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Early Detection of Chronic Kidney Disease: The Diagnostic Value of Ultrasonography



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Chronic kidney disease (CKD) refers to a lasting decline in kidney function, which is characterized by a glomerular filtration rate (GFR) lower than 60 mL/min per 1.73 m² or tracers of kidney injury at least three months [1]. Kidneys are important wedge-shaped organs located on both sides of the spine and eliminate waste products and surplus fluid in the body. CKD is a major health issue in the world, which is directly associated with the enhanced morbidity, death, and cardiovascular risk.

Increased renal echogenicity, a decreased parenchymal and cortical thickness, and a decrease in renal length are ultrasound changes which are signs of permanent structural injury. With the development of CKD, echogenicity increases whereas the size and cortical thickness of the kidney decrease. The length of the kidneys less than 10 cm is usually an indication of irreversible permanent damage[2].

CKD is classified based on GFR into five levels including Stage 1 (GFR 90 or more), Stage 2 (60 to 89), Stage 3a (45 to 59), Stage 3b (30 to 44), Stage 4 (15 to 29), and Stage 5 (below 15 mL/min/1.73 m²)[3]. Other complementary biomarkers like plasma 2-trace protein (BTP) and cystatin C enhance the precision of the diagnosis and help in determining the risk of progression [4]. Ultrasonography improves early detection and accuracy in staging that allows kidney failure to be prevented and intervened in time.

Social and lifestyle determinants such as alcohol and tobacco consumption, nutritional habits, sedentary habits, stress, social isolation, and financial constraints are significant risk factors of CKD. The major causes of end stage renal failure are hypertension and diabetes. Ultrasonographic evaluation is essential in the detection of irreversible changes in the kidney early and in the management of the condition as CKD progresses. Widespread adoption of ultrasound screening, accompanied by health education of the population, would help to considerably decrease the CKD burden. Promoting better health lifestyles and regular renal examination is an effective and realistic way of preventing kidney failure and enhancing the health outcomes of populations[5,6].

Management of the CKD associated with diabetes should be well-monitored both in terms of kidney damage and kidney functioning. Prevention of hypertension, diabetes, and obesity is thus important through the use of public health initiatives. Sufficient management of these conditions can greatly decrease CKD-incidence, whereas lifestyle changes such as balanced diet, physical exercise, and smoking should be taken into consideration as preventive measures.

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Original Article

Production and Evaluation of Polyclonal Antibodies Against Surface Protein of *Mycobacterium tuberculosis*: Diagnostic and Vaccine ImplicationsAmna Bibi¹, Mateen Ur Rehman^{1*}, Sheheryar Ahmad Khan¹, Muhammad Abu Baker¹, Barira Amir Ghauri¹ and Muhammad Fakhar Ghaffar²¹Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Pakistan²Department of Health Promotion and Public Health, Ulster University, Birmingham, United Kingdom

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ABSTRACT

Mycobacterium tuberculosis (MTB), which causes tuberculosis (TB), is a significant health threat in the world. The diagnosis of early active and latent TB is still a problem. Serological methods have potential benefits of offering a fast and cost-effective diagnosis, although they need to be further refined to become clinically reliable. **Objectives:** To generate and test polyclonal antibodies (pAbs) of surface proteins of MTB as a potentially useful immunodiagnostic application. **Methods:** Extraction of surface proteins of MTB was done with the use of PBS and Tween-20, and rabbits were immunized with antigen-adjuvant mixtures. The serum was gathered, and titers of antibodies were determined by an indirect ELISA. **Results:** Rabbits vaccinated against the surface proteins of MTB generated high-titer antibodies, and the median absorbance of the 1:1600 dilution was 1.42 0.15, which was significantly higher ($p < 0.001$) when compared to their controls that were not immunized. The endpoint titer, which was 1:12,800, was geometric, indicating that immunization and antibody were successful. **Conclusions:** The paper shows that it is possible to produce polyclonal antibodies against the proteins of the surface of *Mycobacterium tuberculosis*, which is going to serve as a basis for the future development of serological diagnostic instruments.

INTRODUCTION

Tuberculosis (TB) is an infectious disease that is transmitted by *Mycobacterium tuberculosis* (MTB) bacterium which is one of the most efficient pathogens in the human population and is mostly associated with the lungs [1-3]. It is a pathogenic bacterium that is a part of the *Mycobacterium* genus, which is characterized by its capacity to survive and reproduce in the human body (particularly the lungs). MTB is an aerosol infection whose primary mode of transmission is through inhaling of coughs or sneezes of an infected individual. The cell wall of MTB is

complex and rich in lipids that render it resistant to most widely used antibiotics, and among other factors, it causes its survival in the host immune system [4]. Nevertheless, TB may also disseminate into other body parts such as the lymph node, kidney, and brain, among many others, in a condition referred to as extrapulmonary TB. As mentioned in the WHO Global TB Report 2021, 25% of the world's population has immunologic evidence of previous infection with Mtb in surveillance testing, and 10 million people developed ATB in 2020. TB is one of the most lethal



infections globally and, together with malaria and HIV/AIDS, has affected human beings socially and economically most severely [5]. According to the latest mortality statistics, 1.4 million people died of TB in 2020, which makes it the second leading infectious cause of death after COVID-19. Despite being discovered over a century ago, an effective vaccine capable of preventing pulmonary TB in adults remains unavailable. Vaccination with *Bacillus Calmette-Guérin* (BCG), an attenuated *M. bovis* strain, protects children against disseminated forms of TB but offers variable protection against adult pulmonary infection. Although BCG remains the only licensed TB vaccine, its limited efficacy in high-burden regions underscores the urgent need for more effective immunization strategies [6, 7]. Recent data indicate a possible underestimated role in TB protection by humoral immunity as well as cell-mediated immunity. The development of antibodies against *M. tuberculosis* proteins has been studied by many researchers, mostly in the field of diagnostic and immunological uses [8, 9]. In the first stages of infection, the immune system is mainly focused on surface and secreted proteins of MTB that are the main virulence factors and host-pathogen interactions [10]. The study made use of surface proteins of *M. tuberculosis* to produce polyclonal antibodies in an animal model. Surface proteins play an important role in virulence, adhesion, and signaling [11]. Host immune modulation has been linked to surface proteins of MTB, including pili, antigen85 complex, hyperpolarized antigenic glycoprotein of 19 kDa size, and Proline-Glutamic acid proteins [12]. The relevance of these surface-exposed proteins in the formation of antibodies can be used in the development of better diagnostic reagents and next-generation vaccines. The suggestion that protective antibodies might be used to block the progression to persistence induced by the primary infection has revived interest in the study of the humoral component of TB immunity [13].

Tuberculosis remains a major global health burden, and despite advances in molecular diagnostics, early, rapid, and cost-effective detection particularly for latent and early active disease continues to be challenging. Current serological assays lack sufficient sensitivity and specificity, largely due to suboptimal antigen selection and incomplete understanding of humoral immune responses against *Mycobacterium tuberculosis*. Although surface-exposed MTB proteins play a crucial role in host-pathogen interactions and represent promising immunological targets, their application in reliable immunodiagnostic platforms remains underexplored. In this context, the present study aimed to address this gap by generating and evaluating polyclonal antibodies against MTB surface

proteins and assessing their immunoreactivity using an indirect ELISA, thereby providing foundational evidence for the potential development of serology-based diagnostic tools.

METHODS

The experiment was carried out at the Institute of Molecular Biology and Biotechnology, the University of Lahore, Pakistan, from May 2024 and October 2024. A modified buffer extraction technique was used to isolate surface proteins of *Mycobacterium tuberculosis* H37Rv. In a few words, bacterial pellets were washed with the phosphate-buffered saline (PBS; 10 mM, pH 7.4) twice and resuspended in the PBS with 0.05% (v/v) Tween-20 inclusion (Sigma-Aldrich, USA). The suspension was left rotating at 4°C and 1 hour after incubation, centrifuged at 10000 rpm and 10 minutes at the same temperature. The solubilized surface proteins in the supernatant were then collected, filtered with the use of a 0.22 µm syringe filter, and the concentration of protein was measured through Bradford assay (Bio-Rad, USA). The purified antigen was aliquoted and kept at -20°C until its use. Complete Adjuvant (FCA) and Incomplete Adjuvant (FIA) were procured from Sigma-Aldrich (Cat. No. F5881 and F5506). The choice of these particular adjuvants was based on their established immune-stimulatory activity in the production of polyclonal antibodies in rabbits. To achieve a total of 1 mL in each rabbit, a purified antigen was emulsified with an equal amount of adjuvant (1:1 v/v) in every immunization. The emulsion was made through the gentle mixing with the help of a glass syringe till a stable water-in-oil suspension was obtained. This experiment involved six healthy adults of New Zealand White rabbits (2.0- 2.5 kg, female). The immunized group had three animals, and the controls, or non-immunized, had three animals. All animal experimentations were carried out in accordance with the international animal welfare regulations. Ethical approval was secured at the University of Lahore, according to the ARRIVE and OECD animal care requirements. The immunization mixture was administered subcutaneously (s.c.) at multiple dorsal sites using a sterile 1 mL syringe. The immunization protocol consisted of five injections over two months: Day 0: 100 µg antigen + FCA (primary immunization), Days 14, 28, 42, and 56: 100 µg antigen + FIA (booster doses), and Control rabbits received PBS mixed with the same volume of adjuvant. This schedule was designed to allow sustained antigen release and effective antibody production while minimizing tissue stress. Two weeks after the final booster, blood samples (approximately 5 mL per animal) were collected via cardiac puncture under light ketamine anesthesia (35 mg/kg). Blood was allowed to clot at room temperature for 30 minutes, followed by centrifugation at 5000 rpm for 10

minutes at 4°C. The resulting serum was collected, aliquoted (1.5 mL micro-tubes), and stored at -20°C until further analysis. An indirect ELISA was conducted to establish the levels of antibodies, using the procedures of Grange et al. [14] with some adjustments that were confirmed in the study under the antigen. Overnight incubation of polystyrene 96-well micro-titer plates (Nunc MaxiSorp 3.0, Thermo Fisher) was done with 0.5 0g/well of the purified *M. tuberculosis* surface protein in PBS (pH 7.4). Plates were blocked with 2.5% (w/v) PBS containing 0.05% Tween-20 (PBST) for an hour at room temperature. Rabbit sera were added in serial two-fold dilution (1:100 to 1:51,200) and incubated at room temperature (90 min). Following washing, plates were incubated with goat anti-rabbit IgG-HRP conjugate (1:5000; Sigma-Aldrich, Cat. No. A6154) for 1 hour, and ABTS substrate (2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; Sigma, Cat. No. A1888) was added. Measurement of absorbance was done at 450 nm, using a microplate reader (Bio-Rad iMark™). Validation: On each plate, positive and negative control sera were placed. Three independent assays were conducted to ensure the reproducibility of the assay. Triplicate analysis of serum samples of immunized rabbits (n=3) and control rabbits (n=3) was done. The outcomes were provided as mean + standard deviation (SD). The absorbance surpassing the mean + (3 x SD) of pre-immune sera became the endpoint titer. An unpaired, two-tailed Student t-test was used in Graph-Pad Prism v9.0 to determine statistically significant differences between the groups; p<0.05 was used as a statistically significant value. Sample size justification: The sample size of three animals per group was selected due to the previous literature, which showed that the author has credible immunogenicity data towards polyclonal antibody production with n = 3 animals per treatment group.

RESULTS

The triplicating of the analysis (n=3) was conducted on immunized and pre-immune serum in order to replicate the results. Rabbit anti-*M. tuberculosis* surface protein assays with serum (n=3). The immunogenicity of a fraction of the *M. tuberculosis* surface protein was detected strongly and dilution-dependently in the serum of rabbits immunized with the protein, whereas in the serum of the same rabbits, no binding activity was observed in pre-immune serum (n=3). The median absorbance (SD) of the immunized group at 1:1600 dilution was 1.42 (SD 0.15), which was much greater (p=0.001, unpaired t-test) than that of the pre-immune control (0.08, SD 0.02). Strong antibody formation was evident as a statistically significant increase (p<0.001) was realized with all the dilutions tested. The endpoint of the antibody titer was identified as 1:12,800. The above findings are a quantitative confirmation of the successful

production of polyclonal antibodies that are highly reactive to the *M. tuberculosis* surface protein. Indirect ELISA was done on the serum of immunized rabbits (blue) and controls (red) (serum of pre-immune rabbits). Data points are the standard deviation and mean absorbance of triplicate measurements at 450 nm. The dashed line is used to indicate the cutoff value (mean + 3SD of the pre-immune control). Statistical significance was calculated where an unpaired t-test was used, whereby the statistically significant results were compared to a control group at the same dilution, **, p<0.001. The evident distinction between groups in the ELISA absorbance indicates specific antibody production of high titer (Figure 1).

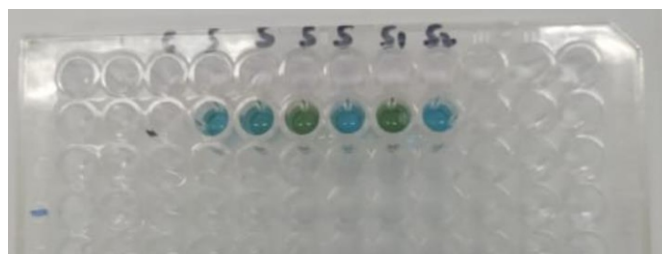


Figure 1: Quantitative Analysis of Polyclonal Antibody Response Against *Mycobacterium tuberculosis* Surface Protein

DISCUSSION

The production and characterization of pABs against surface proteins of *Mycobacterium tuberculosis* (MTB) is an important approach in tuberculosis (TB) research, offering potential applications in diagnostics, vaccine development, and therapeutic interventions [15, 16]. MTB proteins are important virulence factors involved in immune evasion, adhesion, and nutrient acquisition; therefore, they are suitable for the production of antibodies [17]. Any diagnostic test is a keystone of the antigen on the basis of immune reactions that mediate its sensitivity and specificity. In tests involving ELISA, it is important that the antigens used are small enough, uniform, and can attach to the surface of the solid phase of the ELISA plate. Various antigens are typically prepared using high temperature, ultrasound, and chemical methods, but in the process, they alter the structure of the antigen. Thus, milder forms of processing, such as the extraction of surface cellular structures with salt solutions of various ionic strengths with mild detergents, have become more popular. The initial targets of immune response are surface antigens of the pathogens, both of pathogenic mycobacteria and those excreted by the pathogens. Currently, convergent diagnostic kits using serological detection methods have not been established, allowing separation of the humoral response, depending on antibody recognition of *M. TB*, *M. bovis*, BCG vaccine, and other mycobacteria, though this is underway. The *M. tuberculosis* antigens in the serum of infected patients are

not dependent on the intact host immune response, and therefore, they are less variable in patients compared to the TB-related antibodies. Moreover, antigen manifestation following *M. tuberculosis* infection and its removal following the cure are manifested earlier than antibodies. The properties render serologic testing of *M. tuberculosis* antigens a more appropriate method of diagnosis of TB. The production of polyclonal antibodies against surface proteins of *M. tuberculosis* provides valuable tools for studying tuberculosis. In this work, MTB surface proteins key to bacterial survival and host infection were purified and used to immunize rabbits. Antibodies were subsequently isolated from the serum and tested for specificity. In this study, an indirect ELISA demonstrated the successful production of polyclonal antibodies in rabbits immunized with a surface protein fraction of MTB, showing a significant serological response compared to pre-immune controls. ELISA quantitative assays with a mean absorbance of 1.42 /0.15 at a 1:1600 dilution and a geometric mean endpoint titer of 1:12,800 validated the presence of strong immunogenicity of the surface protein extract. These results can be compared to the earlier outcomes of Anderloni [18, 19], who also reported similar antibody titers in rabbits vaccinated with mycobacterial antigens, which proves the applicability of the given method. Although such immunogenicity is encouraging, it has a number of limitations that should be considered. First, a crude antigen fraction was used instead of the purified protein; hence, the antibody response is to a mixture of proteins. This restricts the possibility of ascribing the immune-reactivity to a particular antigen and enhances the possibility of cross-reactivity. The same limitations were observed in previous trials on mycobacterial surface antigens, and it is advised to refine the antigens, followed by diagnostic use. Second, the pilot character of this research, as well as the small size of the sample ($n=3$), implies that the findings confirm a demonstration of the concept but have to be proved in a larger cohort to ensure that the findings are consistent and strong. Conversely, larger-scale antibody generation biomarkers have demonstrated that they can be recapitulated through batch-to-batch reproducible experiments and that they produce species-specific responses, which underscores the need to scale the number of experimental replicates to apply the biomarkers in a translational context [20]. The results indicate that the preparation of antigens is immunogenic and capable of inducing an antibody response that can be measured. Initial research will be required in the future in the purification of the involved antigen(s), rigorous specificity testing against other mycobacterial species, and the analysis of the antibodies using clinically relevant assays. In general, this paper is a step in the right direction as it

confirms the generation of reactive pAbs, yet it emphasizes the importance of additional characterization before any type of application would be a realistic endeavor.

This study has several limitations that should be acknowledged. First, the use of a crude surface protein extract may have resulted in antibody responses against multiple antigens, increasing the risk of cross-reactivity and limiting antigen-specific interpretation. Second, the small sample size ($n = 3$ per group) restricts the generalizability of the findings and reflects the pilot nature of the work. Additionally, antibody specificity was not evaluated against other mycobacterial species or clinical samples, which is essential for diagnostic validation. Future studies should focus on the purification and characterization of individual immunodominant surface proteins, assess antibody cross-reactivity, and validate performance in larger animal cohorts and human clinical samples. Such investigations will be critical to translating these preliminary findings into clinically reliable diagnostic or vaccine-related applications

CONCLUSIONS

This study produced polyclonal antibodies to the surface protein of *Mycobacterium tuberculosis* (MTB) by immunizing rabbits. The resulting antibodies were very reactive in indirect ELISA, which demonstrated specific antibodies to antigens. These findings are evidence of the concept of the immunogenicity of MTB surface proteins. Nevertheless, additional research needs to be done to evaluate the specificity of antibodies, cross-reactivity, and their performance with purified antigens and different strains of *Mycobacterium*. On the whole, the given research is a first step towards creating a set of reliable immunological tools in the sphere of tuberculosis research and paves the further diagnostic and characterization research.

Authors' Contribution

Conceptualization: MUR

Methodology: AB, SAK, MAB

Formal analysis: MFG

Writing review and drafting: MUR, BAG

Review and Editing: MUR, AB, SAK, MAB, MFG, BAG

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

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Original Article



Newborn Care Practices and Perceptions among Mothers in Islamabad, Pakistan: A Mixed Method Approach

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ABSTRACT

The levels of neonatal mortality in Pakistan are also among the highest in the world, and the factors that define the practice of newborn care are strong and culturally determined.

Objectives: To explain maternal attitude and practice of newborn care in Islamabad, focusing on cultural beliefs and socio-economic factors affecting the health conditions of neonatal care.

Methods: Mixed-method design was used, where quantitative data were collected by use of 384 mothers of infants aged 0-6 months on quantitative questionnaires, and qualitative data on 10 in-depth interviews. Data analysis was done through thematic analysis on qualitative results and descriptive and inferential statistics, i.e., ANOVA and chi-square on the quantitative results.

Results: Traditional practices, the views of the elders, and old cultures were mostly prevalent in medical advice. The quantitative data showed significant associations between demographic factors, such as maternal age, education, parity, and delivery place, with newborn care practices, i.e., the initiation of breastfeeding, colostrum usage, and skin-to-skin contact. The interviews also revealed that thematic analysis of the interviews pointed to the role of family customs and societal norms in such practices. **Conclusions:** The evidence taken altogether indicates that there is an immediate necessity to have evidence-based and culturally responsive interventions that are context-specific.

INTRODUCTION

The twenty-eight days of neonatal life are a critical period in the life of the child [1]. Pakistan is ranked among the countries with a high neonatal mortality rate of 41 deaths per 1,000 live births and the under-five mortality rate of 67 deaths per 1,000 live births [2]. The World Health Organization (WHO) identifies five main recommendations of early newborn care (ENC) that include hygienic cord care, optimum thermal regulation, infection prevention, feeding, and the early initiation of breastfeeding [3]. The introduction of these new vital practices of caring for the

newborn would help as much as decrease neonatal mortality by up to 30 per cent [4]. In the guidelines offered by WHO, in cases when there is no appropriate antiseptic to apply, it is better to use dry cord care to preserve hygiene and avoid umbilical stump infections [5]. Lack of sanitary cord care practices also leads to the increased rate of cord infection in developing countries [6]. Some of the early care practices that are included in thermal care are skin contact, delaying bathing in the first 24 hours, warming the baby, using a clean towel, and wrapping the baby. The first

bath on a newborn eliminates vernix and maternal bacteria but interrupts the connection between skin and mother that causes hypothermia and hypoglycemia. It can also interfere with the crawling reflex, which might slow down the establishment of good breastfeeding [7]. Other international organizations, such as the WHO, propose the early initiation of breastfeeding (EIBF) during the first hour of birth. Late breastfeeding initiation may greatly affect the probability of neonatal death, and the probability multiplies by 33 per cent upon breastfeeding initiation being improper [8]. Another problem faced by the country is the inability to conduct routine newborn screening and parental counseling programs, which are critical in enhancing the neonatal outcomes [9]. Although the WHO recommends that at least 5 percent of the GDP should be spent on health, the Government of Pakistan has always spent about 0.5 percent of its GDP on the same sector [10]. To minimize the death of newborns, improve the rate of facility delivery, and provide improved care to the premature babies and those born with an infection. Enhancing prenatal nutrition and practicing breastfeeding and neonatal resuscitation, as well as enabling maternal education and managing health facilities, will prove to be useful in meeting these goals [11]. In rural communities, most of the mothers still stick to their cultural ways of doing things, including the use of herbal medicines as well as postponing the first bath of the baby because they believe that the practices will safeguard the baby. Therefore, cultural expectations on the care of a newborn baby may undermine the efficacy of contemporary healthcare services and postpone the important steps of neonatal care [12]. The rural setups are especially not spared, as most of them do not have proper medical facilities and skilled medics. The lack of healthcare access, coupled with the presence of socio-cultural dependence on traditional practices, puts the newborn at risk of health complications and further raises the mortality rates among these communities [13]. Studies indicate that maternal education has a close relationship with the heightened level of hygiene, good nutrition, and the need to engage in early and exclusive breastfeeding; these factors reduce cases of neonatal mortality and morbidity [14]. Female educated mothers would be less inclined to rely on untrained traditional birth attendants or follow outdated practices, including refusing to breastfeed the baby colostrum or using unsafe substances on the umbilical cord. Research has shown that maternal literacy interventions in low- and middle-income countries improve neonatal health outcomes through creating awareness and busting myths [15]. Despite global and national efforts to reduce neonatal mortality, Pakistan continues to experience unacceptably

high neonatal death rates, largely driven by suboptimal newborn care practices influenced by deep-rooted cultural beliefs, socio-economic constraints, and limited maternal autonomy. Although WHO-recommended essential newborn care practices such as early initiation of breastfeeding, exclusive breastfeeding, hygienic cord care, thermal care, and timely healthcare seeking are well established, their adoption remains inconsistent in many communities. Existing literature has largely focused on either quantitative assessment of newborn care practices or isolated qualitative insights into cultural norms, creating a gap in comprehensive, context-specific evidence that integrates both dimensions. Furthermore, limited data are available from urban-rural mixed settings like Islamabad that explore how cultural perceptions, elder influence, and maternal knowledge collectively shape neonatal care behaviors. This study addresses this gap by employing a mixed-method approach to systematically examine maternal practices, perceptions, and socio-cultural determinants of newborn care, thereby generating evidence to inform culturally responsive interventions. This study aimed to analyze the common practices of newborn care among mothers. To gain insights into the cultural and personal beliefs that determine the choice and practice of mothers in terms of newborn care, and also to determine the extent of awareness of mothers on the necessity of newborn care practices.

METHODS

The proposed study was a mixed-methods approach that used both qualitative and quantitative methods in the Health Services Academy, Islamabad. The research period was between September and December 2024. Written informed consent was taken. The Health Services Academy, Islamabad, gave ethical approval. Complete information regarding the study was supplied to all the participants. In-depth interviews were conducted to obtain qualitative data with the help of the Theory of Planned Behavior as the guiding model. Purposive sampling was used to gather qualitative data, which was that of mothers in Islamabad who had a baby aged between 1 and 6 months. Data saturation was the concept that was used to determine the sample size of this study. To determine the sample size in the quantitative part of the research, the Cochran formula will be used: $n = z^2(p)(1-p)/e^2$. The sample size needed to conduct this study, at a 95% confidence level and a 5% margin of error, will be around 384 participants. The assumption of a large population has been used in this calculation, with the proportion (p) being estimated to be 0.5. The study has chosen to use in-depth interviews because they would explore cultural and personal beliefs and thorough understanding of the

thoughts and feelings of participants, as well as personal experiences in their own words. Also, a structured questionnaire was used to acquire quantitative data. Thematic analysis: Braun and Clarke presented a six-stage thematic analysis framework on which qualitative data were analyzed. In this study, SPSS version 25.0 was the statistical package used in analysing data in terms of quantitative analysis.

RESULTS

Theme 1: Cultural Beliefs and Traditional Practices addressing Newborn Health; Families tend to adhere to the traditional practices, such as applying desi ghee or using

spiritual healing, on the belief that the traditional practices will protect and cure babies. Theme 2: The power of the Elders, particularly mothers-in-law, is very influential in newborn care, and their traditional advice is not subject to question or even medical care. Theme 3: Cultural Perceptions of Illness and Mortality; Illness is often linked to fate or divine punishment, and many parents distrust modern medicine, preferring home remedies instead. Theme 4: Cultural Barriers to Healthcare Access; Dependence on traditional healing and limited decision-making power for mothers delays medical treatment, sometimes leading to serious outcomes for newborns (Table 1).

Table 1: Qualitative Analysis

Theme 1: Cultural Beliefs and Traditional Practices Impacting Newborn Health	
Traditional Practices and Beliefs	"I applied desi ghee on my son's navel because my mother-in-law said that applying desi ghee helps the wound heal faster."
Spiritual and Religious Practices	"Such a small baby should not be taken to the hospital. Treatment should be done at home. When my baby used to fall sick, I would get spiritual healing (dum) from the local religious teacher (Qari Saab)."
Theme 2: Influence of the Elders	
Dominance of Elders' Opinions	"I don't argue with my mother-in-law. If she says that mustard oil will make the baby better, I let her do the massage."
Generational Transmission	"In our family, it is a tradition to place a heated brick near the baby during winter to protect them from the cold."
Theme 3: Cultural Perceptions of Illness and Mortality	
Fatalistic Attitudes towards Newborn Illness	"When my child fell ill, our neighbor said it was a punishment for past sins and that I should seek forgiveness so the child would get better."
Stigmatization of Modern Medical Practices	"Medicines can be very harmful for small children. Instead of helping, they can cause more harm. That's why, when my child falls ill, I don't go to the doctor. I use home remedies instead."
Theme 4: Cultural Barriers to Healthcare Access	
Reliance on Traditional Healing	"Once, my daughter had trouble breathing. My mother-in-law burned some herbs and placed them near her. When her condition didn't improve, we went to the hospital."
Gender Dynamics and Decision-Making	"My child cried continuously for two days, but my husband and mother-in-law said that babies cry all the time. Because of that negligence, I lost my child."

Quantitative results were given that describe the demographic characteristics of the study population (Table 2).

Table 2: Demographic Characteristics of the Study Population (n=384)

Characteristics	n (%)
Mothers' Age in Years	
Mean ± SD	30 ± 4.7
20-25 Years	41 (10.7%)
26-30 Years	170 (44.3%)
31-35 Years	114 (29.7%)
36 and Above	59 (15.4%)
Number of Children	
Mean ± SD	2 ± 0.94
One Child	127 (33.1%)
Two Children	155 (40.4%)
Three Children	64 (16.7%)
More Than Three	38 (9.9%)
Age of Baby in Months	
1-3 Months	155 (40.4%)

4-6 Months	229 (59.6%)
Place of Delivery	
Hospital	168 (43.8%)
Home	216 (56.3%)
Education Level	
Illiterate	64 (16.7%)
Matriculation	115 (29.9%)
Intermediate	54 (14.1%)
Graduation	151 (39.3%)
Occupation	
Government Employee	52 (13.5%)
Corporate Employee	82 (21.4%)
No Job	250 (65.1%)
Place of Residence	
Rural	224 (58.3%)
Urban	160 (41.7%)

Findings show that the awareness of essential newborn care practices among mothers in the study reveals significant gaps. Less than half (43.2%) recognized the importance of initiating breastfeeding within the first hour.

Exclusive breastfeeding for the first six months had relatively higher support (53.4%). Only 38.0% supported delaying the first bath for 24 hours (Table 3).

Table 3: Percentage Distribution of Awareness on Essential Newborn Care

Characteristics	n (%)
First Breastfeeding Should Be Between 30 minutes and an Hour After Delivery	
Agree	166 (43.2%)
Disagree	65 (16.9%)
Unaware	153 (39.8%)
Newborns Should Be Fed with First Yellowish Milk (Colostrum)	
Agree	172 (44.8%)
Disagree	74 (19.3%)
Unaware	138 (35.9%)
Newborns Should be Exclusively Breastfed from Birth to Six Months	
Agree	205 (53.4%)
Disagree	46 (12.0%)
Unaware	133 (34.6%)
The Cord of Your Baby Must Always be Dressed and Covered	
Agree	179 (46.6%)
Disagree	71 (18.5%)
Unaware	134 (34.9%)
Bleeding, Discharges, Redness, and Swelling in Your Baby's Cord Is Normal	
Agree	62 (16.1%)
Disagree	169 (44.0%)
Unaware	153 (39.8%)
At Least 24 Hours After the Baby's Birth, the First Bath Must be Postponed	
Agree	146 (38.0%)
Disagree	103 (26.8%)
Unaware	135 (35.2%)
The Newborn Must Be Wrapped at All Times and Kept in Close Proximity to the Mother's Skin	
Agree	192 (50.0%)
Disagree	44 (11.5%)
Unaware	148 (38.5%)

The data reveal significant deviations from WHO guidelines in newborn care practices. Only 33.9% of mothers exclusively breastfeed. Additionally, 60.9% of mother's use gripe water, contrary to WHO recommendations. Cord care practices show similar concerns, with 22.1% using knives and 3.1% applying mud or saliva. While 52.9% of mothers delay the first bath (Table 4).

Table 4: Percentage Distribution of Practices on Essential Newborn Care

Characteristics	n (%)
Which Kind of Breastfeeding Do You Use?	
Exclusive	130 (33.9%)
Mixed	254 (66.1%)

How Soon After Giving Birth Did You Start Breastfeeding?	
Within an Hour	86 (22.4%)
After an Hour	125 (32.6%)
After 2 Hours	47 (12.2%)
What Sanitary Procedures Do You Follow Prior to Nursing?	
Three Hours and Above	126 (32.8%)
Bath At Least Once A Day	51 (13.3%)
Apply Lotion or Oils on the Breast	52 (13.5%)
Wash Hands	68 (17.7%)
Clean Breast with Towel	49 (12.8%)
Nothing	164 (42.7%)
What Fluid Is Given to the Baby Besides Breast Milk	
Plain Water	16 (4.2%)
Sugar Water	40 (10.4%)
Gripe Water	234 (60.9%)
Others	94 (24.5%)
What Was Used to Cut the Cord After Delivery	
Scissors	176 (45.8%)
Blade	117 (30.5%)
Knife	85 (22.1%)
Others	6 (1.6%)
What Was Used to Tie the Cord After Birth	
String	92 (24.0%)
Rubber Band	99 (25.8%)
Cord Clamp	132 (34.4%)
Wire	61 (15.9%)
Which Primary Drug or Substance Do You Use for Covering Your Baby's Cord?	
Methylated spirit	171 (44.5%)
Mud	12 (3.1%)
Saliva	16 (4.2%)
Mustard oil	168 (43.8%)
Others	17 (4.4%)
Prior to Tending to the Baby's Cord, Do You Sanitize Your Hands?	
Always	67 (17.4%)
Sometimes	121 (31.5%)
Scarcely	43 (11.2%)
Not At All	153 (39.8%)
When Did You Give the Baby Its First Bath After Birth	
Immediately	65 (16.9%)
6 Hours	41 (10.7%)
24 Hours	203 (52.9%)
48 Hours	70 (18.2%)
Others	5 (1.3%)

A chi-square analysis was performed to examine the relationship between various socio-demographic characteristics and newborn care practices. The findings indicated that most newborn care practices had statistically significant associations with respondents' demographic profiles. Specifically, variables such as education level, number of children, place of delivery, occupation, age group, and place of residence consistently influenced caregiving behaviors (Table 5).

Table 5: Chi-Square Analysis

Outcome Variable	No. of Associations Tested	Significant (p<0.05)	Non-Significant	Key Significant Predictors
Type of Breastfeeding	6	4	2	Age, No. of children, Education, Residence
Timing of Breastfeeding Initiation	6	6	0	All variables (e.g., age, place of delivery, occupation)
Reason for Delayed Breastfeeding	6	6	0	All variables
Colostrum Feeding	6	5	1	Education, Place of delivery, Occupation, Residence
Cord Cutting Method	6	6	0	All variables
Cord Tying Material	6	6	0	All variables
Cord Dressing Substance	6	6	0	All variables
Time of First Bath	6	6	0	All variables
Belief: Baby Should Be Wrapped and Held	6	5	1	Age, Education, Residence, Occupation

DISCUSSION

This paper offers important observations regarding the cultural beliefs and practices of newborn care in Islamabad, which will serve as important determinants of the ever-brooding high neonatal mortality rate in Pakistan. It has been revealed that the practices such as the use of ghee on the umbilical stump and postponement of the first bath are culturally based yet can be very dangerous and prone to health complications including infections and death of the child [16]. The results revealed that spiritual and religious beliefs, such as the use of spiritual healers, amulets and prayers, provide emotional support, but postpone important medical procedures, which raise the mortality rate of newborns. The same idea was expressed in another study that claimed that religious beliefs have been associated with the dependency on the non-official information, distrust to scientific results, and reluctance or avoidance of vaccination [17]. The study findings revealed the level of control of the elders, especially the elder women and male authority figures, on newborn care practices in Pakistan is overwhelming. One of the studies found that the failure of mothers to express their opinions may have a negative effect on their health and the health of their newborn, which may affect their overall well-being [18]. The results showed that there were major obstacles to the use of the modern healthcare services, especially in the rural environment or culturally conservative ones. A large percentage of the research participants raised issues regarding biomedical interventions as they felt that they were not as good as traditional remedies. They felt that biomedical care is prone to more side effects, and they would rather resort to local or traditional medical care [19]. The incorporation of these figures into healthcare campaigns has a possibility of closing the gap between the conventional beliefs and medical practice. These results indicated that fewer than half (43.2) of the mothers appreciated the significance of breastfeeding initiation within 30 minutes to an hour after birth. Breastfeeding is vital to the health of newborns, and colostrum contains vital nutrients and antibodies that decrease the chances of

infections and enhance the latter outcomes [20]. In line with the already available literature, the large percentage (39.8) of mothers who were either not aware (unaware) or highly opposed (16.9) to this practice indicates that there was a strong cultural and informational blockage [21]. The study results showed that 53.4% of mothers supported exclusive breastfeeding during the first six months, which is a rather high level of awareness in comparison with other practices yet still desirable. This practice variance to the WHO recommendations where most (66.1) were involved in mixed feeding is attributed to the issues like the traditional modes of feeding, ignorance and possibly insufficient support mechanisms to mothers. These findings are also similar to the other study, which indicates that preference to exclusive breastfeeding was observed as 20.47% (95% CI: 18.84-23.63)[22]. The study results revealed that the use of less common traditional methods like the application of mud or saliva, although less common (3.1% and 4.2, respectively), still exists, and subject's newborns to the risks of neonatal tetanus and sepsis. The same tendencies have been reported in the researches that revealed that over 50 percent of young mothers 196 (62.2%) were well informed about cord care, yet, the practice of cord care was very poor with only 21 percent of them reporting good practice of cord care [23]. Chi-square analysis outcomes in the study revealed the significance of demographic factors and numerous newborn care practices. This is in line with the studies that have indicated that there are strong associations between several factors and the outcome which include the place of residence, the level of education of the mother, number of antenatal care visits, family size, mode of delivery and the place of delivery [24]. Additionally, the educational background of mothers and the location of delivery had a strong impact on the kind of material to cut and tie the cord, as it is an indicator of the inequality of access to safe and standard delivery procedures in rural and urban areas. These results are in line with the past researches that mothers of higher education are more inclined to demonstrate positive

health practices, have better hygiene practices and obtain correct healthcare information, which, altogether, lead to the effective cord management and the minimized chances of acquiring neonatal infections [25].

This study has certain limitations that should be considered when interpreting the findings. First, the cross-sectional design limits causal inference between socio-demographic factors and newborn care practices. Second, self-reported data may be subject to recall bias and social desirability bias, particularly for culturally sensitive practices such as cord care and breastfeeding. Additionally, qualitative interviews were limited in number and conducted within a single geographic setting, which may restrict broader generalizability. Future research should incorporate longitudinal designs to assess changes in practices over time and evaluate the effectiveness of targeted interventions. Expanding qualitative inquiry to include fathers, elders, and healthcare providers would provide a more holistic understanding of household decision-making dynamics. Moreover, community-based, culturally tailored education programs that actively engage elders and address misconceptions should be developed and rigorously evaluated to improve adherence to WHO-recommended newborn care practices and ultimately reduce neonatal mortality.

CONCLUSIONS

The mixed-method approach made the research have a holistic perspective of the role socio-cultural norms, and knowledge gaps play in shaping neonatal care practices. Cultural background and family factors dominate the decision making process thus resulting in poor care choices.

Authors' Contribution

Conceptualization: AN, TS, SEK, MLK

Methodology: AN¹, TS, AQ, MN, AN², IG

Formal analysis: AN, TS, MLK

Writing and Drafting: AN, TS

Review and Editing: AN¹, TS, SEK, MLK, AQ, MN, AN², IG

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article



Study of Rheumatic Mitral Valve Stenosis Using Echocardiography

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ABSTRACT

Rheumatic mitral valve stenosis (RMVS) is one of the primary causes of cardiovascular morbidity in developing nations, which typically leads to severe hemodynamic phenomena in the case of non-prompt diagnosis. Echocardiography is used to determine the severity of the disease and treatment. **Objectives:** To assess the echocardiographic appearances of patients with RMVS, as well as to estimate the rate and intensity of mitral valve involvement by various demographic factors. **Methods:** 50 patients aged 21- 60-year-old diagnosed with RMVS were studied with transthoracic echocardiography. Mitral valve area (MVA), mean pressure gradient (MPG), and pressure half-time (PHT) were read. Descriptive statistics were done to analyze the data and present the data in the form of mean SD. **Results:** The sample size was 74 female and 26 male, and the average age of the study population was 36.8%, aged 9.4 years. The average MPG was 11.2-3.8 mmHg, average PHT was 198-54 ms, and average MVA was 0.96- 0.21 cm². Severe stenosis (MVA < 1 cm²) was noted in 46% of patients, and 68% of patients demonstrated an MPG greater than 10 mmHg. This affected women more than men because women demonstrated more severe disease profiles. There were no major differences between the age and severity of the disease. **Conclusions:** A Large percentage of patients reported with severe RMVS, with women leading. MPG, MVA, and PHT are parameters of the Echocardiography that show the severity of the disease.

INTRODUCTION

Mitral valve stenosis (MS) is another disorder that is manifested with the narrowing of the mitral orifice that obstructs the flow of blood between the left atrium and left ventricle and elevates the left atrial pressure, resulting in left atrial congestion and heart failure [1, 2]. The overall prevalence of MS in developing countries is still caused by rheumatic fever, even though congenital etiology and degenerative etiology are relatively rare [3-5]. Rheumatic fever remains a significant health problem in low- and middle-income countries where it is caused by the lack of preventive medical care and socioeconomic barriers [6-8]. Even though the cases of rheumatic heart disease (RHD) have been reduced considerably since 1960s in developed countries [9, 10], it remains as a major cause of cardiovascular morbidity and mortality in some parts of the world like South Asia, where the disease is not diagnosed in

its early stages, and can cause irreparable cardiac damage [11-13]. Echocardiography is one of the imaging modalities that is studied and remains the gold standard in the diagnosis and severity of mitral stenosis, as it provides accurate, non-invasive measurements of the mitral valve area (MVA), the mean pressure gradient (MPG), and the pressure half-time (PHT) [14-16]. Earlier research has been able to offer good epidemiological information, but generally has not contained region-based analysis or comparison of echocardiographic indices. As an example, two-dimensional echocardiography was considered in 50 patients by Silbiger *et al.* who found a severe stenosis in half of the cases, and more prevalent in female [9]. On the same note, Abdelgawad *et al.* acquired 250 patients and developed 49% severe stenosis, once again in most women [8]. But these studies were mainly descriptive without



examining the variability in each of these demographic subgroups or pointing out any trend in disease development in particular populations. The value of echocardiography in the diagnosis, evaluation, and treatment of rheumatic mitral stenosis (RMS), progressive valvular heart disease. The most critical parameters that are assessed and determine the severity of RMS and abnormalities of valvar nature [17, 18]. The paper also discusses how multimodality imaging, e.g., trans-esophageal echocardiography, can be used to enhance the diagnostic accuracy and therapeutic decision-making. Practical interpretation of the echocardiographic results plays a significant role in the clinical field, and it helps to identify the risk of diseases, organize treatment, and monitor their progression. One of the most common non-invasive techniques to analyze the disease and monitor its progression, as well as assess the effectiveness of the treatment, is the integration of echocardiography [19, 11]. Rheumatic mitral valve stenosis (RMVS) remains a major contributor to cardiovascular morbidity in developing countries, where delayed diagnosis and limited access to advanced cardiac care often result in severe disease at presentation. Echocardiography is the gold-standard, non-invasive modality for assessing RMVS severity through parameters such as mitral valve area (MVA), mean pressure gradient (MPG), and pressure half-time (PHT). Although several studies have documented the prevalence of severe RMVS and its female predominance, there is a lack of region-specific evidence from Pakistan that systematically correlates echocardiographic indices with disease severity and demographic variables. Moreover, many existing studies remain largely descriptive and do not statistically establish the strength of association between echocardiographic parameters and stenosis severity. This study addresses this gap by evaluating the relationship between MVA, MPG, and PHT and the degree of RMVS, while also examining demographic influences, thereby reinforcing the clinical utility of echocardiography in severity assessment. This study aims to assess the severity of rheumatic mitral valve stenosis based on echocardiographic parameters, namely mitral valve area (MVA), mean pressure gradient (MPG), and pressure half-time (PHT), to establish the prevalence and trend of disease severity in the affected individuals.

METHODS

It was a cross-sectional descriptive study that was carried out in a Government Sector Hospital between January 2022 and August 2022. The retrospective data were obtained by means of available echocardiographic data of patients who had been previously diagnosed with rheumatic mitral valve stenosis (MS). The

echocardiographic values were checked and rechecked to be consistent and full of diagnostic parameters. A total of 50 patients (age 21 to 60 years) whose history of rheumatic heart disease (RHD) was confirmed and had echocardiographic evidence of mitral stenosis were used. The sample was suited to a similar study by Albakheit [13], which provided similar demographic and clinical features. There was no official count of power done, but the selected sample size was considered adequate to carry out descriptive analysis. The patients who were older than 60 years old, had co-existing valvular abnormalities (aortic or tricuspid valve disease), and those who had congenital heart defects or undergone past cardiac surgery were excluded to reduce the confounding factors. A convenient sampling technique was used to collect the data; thus, there might be selection bias, and this constraint was accepted in the discussion. The assessments were conducted in the form of echocardiographs with a 4 MHz phased-array transducer that has two-dimensional (2D), M-mode, and Doppler imaging capabilities. The left lateral decubitus position of examination enhanced cardiac structure visualization by examining all patients in this position. A comprehensive evaluation was done on standard parasternal long-axis (PLAX), parasternal short-axis (SAX), and apical four-chamber and two-chamber views. Mitral valve area (MVA) was estimated by pressure half-time (PHT), where $MVA = 220/PHT$, and mean pressure gradient (MPG) was estimated by continuous-wave Doppler across the mitral valve. The time taken between Doppler tracing peaks in gradient of pressure was recorded as pressure half-time (PHT). All of the scans were conducted or checked by one trained cardiologist based on standardized echocardiographic guidelines: mild MS was considered MVA larger than 1.5 cm², MPG less than 5 mmHg, and PHT less than 150 ms; moderate MS was considered MVA between 1.0- 1.5 cm², MPG between 5-10 mmHg, and PHT between 150-219 ms; severe MS was considered MVA smaller than 1.0 cm², MPG larger. The study obtained ethical approval from the institutional review committee and verbal informed consent of all of the participants, whose data, i.e., echocardiographic data, were used in the study. This measure makes sure that all ethical principles were adhered to in the taking and exploitation of participant data. Inclusion Criteria: Patients aged 21-60 years, diagnosed cases of rheumatic heart disease (RHD) with echocardiographic evidence of mitral valve stenosis, and patients with complete echocardiographic profiles, including M-mode, 2D, and Doppler data. Exclusion Criteria: Patients over 60 years of age, individuals with congenital heart disease, non-rheumatic causes of MS, or co-existing valvular lesions (such as significant aortic or tricuspid valve diseases, and patients with previous cardiac surgery, prosthetic valves,

or incomplete echocardiographic records. The data were analyzed using SPSS version 25.0.

RESULTS

50 patients having mitral valve stenosis were studied using echocardiography, out of which 13 were male and 37 were female. The majority of patients (52%) were between 21 and 40 years of age, while 48% were between 41 and 60 years. This indicates that rheumatic mitral valve stenosis predominantly affects individuals in early to middle adulthood. Among the study participants, 74% were female and 26% were male. This demonstrates a clear female predominance in the occurrence of rheumatic mitral valve stenosis (Table 1).

Table 1: Frequency Distribution of Patients' Age, Gender, and Mean Pressure Gradient in Mitral Valve Stenosis

Variables		Frequency (%)
Gender	Male	13 (26.0%)
	Female	37 (74.0%)
	Total	50 (100.0%)
Age	21 to 40 Years	26 (52.0%)
	41 to 60 Years	24 (48.0%)
	Total	50 (100.0%)
MPG	Less Than 5 mmHg	1 (2.0%)
	5 - 10 mmHg	15 (30.0%)
	More than 10 mmHg	34 (68.0%)
	Total	50 (100.0%)

A significant majority (68%) of patients had a mean pressure gradient (MPG) greater than 10 mmHg, reflecting severe stenosis. Only 2% showed mild and 30% moderate elevation in pressure gradient. Pressure half-time (PHT) values indicated that 44% of patients had PHT greater than 220 ms, consistent with severe stenosis, while 32% and 24% showed moderate and mild prolongation, respectively (Table 2).

Table 2: Frequency Distribution of Pressure Half-Time and Mitral Valve Area in Mitral Valve Stenosis

Variables		Frequency (%)
Pressure Half Time	Less Than 150ms	12 (24.0%)
	150-220ms	16 (32.0%)
	More Than 220ms	22 (44.0%)
	Total	50 (100.0%)
Mitral Valve Area	less than 1cm ²	23 (46.0%)
	1.0-1.5cm ²	17 (34.0%)
	more than 1.5cm ²	10 (20.0%)
	Total	50 (100.0%)

Nearly half of the patients (46%) had a mitral valve area less than 1 cm², confirming severe stenosis in most cases, while 34% had moderate and 20% had mild narrowing of the valve area. The statistical comparison indicated that there are significant correlations between echocardiographic

variables and the severity of mitral valve stenosis. Mean pressure gradient ($p=0.002$) and pressure half-time ($p=0.005$) were significantly correlated with the severity of stenosis in chi-square tests, and Pearson correlation indicated that mitral valve area was strongly negatively correlated with severity ($r = -0.82, p < 0.001$). There was also a significant association of gender ($p=0.03$) in that females had a greater probability of having severe stenosis, but age was not significantly correlated ($p=0.25$). Such findings validate the fact that MPG, PHT, and MVA are important parameters used in identifying the severity of mitral stenosis (Table 3).

Table 3: Statistical Association between Echocardiographic Parameters and Severity of Mitral Valve Stenosis (Chi-Square Tests)

Parameter	Test Value	df	p-Value	($p < 0.05$)
Mean Pressure Gradient (MPG) vs Severity	12.84	2	0.002	Significant
Pressure Half-Time (PHT) vs Severity	10.73	2	0.005	Significant
Age vs Severity	1.32	1	0.250	Not significant
Gender vs Severity	4.56	1	0.030	Significant

The correlation of sex and the magnitude of the mitral stenosis shows that women are more commonly affected at all stages of the stenosis severity. The percentage of female patients was higher than male patients in each category. This trend was constant when the total population was taken into consideration and thus, mitral stenosis was more common in female of the analyzed group (Figure 1).

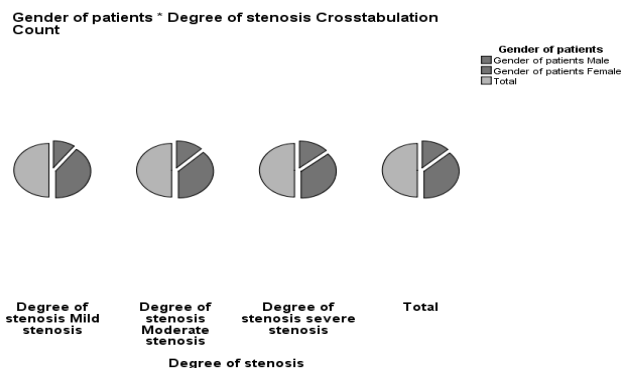


Figure 1: Cross-Tabulation of Gender of Patient and Degree of Mitral Valve Stenosis

The findings present the correlation between the mitral valve area (MVA) and the extent of stenosis. It was evident that these two variables have an inverse relationship, as the more pronounced the stenosis was, the smaller the mitral valve area. When there is mild stenosis, a good majority of the patients possess a valve area that exceeds 1.5 cm², which means that there is very little obstruction. In moderate stenosis, most patients are between the range of 1.0-1.5 cm², whereas in severe cases, most of the patients

have an area of the valve less than 1.0 cm². In general, mild cases of stenosis are the most common in the whole distribution of the patients analyzed. These results were in line with clinical expectations and show that the detectable narrowing of the mitral valve area was directly proportional to the degree of stenosis severity (Figure 2).

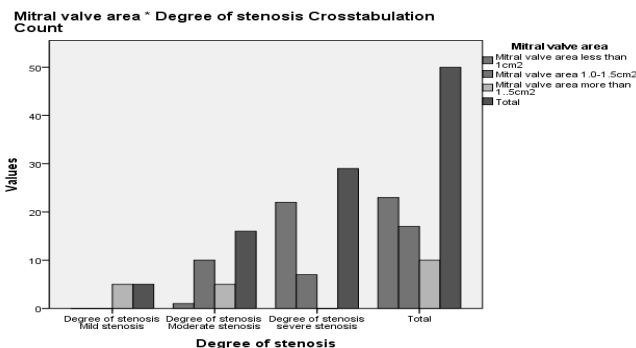


Figure 2: Cross-Tabulation of Mitral Valve Area and Degree of Stenosis

The research shows the association between pressure half-time (PHT) and the extent of mitral stenosis. The coloured bars represent various ranges of PHT, which were less than 150 ms, 150-220 ms, and over 220 ms. The higher the stenosis severity, the higher the pressure half-time. In mild stenosis, the majority of patients possess a PHT of less than 150 ms, whereas in moderate stenosis majority of patients are in the 150 to 220 ms bracket. In acute stenosis, most patients have a PHT of more than 220 ms, which is in agreement with the anticipated hemodynamic alterations in mitral valve constriction. The overall counts affirmed that the average number of patients with a prolonged pressure half-time was more widespread, which implies that the case of moderate to severe stenosis prevails in the data (Figure 3).

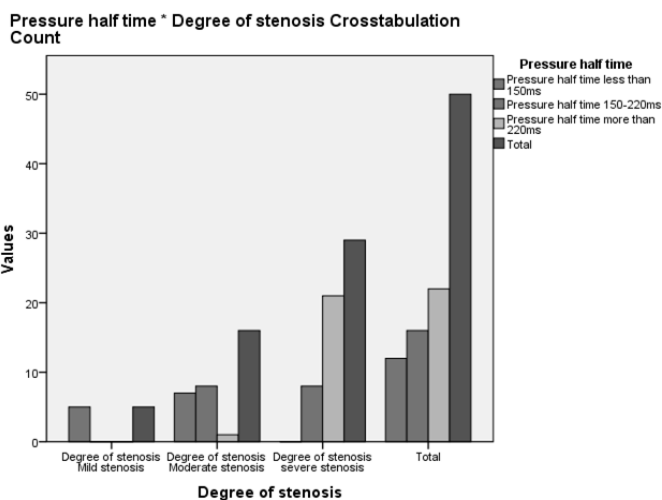


Figure 3: Cross-Tabulation Between Pressure Half Time and Degree of Stenosis

The paper is a study to evaluate the relationship between mean pressure gradient (MPG) and the extent of stenosis.

The color legend splits the MPG values into three categories which were below 5 mmHg, 5-10 mmHg, and above 10 mmHg. The statistics indicate that there was an evident increasing trend in MPG as the stenosis level increased. Most patients in mild stenosis have MPG less than 5 mmHg, with moderate stenosis having MPG values between 5-10 mmHg. In acute stenosis, most of the patients experience an MPG of more than 10 mmHg, which implies very high pressure gradients across the mitral valve as a result of constriction. The overall column indicates that there were more patients with higher MPG values (>10 mmHg) in general, which was anticipated by the physiological facts that the higher the gradient, the more severe the obstruction (Figure 4).

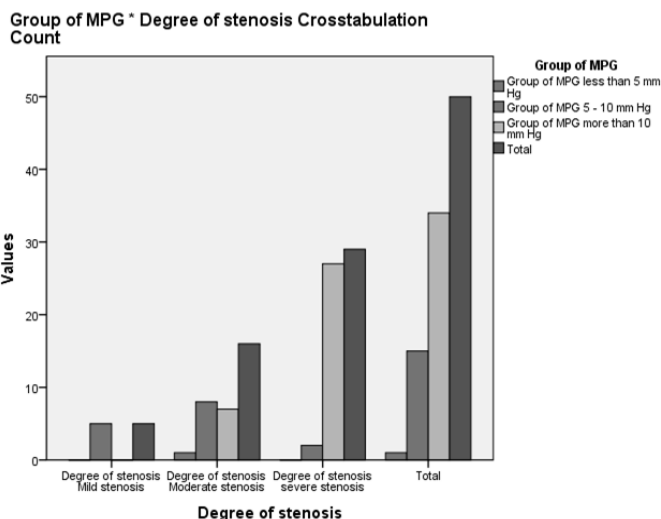


Figure 4: Cross-Tabulation of MPG and Degree of Stenosis

DISCUSSION

The current study sought to determine the severity of rheumatic mitral valve stenosis (MS) based on the echocardiographic parameters, mean pressure gradient (MPG), pressure half-time (PHT), and mitral valve area (MVA), and also compare them with the demographic variables. Among 50 patients who were studied, most of them were female (74%), which suggests a high gender disposition to rheumatic mitral stenosis. The result is in agreement with other previous findings by Manjunath *et al.* who found the same predominance of female patients with rheumatic valvular heart disease [20]. Even though the frequency analysis showed that 68 percent of respondents had an MPG of 10 mmHg and above and 46 percent had an MVA of less than 1cm² or less, inferential tests were done in order to determine the strength of these relationships. Chi-square analysis showed that MPG and MVA and PHT and MVA were statistically related, and hence, it was established that the increase in pressure gradient and half-time were closely related to the decrease in valve area. This trend was also supported by the correlation coefficient between

Pearson, where MVA and MPG were significantly inversely correlated ($r = -0.82$, $p < 0.01$), which is also in line with the pathophysiological mechanism of mitral narrowing. The fact that there is no significant correlation between age and severity of stenosis ($p > 0.05$) would imply that rheumatic MS development is not largely age-dependent as valvular involvement develops. Nonetheless, the analysis based on gender revealed a high correlation ($p < 0.05$) and female displayed more advanced levels of stenosis than males, which is also correspondent with the results of Mulugeta *et al.* and Paolisso *et al.* [5, 21]. The present results also correspond to the results described in the article by Abdelgawad, who proved that patients with severe MS tend to have MVA less than 1.0 cm² and MPG over 10 mm Hg [8]. Equally, our findings support the American Society of Echocardiography (ASE) diagnostic limits, which support the accuracy of echocardiographic parameters in determining the severity of the disease. These concurrences with the previous literature support the diagnostic power of MPG, PHT, and MVA as correlated and clinically significant indicators of rheumatic mitral stenosis. Although these parallels can be seen, the study had weaknesses in terms of limited sample size and descriptive design. Future studies must use bigger multicenter samples and use regression or survival analysis to examine predictive relationships among echocardiographic indices and clinical results, including symptom progression or surgical intervention rates [22]. This study has certain limitations that should be considered when interpreting the findings. The cross-sectional and descriptive design limits causal inference and does not allow assessment of disease progression or clinical outcomes over time. The relatively small sample size from a single center and the use of convenient sampling may restrict the generalizability of the results to broader populations. Additionally, the absence of advanced imaging modalities, such as transesophageal or three-dimensional echocardiography, may have limited comprehensive structural assessment. Despite these limitations, the study provides valuable baseline data and supports the reliability of standard echocardiographic parameters in evaluating the severity of rheumatic mitral valve stenosis. Future studies must use bigger multicenter samples and use regression or survival analysis to examine predictive relationships among echocardiographic indices and clinical results, including symptom progression or surgical intervention rates [22].

CONCLUSIONS

In conclusion, it can be stated that Echocardiographic examination is a crucial and valid tool for measuring the degree of rheumatic mitral valve stenosis. The research also establishes that increased MPG and PHT values are

strongly associated with reduced mitral valve areas, which are a result of severe stenosis. The female preponderance of patients is an important signal of the possible necessity to implement gender-specific prevention measures and screening with the help of echocardiography at an earlier age in the risk groups. Increasing screening measures and enhancing access to early diagnostic imaging may help to relieve the progression of the disease and the burden of rheumatic heart disease in developing areas.

Authors' Contribution

Conceptualization: AA¹

Methodology: AA¹, NT

Formal analysis: AA¹, RR

Writing and Drafting: AA¹, AA²

Review and Editing: AA¹, NT, RR, AA²

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article

Prediction of Fetal Gender Through Fetal Biometry



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ABSTRACT

An important part of prenatal care is gender determination, which shows expectant parents and medical professionals significant personal and medical insights. It becomes extremely important to determine early fetal gender for enhancing treatment approaches for sex-chromosome-related illnesses, like X-linked disorders. **Objectives:** To investigate the relationship between fetal gender determination during the second and third trimesters of pregnancy and ultrasound-based fetal biometric parameters, including estimated fetal weight (EFW), femur length (FL), abdominal circumference (AC), head circumference (HC), and biparietal diameter (BPD). **Methods:** This cross-sectional comparative study was conducted at the Radiology Department of Meer Khan Children and Family Hospital. Women between 14–40 weeks of gestation undergoing ultrasound for fetal assessment were included. Data were analyzed using SPSS version 26.0, applying an independent samples t-test and one-way ANOVA with a significance level set at $p < 0.05$. The Shapiro-Wilk test was used in order to evaluate the data distribution normality. **Results:** The study found biometric ultrasound measurements of 125 fetuses (63 male and 62 female). Three important metrics showed higher values were found in male fetuses: Male had significantly higher FL ($p = 0.002$, 95% CI: 3.16–14.28 mm). The test also indicated male with significantly higher AC, $p = 0.005$, 95% CI: 10.77–58.44 mm, also significantly higher HC ($p = 0.016$), 95% CI: 5.06–48.10 mm, in male was observed. **Conclusions:** Male fetuses had increased BPD and EFW compared to the female fetuses, whereas EFW and BPD showed non-significant trends toward higher values in male. Moreover, male fetuses have significantly greater AC, FL, and head circumference HC than female fetuses.

INTRODUCTION

An essential part of prenatal care is identifying the fetus's gender, which offers both expectant parents and medical professionals important personal and medical insights [1]. For diseases linked to sex chromosomes, like X-linked disorders, early evaluation of fetal sex can enhance treatment planning. Clinicians can also foresee and handle possible issues linked to sex-specific health risks when they can accurately predict the gender of the fetus. In addition, a lot of parents say they want to know the gender of the fetus for cultural and family planning reasons. Psychologically speaking, knowing the baby's gender could improve parental bonding and emotional readiness for the baby's birth [2]. Cultural and religious beliefs greatly

influence how much weight is given to fetal gender determination in nations like Pakistan. Long-standing social traditions frequently influence family planning decisions, cultural preferences for male children in particular communities, and preparations for the child's upbringing. Healthcare providers must be aware of these cultural considerations to deliver prenatal care services that are appropriate, considerate, and sensitive [3, 4]. When it comes to treating gender-influenced medical conditions and complications, knowing the fetal sex is helpful. Given that male fetuses are occasionally linked to particular risks, early detection is especially crucial. Fetal sex is ascertained using a variety of ultrasound techniques.



Fetal heart rate (FHR) may provide early indicators, but its precision and reliability are still restricted [5]. The treatment of genetic disorders also heavily relies on the early identification of fetal gender. While ultrasound examinations in the later trimesters can visualize fetal genital anatomy for more precise gender determination, the orientation of the genital tubercle can be used to determine the fetal gender as early as the eleventh week of gestation [6]. The potential of biometric parameters in predicting fetal gender has been highlighted by studies showing that male fetuses typically have significantly larger biparietal diameter (BPD) measurements than female fetuses during ultrasounds performed between 16–21 weeks and 31–35 weeks of gestation [7, 8]. With advancements in medical technology, non-invasive and highly accurate techniques (i.e., ultrasound imaging, Magnetic Resonance Imaging (MRI)) have become increasingly popular for determining fetal gender. Genetic testing is still the most accurate method, but it can be expensive and has risks, such as the potential to cause miscarriage. On the other hand, ultrasound evaluations are generally considered to be safer, more accessible, and less expensive methods of determining a person's gender, especially in the second and third trimesters [9, 10]. Accurate determination of the fetal gender is extremely necessary to treat many medical disorders. Yet some techniques as chorionic villus sampling, the transabdominal method, or the transcervical method, which are employed at 11–12 weeks, provide quick results, being invasive with procedural risks. The early gender identification precision has increased with the advancement of the technology of two-dimensional ultrasound, although this method is not fully sufficient to replace invasive techniques fully. With the development of three-dimensional ultrasound imaging, reliability has been further improved, which makes it analysable volumetric, fully detailed data from multifarious viewing angles [11, 12]. Fetal gender identification, beyond parental curiosity, is important in prenatal care. The precise and early detection enhances health results both for mother and child, with great support to the congenital abnormalities diagnosis and treatment, fetal growth variations, and sex-linked genetic disorders [13, 14]. Early and accurate prediction of fetal gender using non-invasive ultrasound techniques can support prenatal assessment and enhance understanding of fetal growth patterns. Since limited local data exist regarding gender-based variations in fetal biometric parameters in the Pakistani population, this study was conducted to determine whether routine ultrasound measurements can reliably predict fetal gender. Accurate determination of fetal gender is an important

component of prenatal care, particularly for the early identification and management of sex-linked genetic disorders and for understanding sex-specific growth patterns. While direct visualization of fetal genitalia remains the standard method for gender identification, it can be limited by fetal position, gestational age, and operator dependency. Ultrasound-based fetal biometric parameters offer a non-invasive alternative that may assist gender prediction; however, existing evidence on gender-related differences in fetal biometry remains inconsistent and is largely derived from non-local populations. In Pakistan, there is a notable lack of empirical data evaluating the association between fetal gender and routine biometric measurements during the second and third trimesters. Therefore, this study aimed to assess whether commonly used fetal biometric parameters biparietal diameter, femur length, abdominal circumference, head circumference, and estimated fetal weight differ significantly between male and female fetuses, and to determine their potential role in predicting fetal gender in a local clinical setting.

METHODS

In this case control cross-sectional comparative study, pregnant women between 14 and 39 weeks of gestation who underwent ultrasound examinations at the Radiology Department of Meer Khan Children and Family Hospital in Lahore, from October 2024 to June 2025 (MKCFH/LHR/2024-235), who had participated in this research. The ethical approval was taken from Green International University with Ref no: GIU/REC/25-09. The research team first conducted a detailed, pertinent literature review to find out prenatal biometric markers pertinent to gender prediction. The five ultrasound-based fetal measurements were chosen for examination in light of the review, such as abdominal circumference (AC), biparietal diameter (BPD), estimated fetal weight (EFW), femur length (FL), and head circumference (HC). The sample size was determined by using Open Epi software (Version 3.01), with 80% power and a 95% confidence level. Using Galjaard et al. research, conducted on mean BPD differences between male and female fetuses, considering it as a guiding study, a minimum sample size of 120 was required for this pertinent study [7]. Yet, the researchers recruited a total of 125 participants for this research to consider potential dropouts. The purposive sampling technique was used to collect the data for the specific purpose of the research. For this purpose, the researchers considered pregnant women with an age between 14 and 40 weeks of gestation willing to undergo standard prenatal ultrasound examination. The pregnant women who were undergoing routine ultrasounds were taken as the general population,

and served as controls, while the researchers designated the cases as fetuses with proven gender (male or female) the considering either at birth or through a second-trimester anatomy examination. Before the sample was included in the research, all the respondents were given a briefing about the study and were provided verbal informed consent. Also, women with known fetal anomalies or with multiple pregnancies were excluded from the study. The data were collected by qualified sonographers, with a follow-up of established ultrasonography protocols. Whereas the fetal biometric data were collected according to the recognized clinical procedures. Biparietal diameter (BPD), which was assessed at the level of thalami and cavum septum pellucidum, was measured following the same level protocol as the head circumference (HC). At the level of the fetal stomach and umbilical vein, abdominal circumference (AC) was measured, but the femur length (FL), considering the largest axis, was measured for the ossified femoral diaphysis. The estimated fetal weight (EFW) was calculated using Hadlock's method, in which BPD, HC, AC, and FL were taken into consideration. The researchers documented both breech and cephalic fetal presentations. The researchers used Statistical Package for the Social Sciences (SPSS) version 26.0 for the analysis of the data collected from the pregnant female. Descriptive statistics of all the variables were calculated in the study to find frequencies, percentages, and other descriptive details. The researchers presented categorical variables with frequencies and percentages; whereas, for the measurement of the continuous variables were measured with their Mean and SD (i.e., mean \pm standard deviation). To check the normality of the data, the Shapiro-Wilk test was used. Independent samples t-tests were utilized to compare mean biometric measurements between male and female fetuses; while One-way ANOVA was utilized to find differences between fetal presentation groups, more than 2. Before the performance of parametric tests, Levene's test was used to verify the homogeneity of variances; while, for all comparisons, 95% CIs were used at a level of significance, $p < 0.050$. No post-hoc test was required, as group comparisons did not involve more than two categories.

RESULTS

Out of 125 participants was almost equal, with 62 male (49.6%) and 63 female (50.4%). The breech presentation accounted for 12.8%, whereas, majority of fetuses were in cephalic presentation (87.2%). The findings reflect a mean maternal age reported 28.50 ± 6.75 years, with the indication of women in their late twenties. An average biparietal diameter (BPD) of 71.54 ± 17.47 mm was shown by Fetal biometric parameters, reflecting femur length (FL) as 55.28 ± 16.24 mm. The HC averaged 270.46 ± 61.98 mm,

whereas, average abdominal circumference (AC) was 248.40 ± 69.25 mm. With the coverage of both early and later stages of pregnancy, the gestational age (GA) had a broad range, with having mean of 29.02 ± 6.64 weeks, and with a mean of 1778.61 ± 1234.27 grams, the EFW showed marked variation, by showing growth differences among gestational periods (Table 1).

Table 1: Baseline Demographic Variables with Fetal Biometric Characteristics (n=125)

Variables	Category	Descriptive Statistics
Gender	Female	63 (50.40%)
	Male	62 (49.60%)
Presentation	Breech	16 (12.80%)
	Cephalic	109 (87.20%)
Age (Years)	–	28.50 ± 6.75
BPD (mm)	–	71.54 ± 17.47
FL (mm)	–	55.28 ± 16.24
AC (mm)	–	248.40 ± 69.25
HC (mm)	–	270.46 ± 61.98
GA (Weeks)	–	29.02 ± 6.64
EFW (Grams)	–	1778.61 ± 1234.27

Results show 125 fetuses, by reflecting 62 female and 63 male in the above analysis. Although, non-significant statistically significant difference was found, the results show mean females' greater biparietal diameter (BPD) (68.55 ± 17.59 mm) ($t(123) = 1.92$, $p = 0.057$, 95% CI: -0.19 to 12.05), as compared to male fetuses (74.49 ± 16.97 mm). Conversely, the average femur length (FL) reported to be longer as 8.72 mm, in males (59.61 ± 14.69 mm), compared with females (50.89 ± 16.68 mm). Also, there was found a significant difference ($t(123) = 3.10$, $p = 0.002$, 95% CI: 3.16–14.28) was found in the t-test while comparing both genders. There was also a larger abdominal circumference (AC) in male than female (265.57 ± 64.91 mm vs. 230.96 ± 69.66 mm), showing a mean difference of 34.61 mm ($t(123) = 2.87$, $p = 0.005$, 95% CI: 10.77–58.44). Female had lower HC values (257.06 ± 66.60 mm), compared with male with higher values 283.64 ± 54.43 mm which resulting in a mean difference (26.58 mm) ($t(123) = 2.45$, $p = 0.016$, 95% CI: 5.06–48.10). Yet, males reported a higher EFW (1984.04 ± 1249.23 g) as compared to female (1569.87 ± 1192.80 g). Furthermore, the analysis did not show a statistically significant difference ($t(123) = 1.90$, $p = 0.060$, 95% CI: -18.39 to 1984.74) (Table 2).

Table 2: Comparison of Fetal Biometry Between Male and Female Fetuses

Parameters	Gender	N	Mean ± SD	Mean Diff. (Male and Female)	t (df)	p-Value	95% CI of Difference
BPD (mm)	Male	63	74.49 ± 16.97	5.93	1.92 (123)	0.057	-0.19 to 12.05
	Female	62	68.55 ± 17.59				
FL (mm)	Male	63	59.61 ± 14.69	8.72	3.10 (123)	0.002*	3.16 to 14.28
	Female	62	50.89 ± 16.68				
AC (mm)	Male	63	265.57 ± 64.91	34.61	2.87 (123)	0.005*	10.77 to 58.44
	Female	62	230.96 ± 69.66				
HC (mm)	Male	63	283.64 ± 54.43	26.58	2.45 (123)	0.016*	5.06 to 48.10
	Female	62	257.06 ± 66.60				
EFW (g)	Male	63	1984.04 ± 1249.23	414.18	1.90 (123)	0.060	-18.39 to 846.74
	Female	62	1569.87 ± 1192.80				

*p<0.050 considered statistically significant

There was no significant difference between breech and cephalic presentations regarding fetal biometric parameters. Almost identical values were shown by the femur length (FL) (55.59 mm in breech vs. 55.24 mm in cephalic, p=0.94), and in both groups, the mean BPD was reported to be similar (71.54 mm, p=1.00). Also, similar results were shown in both groups regarding HC averaged 261.71 mm in breech and 271.74 mm in cephalic fetuses (p=0.55) and AC (254.76 mm vs. 247.47 mm, p=0.70) impacted by fetal presentation (Table 3).

Table 3: ANOVA Results by Fetal Position and Fetal Biometric Parameters

Variables	Fetal Position	N	Mean ± SD	F (df=1, 123)	Sig. (p-Value)
BPD (mm)	Breech	16	71.54 ± 22.47	0	1.00
	Cephalic	109	71.54 ± 16.74		
	Total	125	71.54 ± 17.47		
FL (mm)	Breech	16	55.59 ± 18.47	0.01	0.94
	Cephalic	109	55.24 ± 15.99		
	Total	125	55.28 ± 16.24		
HC (mm)	Breech	16	261.71 ± 76.40	0.36	0.55
	Cephalic	109	271.74 ± 59.89		
	Total	125	270.46 ± 61.98		
AC (mm)	Breech	16	254.76 ± 83.93	0.15	0.70
	Cephalic	109	247.47 ± 67.24		
	Total	125	248.40 ± 69.25		
EFW (g)	Breech	16	1795.59 ± 1397.80	0	0.95
	Cephalic	109	1776.12 ± 1215.60		
	Total	125	1778.61 ± 1234.27		

DISCUSSION

Fetal gender determination during prenatal care has significantly improved with the continuous growth in Ultrasound Imaging Technology. The accuracy of first-trimester gender prediction varies compared with the previous research's reflection, with influenced gestational age success rates, primarily, and by means of imaging methods [15, 16]. The present study aimed at investigating the association between gender (calculated by ultrasound)

and fetal biometric parameters in the second and third trimesters of pregnancy. In many countries, ultrasonography and imaging technology have become popular techniques to determine fetal gender [17]. Generally, it allows High-resolution visualization of the fetal genitalia between 16 and 20 weeks of gestational age. Determination of fetal gender has become a very popular clinical implication, particularly in genital abnormalities, X-linked genetic disorders, and presumed ovotesticulars are included. Also, BPD keeps a close association with gestational age; according to research, including crown-rump length (CRL) is considered the most effective way of gestational age estimation during the first trimester. Following the discussion, BPD readings are obtained between 12 and 18 weeks of gestation age for the prediction of the estimated delivery date. Such readings are considered statistically beneficial than those of CRL measurements, which are obtained 14 weeks before [18, 19]. Khalid et al. found a significant correlation between fetal gender and placental location, reporting posterior placentas more common in male fetuses (p<0.05). Whereas, in female fetuses, anterior placentas were reported to be significantly more common [4]. Subtle anatomical and physiological differences are implied through these results, beyond genital visualization that may show fetal gender, excluding placental position in this research. Gender prediction models' accuracy can be enhanced by combining placental positioning by means of biometric indicators. Anogenital distance (AGD) and genital tubercle angle (GTA) were identified as trustworthy early indicators for fetal gender identification in research by Alfuraih et al. and Elanwar et al. [14, 20]. For early and precise gender determination, these indicators are considered supplementary techniques, even though they are different from growth-based metrics like FL, HC, and AC. AGD's predictive value in early gestational assessments has been reinforced by studies that continuously demonstrate male fetuses with longer AGD measurements

than female. Fetal gender and biometric measurements were found to be significantly correlated in this 125-person study. The biometric values of male fetuses were consistently higher than those of female fetuses. Abdominal circumference (AC) (male > female, $p=0.005$), head circumference (HC) (male > female, $p=0.016$), and femur length (FL) (male > female, $p=0.002$) all showed statistically significant differences. Males also had higher estimated fetal weights (EFW) and biparietal diameters (BPD), but these differences were not statistically significant ($p=0.057$ and $p=0.060$, respectively). Male fetuses showed larger biometric parameters during the second and third trimesters, which is in line with the findings of Broere-Brown *et al.* who also reported sex-specific growth variations [3]. It is also indicated by the studies that ultrasound-based gender determination achieved 100% sensitivity in accurately identifying fetal gender, during the second and third trimesters, especially having direct visualization pertains to the genitalia is possible, ultrasound-based gender determination achieves 100% sensitivity by precisely identifying fetal gender [1, 10]. Additionally, the observed biometric trends reinforce the value of objective fetal measurements as complementary tools for gender determination, particularly in cases where suboptimal fetal positioning obscures genital visualization.

Despite its valuable findings, this study has several limitations. The cross-sectional design limits the ability to establish causal or longitudinal growth patterns across gestation. The use of purposive sampling and a single-center setting may restrict the generalizability of the results to broader populations. Additionally, gestational age variability and the absence of trimester-wise subgroup analysis may have influenced biometric comparisons. Operator dependency in ultrasound measurements and the lack of adjustment for maternal factors such as body mass index and parity may also introduce measurement bias. Future research should incorporate larger, multicenter cohorts with longitudinal follow-up and trimester-specific analyses to validate these findings. Incorporating advanced imaging techniques and developing predictive models that combine biometric parameters with other sonographic markers may further enhance the accuracy of non-invasive fetal gender prediction.

CONCLUSIONS

The current research extends the effective use of routine ultrasound biometry as a reliable, non-invasive, and cost-efficient approach in the prediction of fetal gender, especially during the later stages. Significantly greater femur length was shown by male fetuses' head circumference, and abdominal circumference compared to female fetuses, while estimated fetal weight and

biparietal diameter were higher in males without being statistically significant. Adopting standardized ultrasound protocols by continuously investigating biometric indicators can improve prenatal care planning, which relies on less invasive diagnostic methods, and early gender prediction is precisely improved. Finally, significant differences in AC, FL, and HC between male and female fetuses emphasize the significance of biometric parameters as valuable predictors of fetal gender.

Authors' Contribution

Conceptualization: MIUH

Methodology: MNA, SMYF, ZN

Formal analysis: MU

Writing and Drafting: MNA, MU, SMYF, MIUH, SM, ZN, AM

Review and Editing: MIUH, MNA, SMYF, ZN, MU, SM, AM

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Article



Prevalence and Risk Factors of Rheumatoid Arthritis among Women in Sahiwal District of Pakistan: A Cross-Sectional Study

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ABSTRACT

Rheumatoid arthritis (RA) starts as a chronic autoimmune disease and gradually destroys joints and representing a systemic complication. **Objectives:** To identify the prevalence of RA and to investigate demographic, clinical, and lifestyle-related factors of the disease in the Sahiwal District of Pakistan. **Methods:** 288 patients who had known RA were recruited. Categories such as demographic, socioeconomic, and lifestyle were used to gather data using a structured questionnaire. There was also a review of clinical records and lab parameters such as ESR and anti-CCP. **Results:** The most prevalent seem to be between the ages of 43-48 years, with RA being 38.2% ultimate, which implies that middle age is the riskiest age group. A large percentage of respondents were experiencing coexisting polycystic ovary syndrome (36.1%), and almost half (48.6%) of the respondents had a family history of autoimmune disease. Over 52.8 percent of them were in low-income households. Although 87.5% were on RA treatment, more than half (54.2%) said they had no regular exercise, even though obesity was found in 43.4%. There were high rates of comorbidities, such as diabetes (39.2%), other autoimmune connective tissue disorders (53.1%). The level of inflammatory activity was high, and 82.6% had an elevated level of ESR, and 94.8% an abnormal result of anti-CCP. **Conclusions:** The findings are a confirmation of the multifactorial nature of RA, which is a combination of the impact of age, genetic factors, hormonal imbalance, lifestyle, and socioeconomic status.

INTRODUCTION

Rheumatoid arthritis (RA) is a combination of various factors, which are mostly categorized as non-modifiable and modifiable factors. These factors are also known as modifiable personal factors, and some of them include tobacco, overweight, and nutrient-deficient diets. These are some of the intrinsic factors, such as age, sex (female), and genetic background/predisposition to RA. Other elements of vulnerability to RA are environmental, including socioeconomic status and infection exposures [1, 2]. The less socioeconomically developed or less

educated people are more vulnerable to RA, especially the antibody-positive type [3, 4]. RA diagnosis depends on clinical manifestation and laboratory tests in accordance with the classification criteria, which were developed by the European League against Rheumatism and American College of Rheumatology. They are categorized by systemic inflammation markers (i.e., high C-reactive protein or high sedimentation rate) and by RA symptoms: joint pain, swelling, and stiffness. Most often, joint damage is examined by means of imaging techniques, including X-ray



and MRI [5]. Rheumatoid arthritis nowadays is treated more with a combination of medication and supportive care. The supportive methods, including physical and occupational therapy, are used to enhance mobility and positively stimulate the work of joints and muscles. Pharmacologically, patients can be treated with a variety of options that include traditional anti-inflammatory medications and the disease-modifying antirheumatic drugs (DMARDs), or more recent agents, including Janus kinase (JAK) inhibitors, used to address the inflammatory pathways to control the symptoms and prevent or slow the progression of the disease [6]. Rheumatoid arthritis (RA) is not new in the world, and its increased prevalence in developed nations could be explained by genetic inclination as well as environment. The prevalence of RA among adults is approximately 1 per cent across the globe, and among women, the disease affects a significantly greater proportion, especially those in the 40 to 60-year-old category. The growing prevalence is likely to result in an aging population, changes in the epidemiology of lifestyle, and improved diagnosis [7]. Lifestyle change is a major component of prevention, and to a certain degree, there exists evidence that breastfeeding is protective. The overall treatment of RA is pegged on the application of the disease-modifying antirheumatic drugs (DMARDs), which selectively attack the underlying inflammatory processes. It seems that the first DMARDs (methotrexate and sulfasalazine) still seem to be the center around which the treatment plans keep rotating, and even the biologic and more modern synthetic agents (the Janus kinase (JAK) inhibitors) have proven to be effective in controlling the disease activity [8, 9]. Although significant progress has been made in efforts to treat rheumatoid arthritis (RA), high inconsistencies in defining treatment and response have still been reported by patients, thus making it worthwhile to understand the biological mechanisms involved to pursue individual management [10]. The more recent forms of treatment include biologic therapies such as interleukin-17 (IL-17) and interleukin-23 (IL-23) and targeted synthetic DMARDs such as Janus kinase inhibitors, which may be useful in the management of symptoms of RA and also in evaluations of the activity of RA [11]. Simultaneously, the improvement of genomics made it possible to identify the polymorphisms related to RA risk, including polymorphisms in human leukocyte antigen (HLA) and other genes related to immunology, which can enhance the risk prediction and provide the possibility of individualized treatment [12]. Even though rheumatoid arthritis is a well-known health issue in the global community, there has been little evidence on the same in Pakistan, particularly in semi-urban areas like Sahiwal. Earlier literature has largely

focused on the urban population, and there is a gap in the literature to comprehend how demographic, lifestyle, and socioeconomic status affect the disease patterns in smaller districts. Clinically, the need to fill this gap can facilitate the earlier diagnosis, risk assessment, and more effective management approaches that help address local needs. The recent years have been marked by the efforts aimed at the implementation of patient-centered care to include shared decision-making with patients in their management to meet the unique needs and preferences [11]. Although rheumatoid arthritis presents a heavy burden, new research and new treatments are being developed, and this will mark the beginning of the era of better care and disease outcome, and improved living [13, 14]. Complementary and alternative medicine (CAM), such as dietary supplements, massage, herbal preparations, and acupuncture, has also elicited interest in addition to standard treatment. Certain literature indicates that some CAM can be of assistance, although findings have been variable even within the same CAM approach, and patients are never forgetting to consult his/her health provider or primary provider before introducing a given approach, taking into account his/her risks and benefits [15]. Exercise is also part of RA treatment. For some people, individualized programs that include aerobic activity, strength exercises, or stretching can also be useful in relieving pain and enhancing normal functioning in everyday life. It is indicated that medical staff or exercise specialists are also to cooperate with the persons on how to properly organize safe activity [16]. Rheumatoid arthritis (RA) may severely affect the quality of life of a person, as it may not only be physical, but also psychological. As an example, RA patients will tend to experience anxiety and depression, which could exacerbate the disease. In line with these, holistic care through medical intervention and psychological assistance (e.g., promoting engagement with mental health specialists, or access to RA patient support groups) should be suggested [17]. The presence of RA in women is partially a complicated mix of hormonal changes in females, hereditary inclinations, and exposure to the environment. In the prevention strategies, they can be based on knowledge and alterations of certain hormonal components. In other countries like Pakistan, further studies are justified to comprehend the effects of socioeconomic factors, individual factors (e.g., demographic attributes like race, gender, and age), environmental exposures, dietary exposures, and treatment on the experiences and outcomes of people with RA. Despite the extensive research on rheumatoid arthritis, very few studies have been conducted to find the prevalence of the disease and the risk factors of the

disease among women in the Sahiwal region. These local trends should be understood to aid in the early detection of the disease and to aid intervention programs that have the potential to enhance patient care and outcomes within this community.

Rheumatoid arthritis (RA) is a chronic autoimmune disease that disproportionately affects women and poses a substantial public health burden due to its impact on physical function, quality of life, and socioeconomic productivity. Although global evidence highlights the role of demographic, hormonal, lifestyle, and socioeconomic factors in the development and progression of RA, there remains a paucity of region-specific data from Pakistan, particularly from semi-urban districts such as Sahiwal. Most existing national studies focus on urban populations or lack comprehensive evaluation of hormonal, metabolic, and lifestyle-related comorbidities among women. This gap limits the development of contextually relevant prevention and management strategies. Therefore, the present study aimed to determine the prevalence of rheumatoid arthritis among women in the Sahiwal District of Pakistan and to investigate associated demographic, socioeconomic, clinical, hormonal, and lifestyle-related risk factors to provide localized evidence for improved disease recognition and targeted interventions.

METHODS

The cross-sectional study was conducted at several hospitals in the Sahiwal District. A convenience sampling method was used to recruit the participants. The research was conducted between April 27, 2024, and October 27, 2024. Permission to conduct the study was sought and given by the Medical Superintendent of District Headquarter Hospital, Sahiwal (Diary No. 2870). Informed consent was given by all participants in writing, and they were assured that their details would be kept confidential. A hierarchical questionnaire was employed in the study to study demographics, clinical symptoms, diagnostic evaluation, prevalence, and risk determinants of rheumatoid arthritis (RA) in the context of hormones and reproductive health. Ultrasound, MRI, and blood test evidence to be used in diagnosis were also examined. The preliminary results indicated that arthritis is rampant in the community, yet no detailed examination of the occurrence of RA and its risk factors had been done. Therefore, this research was conducted in order to determine the prevalence and possible contributory factors. A total of 288 subjects were enrolled in the study; the majority of them were enrolled in the study at hospitals located in Sahiwal, with the District Headquarters (DHQ) Hospital being the primary recruitment site. The recruitment plan was aimed at recruiting individuals with diverse medical and

demographic backgrounds and representative of the healthcare-seeking population in the local setting. Patients and their families were different in their clinical condition to reflect a picture of the community's health. The necessary size of the sample was calculated through the formula of cross-sectional studies: $n = z^2 \cdot p(1-p) / c^2$, where $Z = 1.96$ critical value of a 95% interval, $p =$ expected prevalence of rheumatoid arthritis (20% of the studies by the region), and $d = 0.05$, the level of error. Based on these values, a minimum of 246 participants was determined to be the minimum sample size. A total of 288 participants were eventually recruited to guarantee a robust study, as well as to take into consideration the potential non-response. The recruitment was done predominantly in Sahiwal, and the District Headquarters (DHQ) Hospital was the main location. The sample of this study entails female participants aged 25–60 years having moderate to severe rheumatoid arthritis and having abnormality due to hormonal factors. A particular population was chosen in order to measure the interplay between hormonal factors and RA and identify the particular issues the patients face in managing the disease. This population facilitated this research to narrow down and generate evidence that can be used to develop effective interventions that can be used in controlling RA and hormonal factors associated with it. Explicit exclusion criteria were used in the study to minimize bias and conflicting information. Women aged below 19 years and above 60 years were not included. Women who had a previous diagnosis of tuberculosis, who are hypersensitive to any medication, or who have any allergy were also excluded. Overall, individuals who had a previous infection diagnosis, including HIV or Hepatitis B or C, or whose immunity was compromised, were excluded without an indication of exclusion, since these factors can or cannot influence the conclusion of the research study. Certified raters, through a standardized and validated questionnaire, collected data [18]. The instrument consisted of four major parts: (1) Demographics, age, body mass index, family history of disease, and past medical history; (2) Socioeconomic Background, including the household setting, education, occupation, income, use of medication, and self-reported physical activity, and (3) Dietary Assessment, measuring the number of meals per day, eating habits, and consumption of fruits and vegetables; and (4) Laboratory Tests, which included clinical assessment of factors such as complete blood count, rheumatoid factor and erythrocyte sedimentation rate. This was a systematic method of collecting data that was complete and dependable to the study. The analysis of the data was done with SPSS version 25.0. A descriptive analysis was undertaken in order to summarize numerical variables, and cross-tabulations were generated to determine ratios of the dependent variable. Statistical

tests were performed through chi-square to find out statistical associations between independent variables (that can affect the dependent variable) with respect to the prevalence of rheumatoid arthritis. To further investigate the predictors that are linked to rheumatoid arthritis (RA), the binary logistic regression was used. The research design was aimed at developing the comprehension of aspects concerning the occurrence and treatment of rheumatoid arthritis among this specific group of people.

RESULTS

The sample described the demographic and clinical features of the patients with the background of rheumatoid arthritis (RA). The factors that were included were polycystic ovary syndrome (PCOS), family history, socioeconomic status, medications, physical activity, menopause status, and obesity. The study of 288 patients showed that RA progressed significantly with age, and the highest prevalence rate was found in the group of patients aged between 43-48 years, showing 110 cases (38.2%), which depicts a risk presented in middle age. The highest prevalence was recorded in the cohort between 49-54 years of age and had 54 cases (18.8%), as the second-highest prevalence. Prevalence was lower in younger age groups, where the cohort of age between 37-42 years gave a threshold of 39 cases (13.5%), the cohort of age between 55 and 60; prevalence further declined in persons aged between 25-30 years, with total cases of 17 cases (5.9%). Cumulative percentages are based on percentage of the proportion of participants with RA at each age group, not on incidence over time. Furthermore, the researchers determined the prevalence of Polycystic Ovary Syndrome (PCOS) among the RA patients and concluded that 184 (63.9%) of them had no PCOS, and the other 104 (36.1%) had PCOS, which appears to be an interesting correlation between RA and PCOS. Also, 140 patients (48.6%) indicated that they had a family history of autoimmune diseases, whereas 148 patients (51.4%) did not. The given statistic stressed the relevance of the family in the case of RA because a considerable number of them can be predisposed to autoimmune diseases (Table 1).

Table 1: Frequency of RA According to Age, Frequency of PCOS, Family Disease History

Valid	Frequency (%)	Cumulative Percent
Age		
25-30	17 (5.9%)	5.9%
31-36	29 (10.1%)	16.0%
37-42	39 (13.5%)	29.5%
43-48	110 (38.2%)	67.7%
49-54	54 (18.8%)	86.5%
55-60	39 (13.5%)	100.0%
Total	288 (100.0%)	—

Frequency of PCOS		
No	184 (63.9%)	63.9%
Yes	104 (36.1%)	100.0%
Total	288 (100.0%)	—
Family Disease History		
No	148 (51.4%)	51.4%
Yes	140 (32.8%)	100.0%
Total	288 (100.0%)	—

The socioeconomic background of the patients revealed that most of them are within the low-income bracket, with 152 patients (52.8%). It might mean that socioeconomic conditions might contribute to the control and the course of RA, since a lower socioeconomic background is traditionally linked to the inability to access health care resources. A large proportion of patients with RA (252 or 87.5%) were using medication to manage their disease, with only 36 patients (12.5%) not doing so. This highlights the chronicity of RA and the need to have continued medical care in the majority of patients. Additional research will provide the data on physical activity, and it was found that 156 patients (54.2%) were sedentary, 93 patients (32.3%) were involved in limited physical activity, and only 39 (13.5%) people were moderately active in their physical activity. This implies that a high percentage of RA patients are possibly not exercising enough, which is essential to handle the symptoms and enhance quality of life. The status of the patients in terms of menopause indicates that 133 (46.2%) of the patients were postmenopausal and 155 (53.8%) were not. This data was pertinent because menopause hormone transformation may alter the course of RA and its intensity. Obesity has been a major issue among the RA patients, and 125 (43.4%) patients were found to be obese as opposed to 163 (56.6%) who were not. Obesity is prevalent in this population and may also make management of RA more challenging, as well as worse. The data analysis of the information obtained from RA patients showed some considerable data about the demographic features, health status, and lifestyle of the patients. Most of the RA patients are in a rural setting, where 178 (61.8%) of the respondents live in rural settings, with 110 (38.2%) in urban settings. The distribution of RA shows that there is a significant prevalence of this condition among people living in rural environments (Table 2).

Table 2: Frequency of Socioeconomic Status, Taking Medicine, Physical Activity, Post Menopause, Obesity, and Area of Residence with RA patients

Valid	Frequency (%)	Cumulative Percent
Socioeconomic Status		
High	46 (16%)	16.0%
Low	152 (52.8%)	68.8%
Middle	90 (31.3%)	100.0%

Total	288(100.0%)	–
Taking Medicine		
No	36(12.5%)	12.5%
Yes	252(87.5%)	100.0%
Total	288(100.0%)	–
Physical Activity		
Little	93(32.3%)	32.3%
Moderate	39(13.5%)	45.8%
Sedentary	156(54.2%)	100.0%
Total	288(100.0%)	–
Post Menopause		
No	155(53.8%)	53.8%
Yes	133(46.2%)	100.0%
Total	288(100.0%)	–
Obesity		
No	163(56.6%)	56.6%
Yes	125(43.4%)	100.0%
Total	288(100.0%)	–
Area of Residence		
Rural	178(61.8%)	61.8%
Urban	110(38.2%)	100.0%
Total	288(100.0%)	–

A huge percentage of 199 (69.1%) of the participants claimed that they are facing insomnia, and only 89(30.9) of them do not experience insomnia. This implies that one of the typical comorbidities in people with RA is insomnia. The dietary habits of the RA patients revealed that a big percentage of the sample population eats twice a day, with 143(49.7%) constituting the percentage. In the meantime, 57(19.8%) indicated that they ate once a day, and 88(30.6%) were eating thrice a day. Such a distribution implies inconsistency in the frequency of meals, which can influence the general well-being and treatment of RA. The correlation between RA and thyroid diseases showed that 212 (73.6%) of RA patients did not have a thyroid disease, whereas 76 (6.4%) had a thyroid disease. It indicates that although thyroid diseases do exist in a significant proportion of patients with RA, they are not the most common one. The incidence of diabetes is interesting among patients with RA. 175 (60.8%) of the respondents had no diabetes diagnosis as compared to 113(39.2%) who had a diagnosis of diabetes. This suggests that diabetes is a major prevalence in the RA patients. The infection rate of the autoimmune connective tissue disease (ACD) among the RA patients suggested that 153 (53.1%) of the respondents have ACD, and 135 (46.9%) do not. This brings out a significant overlap between ACD and RA (Table 3).

Table 3: Frequency of Insomnia, Taking Meals, Thyroid Disorder, Diabetes, and ACD in RA Patients

Valid	Frequency (%)	Cumulative Percent
Insomnia		
No	89(30.9%)	30.9%

Yes	199(69.1%)	100.0%
Total	288(100.0%)	–
Taking Meals		
1 Time	57(19.8%)	19.8%
2 Times	143(49.7%)	69.4%
3 Times	88(30.6%)	100.0%
Total	288(100.0%)	–
Thyroid Disorder		
No	212(73.6%)	73.6%
Yes	76(26.4%)	100.0%
Total	288(100.0%)	–
Diabetes		
No	175(60.8%)	60.8%
Yes	113(39.2%)	100.0%
Total	288(100.0%)	–
ACD		
No	135(46.9%)	46.9%
Yes	153(53.1%)	100.0%
Total	288(100.0%)	–

The given results of erythrocyte sedimentation rate (ESR) in the patients with RA demonstrate that an impressive 238 (82.6%) of them had high positive ESR, and 50 (17.4%) had moderate positive levels. This indicates that there is a high inflammatory reaction in most RA patients. Another important result is that the percentage of participants with abnormal anti-CCP levels is rather high (273, or 94.8%), which means that the correlation between such antibodies and RA is strong, and 15 (5.2%) participants have normal levels (Table 4).

Table 4: Frequency of ESR and Anti-CCP in RA Patients

Valid	Frequency (%)	Cumulative Percent
ESR		
High Positive	238(82.6%)	82.6%
Moderate Positive	50(17.4%)	100.0%
Total	288(100.0%)	–
Anti-CCP		
Abnormal	273(94.8%)	94.8%
Normal	15(5.2%)	100.0%
Total	288(100.0%)	–

DISCUSSION

Rheumatoid arthritis (RA) is a long-term autoimmune disease, which is marked by inflammation of the joints resulting in serious morbidity and loss of quality of life. The purpose of the discussion is to place the results of the present study on the prevalence of RA and the risk factors related to it in the environment of the existing literature to indicate the epidemiological patterns, socioeconomic determinants, comorbidities, and implications of research on healthcare. It was significantly age-related, and rheumatoid arthritis (RA) was common, with the highest prevalence occurring between the ages of 43 and 48. The

same trends have been observed in Pakistan, where RA was observed mostly in middle-aged women. Research has indicated that RA was observed more in women compared to men across the globe, usually between the ages of 40-60 years as a result of hormonal and genetic effects [1]. In this study, it was indicated that RA was 29.5% at the age of 42 and at its highest by the age of 60, which was 100. Thus, age is a significant factor that determines the disease onset and outcome. The financial condition of the RA patients in Sahiwal identified that the majority of the patients belonged to low-income families. It is common to associate the socioeconomic position with ill health and inaccessibility to health services [3]. Health infrastructure has its threshold in Pakistan, and the patients who have limited resources will find it harder to deal with the conditions that are related to RA. The majority of the participants were generally poorly educated, and this could be a constraint on their knowledge of the disease and the capacity to comply with medications. Although 87.5 percent of the participants had been taking medicine, more than half (54.2%) respondents said they were of a sedentary lifestyle, indicating how lacking awareness of the disease and self-management is. The comorbid conditions among patients in the study were also of great burden, with 43.4, 39.2, and 69.1 being obesity, diabetes, and insomnia, respectively. The current literature has also shown similar tendencies in which it is possible to complicate treatment and thus impair health outcomes when multiple co-existing conditions are involved [11]. The insomnia prevalence rates are also additional examples of why the integrative care approaches should be considered to cover both physical care and mental care. Hormonal factors - especially in women were also checked in connection with RA. A high percentage of RA patients were stated to experience polycystic ovary syndrome (36.1%), and therefore, there might be a relation between hormonal factors and autoimmune diseases. Previous studies have shown that hormonal changes affect immune performance and predispose to autoimmune or autoimmune diseases such as RA [7]. These are also key factors to include hormonal wellness in RA treatment, especially among women. The studies also indicated that there was an urgent demand for healthcare provision tailored to the region, in this case, Sahiwal, Pakistan, and other regions that are located in a similar location. RA and other risk factors are also a huge burden, and the healthcare system should note the need to evaluate the disease timely, the comprehensive method of care in the disease and the patient, and the requirements linked to the socioeconomic and mental effects of the disease, but not only the physical effects. Education and awareness programs may be an important part of making patients more adherent to the treatment process in order to improve the quality of life of people with

rheumatoid arthritis (RA) [20]. The noted relationships between rheumatoid arthritis and PCOS, menopause, obesity, SES, diabetes, and insomnia are descriptive. These cannot be taken as causal relationships because no regression analysis was done to ascertain. These relationships have to be verified in future analytical research. The results of our studies on the relationship between RA and age, gender, and hormonal factors agree with those done previously. As an example, Koller-Smith *et al.* and Bullock *et al.* found women to be higher in midlife, which is in line with the fact that the majority of cases were identified in women aged 40-55 years in our case [1, 2]. On the same note, our study findings that found an obesity and RA relationship are similar to Tanaka's findings, although our group association was stronger, perhaps because of dietary and lifestyle factors within the region [9]. Nevertheless, the findings of our study were in contrast to those of Tobone *et al.* who discussed the effects of environmental triggers, because there was no significant effect of SES on the prevalence of RA in our study, implying that local population characteristics might alter the known risk factors [3]. These comparisons draw similarities and differences, and it is important to have context-specific studies that would inform prevention and management strategies. The results are descriptive, and they point to the noticeable trends in the population of the study. The only inferential analysis that was not done included logistic regression and others; therefore, associations are supposed to be taken with care. Multivariate models should be used in future studies to establish the independent and causal influence of these factors.

This study has certain limitations that should be acknowledged. The cross-sectional design restricts causal inference between identified risk factors and the development or progression of rheumatoid arthritis. The use of convenience sampling and recruitment from hospital-based settings may limit the generalizability of findings to the wider community, including undiagnosed or untreated individuals. Additionally, the absence of multivariate regression analysis limits the ability to determine independent predictors of RA, and reliance on self-reported data may introduce recall or reporting bias. Future studies should employ longitudinal and community-based designs with probability sampling to better assess causal relationships and disease progression. Incorporating multivariate analytical models and exploring genetic, environmental, and psychosocial determinants would further strengthen understanding. Such research could support the development of targeted screening, early intervention, and comprehensive management programs tailored to women in resource-limited and semi-urban settings of Pakistan.

CONCLUSIONS

The findings of this study indicate that the occurrence rate of rheumatoid arthritis (RA) in females residing in Sahiwal, Pakistan, is very high, and the most vulnerable group is middle-aged individuals, with a range of 43 to 48 years. The research results also showed that age, socioeconomic disadvantage, less education, and sedentary lifestyle are the risk factors that are linked to RA, as well as the fact that the environmental and personal variables are at times confounding in the development and progression of the disease. Similarly, common obesity, diabetes, insomnia, and other comorbidities contributed specifically to the difficulty in managing the disease and reminding them of their necessity of comprehensive care. The role of hormones, especially polycystic ovary syndrome (PCOS), was observed among a significant proportion of patients, and consequently, there has been speculation as to the link that exists between the endocrine and the autoimmune functions.

Authors' Contribution

Conceptualization: SA

Methodology: KR, MAN, J, A, TI

Formal analysis: MS

Writing and Drafting: AI, TI

Review and Editing: SA, KR, MAN, J, A, TI, MS, AI

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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