard	Extremely Hard	Maximal Exertion		
No pain	2	2	3	1	4	3	0	0	0	15	
Mild Pain	4	4	6	6	4	5	0	0	0	29	
Moderate Pain	1	2	3	9	4	7	6	0	0	32	0.007
Severe Pain	0	0	1	3	4	6	4	5	1	24	
Total	7	8	13	19	16	21	10	5	1	100	

Table IV: Association of Pain and Level of Depression

Pain Rating	Depression Level				Total	P-value
	No Depression	Mild Depression	Moderate Depression	Severe Depression		
No pain	15	0	0	0	15	
Mild Pain	29	0	0	0	29	
Moderate Pain	4	21	7	0	32	0.000
Severe Pain	0	0	15	9	24	
Total	48	21	22	9	100	

P value is significant at ≤.01

study, participants have 21% had a hard physical activity exertion level, and 19% had a light physical activity exertion level due to work type and working hours with excessive physical exertion. Since food workers habitually carry foodstuff or supplies and very often pick up cuisine equipment, humeral elevation occurs with high incidence. So there might be Serratus anterior weakness that can lead to overwork of the upper trapezius and causes pain[15].

In this study, 30% had mild depression and severe depression combined, and a P-value = 0.000 indicated a significant association between depression and upper trapezius Pain. P-value has stated that there is a significant association between pain score and depression level reported by participants. Christiane H et al.[16] provided a unique and imperative insight into the association between food timidity, depression and comfortable work among women; this presents a significant public health apprehension, particularly as the high rate of food diffidence dramatically increases the chance of poor health and its development outcomes. In 2018, Pancardo P 2018[17] stated that bodily labor activities should reflect physical condition and environmental factors like hotness, dampness, and altitude; or factors affecting individual physical response within physical activities like breaking in and becoming accustomed. Khal KM 2020[18] observed muscle fatigue in infraspinatus, which was clinically significant because RPE, heart rate and perceived level of fatigue was correlated moderate to strong. This finding was important, as it assessed the sensations of an active shoulder movement during the exercise rather than the whole body response. Falla D 2017[19] undignified that during repetitive lifting activities, the upper trapezius muscle was shifting its activity distribution to the caudal region as a whole during the assessment. This adjustment in the allotment of activity to different muscle regions has crucial implications for the persistence and deterioration of neck-shoulder pain throughout repetitive tasks. Still, further, the middle of skeletal muscle activity was shifted to the caudal within the painful condition. In the current study, only 15% had no pain, but 85% had pain from mild to severe because 40% had hard-to-light exertion of physical activities at their jobs. Kumgai G et al[20] highlighted that neck pain might be a disabling condition in employees due to working hours and other associated factors.

This study was conducted on less sample and was confined to Lahore due to limited time, and all participants were selected from specific areas. Future researchers should take a larger sample from multiple settings and a sample from other cities to generalize the results.

CONCLUSION

This study concluded a significant association between upper trapezius pain with physical activity exertion and depression Level. An increase in pain intensity increases the level of depression and physical exertion.

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AUTHOR CONTRIBUTIONS

Irfan S: Concept & design, analysis, data collection, interpretation of data, drafting & revision

Mahmood W: Concept & design, analysis, interpretation of data, drafting & revision

Mahmood T: Concept & design, analysis, data collection, interpretation of data, drafting, final approval

Shahid HZ: Concept & design, analysis, data collection, interpretation of data, drafting & revision

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Letter to Editor

Addressing the Issue of the Decline in Nurses in Pakistan

Anny Ashiq Ali

Dear Editor,

The shortage of nurses is a global issue harming the international healthcare system. According to the WHO, Health care nurses deficiency is assessed to be 7 million and is anticipated to reach 12.9 million by 2035[1]. Around 57 countries in the mainstream continent of Africa, and Asia, were facing severe healthcare worker crises[2]. Thus policies should be made to cover this ratio of shortfall in Pakistan.

Concerns are articulated by the authors about the shortage in nursing, nursing downsizings, decreasing student admissions, faculty leavings, the ageing workforce, and worldwide hiring. Some of the reasons for these concerns are Poor management and infrastructure of the hospitals, low wages, lack of recognition of the values of nursing as a profession, scarce resources in education and clinical settings, medical dominance, and limited opportunities for career advancement, these all factors contribute to powerlessness and harassment among nurses, working in Pakistan. Thus, these are considered the main reasons for the migration of nurses to our country. In Pakistan, the management has paid little attention to developing an approach for nursing personnel. Thus, the passage of Pakistani-educated nurses overseas has an undesirable effect and has already damaged our healthcare system.

Following are some of the suggestions that are needed at the time to upturn the situation and hold nurses in our country. The first step in this regard is giving the nurses the choice of numerous career paths they can attain in their nursing profession. Besides this, steps should be taken regarding providing a workplace environment for the nurses where they are personally satisfied. Secondly, another initiative I think will substantially affect nurses' uphold, and new staff hiring is giving educational aid to existing employees. Apart from this, offering educational programs helps enhance efficiency, which positively impacts the institution's standards. Additionally, Health care system progression significantly affects nurse maintenance, design of a resilient educational framework, and enrichment of the public's insight towards nursing. Another way is by altering main health guidelines, enlightening unsuitable core dissemination and stopping relocation of nurses, and introducing applicable nursing management opportunities.

According to Maslow's hierarchy of needs, staff dissatisfaction initiates when their needs are in vain. Wages are considered an elementary need in any profession. Sufficient income helps people to fulfil their basic needs like food, shelter, and clothing for themselves and their families. On the contrary, low wages impose difficulty in meeting their basic needs and thus result in employees quitting their institutions. Therefore, organizations should offer fair wages policies, and modest assistance should be given to guarantee staff retention. This may helps the individual move to the next level of mindset when they seek job safety, and they also get freedom from all the anxiety and stress during work.

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Viewpoint

Viewpoint on the Application of Virtual Microscopy in Teaching at a Medical College in Saudi Arabia

Shahid Akhund

Conventional light microscopy (CLM) was the primary technique used to teach histology and pathology for a long time. However, it cannot view slides simultaneously, making it difficult for group discussions and cooperative learning. Multiple microscopes, glass slide production and storage, are expensive and require time-consuming maintenance. The invention of projectors and digital video cameras in the early 20th century made using CLM more effective. However, these tools could only be used by one person at a time, which prevented them from totally replacing CLM.[1] the 1980s, the initial digital images were generated from histological slides, but it wasn't until the availability of personal computers with sufficient memory capacity that digital microscopy advanced rapidly, this led to the development of imaging converter programs and servers that facilitated the uploading of virtual slides to the internet, enabling image viewing and zooming capabilities. Presently, numerous systems can generate high-quality virtual images of histological tissues. Users can browse the images using a mouse or joystick, allowing them to navigate through different areas of the slide and simulate the zooming functionality of an optical microscope[1,2].

Medical education incorporates immersive technologies that allow for human-computer interaction.[3] Virtual Microscopy(V.M.) refers to simulating microscopic glass slides or tissue sample slides using immersive technology. In V.M., a high-resolution scanner or a microscope with a digital camera is used to digitalize glass slides or tissue samples. A computer or server is used to upload the digital image, which may be viewed and edited. The images may be viewed in high definition, and one can navigate around the images and zoom in and out. It offers alternatives for marking up and annotating images for teaching or research. This technology enables sharing and cooperation amongst numerous users, including pathologists, researchers, teachers, and medical students, to see and analyze tissue remotely[2].

The use of V.M. in medical education is significant and has several benefits over CLM. Students can view digital slides using V.M. at any time and from any location, allowing them to study and learn at their speed; this might be extremely helpful in distance learning programs, where students might not have access to a physical lab or classroom environment. In addition, its expanded accessibility, enhanced collaboration and communication between students and teachers has improved learning outcomes[4]. It is simple for students to download digital photographs and annotations, get teacher feedback, and engage in online discussions. To build clinical skills and professional abilities. It is essential to create a learning atmosphere that is more collaborative and active. V.M. enhances student engagement and learning outcomes in medical education. According to studies, students who used V.M. performed better in exams and retained more information than those who used CLM[5-7]. This might be because students can more readily recognize and comprehend essential characteristics of the tissue specimens because of the improved capacity to alter and interpret digital images.

The V.M. is a valuable tool in medical education, offering improved access to tissue slides and enhanced learning outcomes. It has been widely adopted in medical curricula worldwide, with institutions incorporating it into their educational programs[8-10]. However, opinions among medical educators vary regarding its application and potential impact. Supporters acknowledge virtual microscopy's advantages over conventional microscopy, emphasizing features such as the ability to edit and annotate digital images, which enhance student engagement and conceptual understanding.[11] Research indicates that medical students using virtual microscopy perform better on exams and retain information more effectively[5-7]. Nevertheless, concerns exist among some educators. Critics argue that virtual microscopy may not fully replicate the tactile experience of traditional microscopy, potentially impacting the development of clinical skills. They also highlight the limitations of relying solely on digital images, which may restrict exposure to the full range of diseases and abnormalities found in real specimens. Some students have reported feeling distant from the specimens and prefer CLM[7]. As V.M. continues to evolve, further research is needed to optimize its integration, address concerns, and ensure its effective utilization in medical education.

Despite the conflicting opinions, educators and institutions worldwide are using virtual microscopy as a tool in medical teaching. As Gurcan MN et al.[10] discovered, virtual microscopy was well-liked by medical educators, indicating that it may improve uniformity, collaboration, and communication in pathology teaching. Virtual microscopy, according to Amer MG 2020[12], is not only a valuable tool for teaching histology but it can also be used to gauge student performance in online assessments.

In conclusion, medical educators' and teachers' perspectives on using virtual microscopy in medical education are varied and complex. Some see it as a valuable and advantageous tool to improve student

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involvement, understanding, and collaboration, while others worry about its limitations. To keep up with the fast-changing technological world, medical institutions must use virtual microscopy more widely in their curriculum. Virtual microscopy is an appealing alternative for educators of all levels because of developments in digital imaging and related technologies that have made it more available and affordable. Since educators want to employ the most recent techniques and technology to give their students the most effective and efficient educational experiences possible, the usage of virtual microscopy in medical education is expected to continue to develop and expand.

At the College of Medicine, Al Faisal University in Riyadh, Medical students are taught histology using CLM and virtual microscopy. Virtual microscopy is a valuable tool that has the potential to completely change how histology and other subjects like pathology are taught in medical school. I advise using a hybrid approach to teaching and learning with traditional and virtual microscopy combined with self-evaluation. The College possesses an Olympus microscope for scanning glass slides used in histology teaching. A virtual microscope image archive is created from high-quality scanned images. These images have annotations and are saved in a repository in the university's online learning management system accessed by authorized users. The users must download OlyVIA, a free image viewer software. Teachers and students can study the slides at their own pace and convenience. Students bring their digital devices to the histology lab sessions to view the photographs and the same tissue slide under a microscope. They view the tissue glass slides under a microscope at various magnifications while studying digital pictures. The students benefit from this mixed-method experience of real and virtual microscopy. The students are asked to complete an interactive self-assessment at each session's conclusion to gauge their comprehension of essential histological structures. In the self-assessment quiz, students identify different microscopic structures in the photographs and respond appropriately to the questions. Many students have responded favorably to this mixed method experience with self-evaluation exercise. They get the chance to connect more deeply and actively with the histological content, and it also lets them recognize where they still have knowledge gaps.

Our experience suggests that virtual microscopy can potentially change how histology and other topics linked to pathology are taught in medical school. We can improve students' comprehension of essential knowledge and skills by giving them access to CLM and virtual microscopy by providing high-quality, digital photographs and histology tissue slides to observe. Additionally, interactive tests and other stimulating educational activities can help maintain students' interest and motivation, resulting in more significant learning outcomes and enhanced patient care. Our unique mixed-method approach to teaching histology addresses the issue of optimizing the integration of V.M., addresses the concerns, and ensures its effective utilization in medical education.

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