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Original Research

Diagnostic Evaluation of Primary Ovarian Insufficiency in a Cohort of 290 Pakistani Women: Clinical, Hormonal, and Genetic Perspectives

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Abstract

Background: Primary ovarian insufficiency (POI) is a heterogeneous disorder with multifactorial etiologies. Accurate diagnosis requires an integrated clinical, hormonal, and genetic evaluation, yet data from Pakistan are limited, and the burden of idiopathic and genetically predisposed cases remains largely unknown. **Methods**: A total of 345 women under 40 years presenting with amenorrhea or menstrual irregularities were screened. After excluding pregnancy, cases not meeting the European Society of Human Reproduction and Embryology (ESHRE) diagnostic criteria, and incomplete records, 290 women were included. Comprehensive clinical, hormonal, and genetic investigations were performed according to ESHRE guidelines to determine underlying etiologies. **Results**: The mean age at presentation was 33 $\pm$ 4.5 years, with a median symptom duration of 6 months. The mean age at menarche was 13 $\pm$ 1 years, and the mean body mass index (BMI) was 24.5 $\pm$ 3.4 kg/m^2. Most women presented with amenorrhea (80%) or oligomenorrhea (20%). Secondary infertility was reported in 72.8% and primary infertility in 2.4%. A history of miscarriage was documented in 5.9% of participants. Common clinical features included hot flushes (75.9%), depression (72.4%), high stress (65.5%), mood changes (62.1%), vaginal dryness or dyspareunia (55.2%), and night sweats (54.5%). Coexisting comorbidities were observed in 12.4%, most frequently migraines (4.1%). Hormonal evaluation confirmed elevated follicle-stimulating hormone (FSH) levels (>25 IU/L) and low estradiol (<50 pg/mL) in all participants. Etiological classification identified iatrogenic causes in 7.2%, genetic causes in 3.8% (confirmed in women with suggestive genetic features or isolated POI), autoimmune causes in 6.6%, and idiopathic POI in 82.4%. Statistically significant differences in confirmed diagnoses were observed among most etiological groups ($p < 0.0001$), except for women with features suggestive of a genetic cause ($p \approx 0.8500$). **Conclusions**: POI presents with diverse clinical features. Evaluation based on ESHRE guidelines enables identification of iatrogenic, autoimmune, and genetic contributors, and highlights the high prevalence of idiopathic cases, which may have an underlying genetic predisposition.

Keywords: primary ovarian insufficiency; idiopathic POI; genetic predisposition; autoimmune POI; iatrogenic causes; ESHRE guidelines

1. Introduction

Primary ovarian insufficiency (POI) is a heterogeneous condition characterized by impaired ovarian function occurring before the age of 40 years, affecting approximately 1.1% of women of reproductive age [1]. POI is characterized by menstrual irregularities, amenorrhea, infertility, and hypoestrogenic symptoms such as hot flushes, night sweats, and vaginal dryness. It is further associated with a wide range of additional clinical features, including neuropsychiatric, psychological, musculoskeletal, cardiovascular, and lifestyle-related factors [2–4]. POI often coexists with thyroiditis, Addison's disease or other autoimmune disorders, sometimes accompanied by enlarged multifollicular ovaries [2]. In addition, syndromic features of Turner syndrome or blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) or fragile X syndrome and a family history of early menopause or infertility may also be observed with POI [5–7]. Beyond infertility, POI carries substantial health consequences, including increased risks of osteoporosis, cardiovascular disease, metabolic disorders, and diminished quality of life [8,9]. Associated symptoms, clinical findings and comorbidities can be variable due to intermittent ovarian hormone secretion [2]. At presentation, documenting medical and family histories, along with key clinical features, is essential to help identify POI and its possible underlying causes.

According to the 2016 European Society of Human Reproduction and Embryology (ESHRE) guidelines, POI should be considered in women younger than 40 years who present with oligo/amenorrhea or estrogen-deficiency symptoms, with diagnosis confirmed by oligo/amenorrhea lasting at least four months, two elevated follicle-stimulating hormone (FSH) measurements (>25 U/L), and low estradiol levels (<50 pg/mL) obtained 4–6 weeks apart in the early follicular phase [10]. In contrast, the updated 2024 ESHRE guidelines now indicate that a single elevated FSH measurement may suffice to establish the diagnosis. The guidelines also recommend excluding pregnancy and accounting for the effects of hormonal therapy, which can mask amenorrhea or alter FSH levels, before confirming POI [11]. These recommendations outline further testing to determine the cause of POI. Women with a history of chemotherapy, radiotherapy, pelvic or ovarian surgery, or bilateral salpingo-oophorectomy before age 40 are considered to have iatrogenic POI and typically do not require further evaluation. For all non-iatrogenic cases, following genetic counseling and informed consent, chromosomal analysis and *FMR1* premutation (PM) testing are recommended, while broader genetic testing may be considered where feasible. If no genetic cause is identified, screening for 21-hydroxylase autoantibodies (21OH-Abs) and thyroid-stimulating hormone (TSH) levels is recommended to detect potential autoimmune etiologies. Cases with no identifiable cause after these evaluations are classified as idiopathic POI [10,11].

Despite growing knowledge and international guidelines, their implementation is limited in low- and middle-income regions, and data on POI remain scarce. In Pakistan, only a few studies have explored POI, focusing mainly on poor ovarian response, hormonal disturbances, and associated symptoms [12,13], without performing integrated clinical, biochemical, or genetic assessments. Limited awareness and resources have hindered guideline-based studies, leaving most cases unexplained. We hypothesized that comprehensive clinical, hormonal, and genetic evaluation according to ESHRE guidelines would enhance identification of underlying etiologies in POI, including potential genetic contributions among idiopathic cases. Therefore, this study aimed to comprehensively characterize the clinical, hormonal, and genetic features of POI among affected Pakistani women and to classify its etiologies according to ESHRE guidelines.

2. Materials and Methods

2.1 Study Design and Participants

This multicenter cross-sectional study was conducted between August 2023 and February 2025, and participating women were recruited from the obstetrics and gynecology departments of tertiary care hospitals. Women under 40 years of age presenting with amenorrhea or oligomenorrhea persisting for at least four months and meeting the ESHRE diagnostic criteria of FSH >25 IU/L and estradiol <50 pg/mL on two separate measurements taken more than four weeks apart, were included. Women who were pregnant, currently using hormonal therapy, diagnosed with polycystic ovary syndrome (PCOS), had malignant tumors, or had undergone ovarian removal were excluded (according to 2016 and 2024 guidelines), as these conditions can confound menstrual irregularities or hormonal measurements required for POI evaluation [10,11]. A history of prior hormonal therapy was recorded, and participants were included only if therapy had been discontinued for at least three months before recruitment. Written informed consent was obtained, and the study was approved by the Ethical Committee of KUST (REF:/KUST/Ethical Committee/837).

2.2 Clinical Evaluation

Detailed medical and reproductive histories were obtained, including age at menarche, age at symptom onset, type of menstrual disturbance (amenorrhea/oligomenorrhea), reproductive history, miscarriage history, and infertility. Body mass index (BMI) was measured for all participants. Hypoestrogenic symptoms such as hot flushes, night sweats, vaginal dryness, and systemic manifestations were recorded. Features suggestive of Turner syndrome, BPES, autoimmune disorders, or iatrogenic causes (chemotherapy, radiotherapy, ovarian surgery) were carefully recorded. Coexisting comorbidities were documented based on patient history, prior diagnoses, management records, laboratory investigations, imaging, and review of medical records as appropriate. Family history of infertility or early menopause, as well as lifestyle and psychosocial factors, were also recorded. Depression and emotional instability were evaluated using the Patient Health Questionnaire-9 (PHQ-9) [14].

2.3 Diagnostic Evaluation

Following ESHRE recommendations, menstrual disturbance was required to persist for at least four months to support a diagnosis of POI. For classification purposes, amenorrhea was defined as the complete absence of menstruation for $\geq$ six months, while oligomenorrhea was defined as menstrual cycles longer than 35 days or fewer than nine cycles per year. Hormonal testing (FSH and estradiol) was performed in all participants, in the early follicular phase (days 2–5) for women with ongoing menstrual cycles, and on a random day for those presenting with amenorrhea. Thyroid function was assessed in all participants by measuring serum TSH levels, with a reference range of 0.4–4.0 mU/L. After genetic counseling and obtaining informed consent, peripheral blood samples were collected in EDTA tubes, appropriately labeled, and transported under cold-chain conditions. Samples were stored at 4 °C and processed within 24–48 hours. Genomic DNA was extracted using the standard phenol–chloroform protocol or the QIAamp DNA Blood Mini Kit (Catalog No. 51306;

Table 1. Symptoms, clinical features, psychological factors, and lifestyle characteristics in women with POI (N = 290).

Category	Specific symptom(s)/Findings	Frequency (n, %)
Estrogen deficiency	Hot flushes	220 (75.9%)
	Night sweats	158 (54.5%)
	Vaginal dryness/dyspareunia	160 (55.2%)
Neuropsychiatric	Mood changes (mood swings, mental fog, melancholia)	180 (62.1%)
Sleep disturbances	Insomnia/irregular sleep–wake cycle	115 (39.7%)
Sexual dysfunction	Reduced libido, dyspareunia	108 (37.2%)
Fatigue	Generalized fatigue	103 (35.5%)
Dermatological	Hair loss, skin dryness	92 (31.7%)
Ophthalmological	Dry eyes	88 (30.3%)
Endocrine/Metabolic	Cold intolerance	83 (28.6%)
Musculoskeletal	Joint clicking, muscle/joint pain	75 (25.9%)
Neurological	Headaches, vertigo, tingling limbs	72 (24.8%)
Cardiovascular	Palpitations	65 (22.4%)
Psychological	Depression	210 (72.4%)
	High stress levels	190 (65.5%)
	Anxiety	120 (41.4%)
Lifestyle	Social withdrawal	98 (33.8%)
	Poor diet/nutrition	85 (29.3%)
	Low physical activity	65 (22.4%)

Note: Values represent frequency (n) and percentage (%) of participants reporting each manifestation.
POI, primary ovarian insufficiency.

Table 2. Comorbidities observed in women with POI.

Comorbidity	Frequency (n, %)	Documentation/Diagnosis
Migraine	12 (4.1%)	Patient history and documented past diagnosis and management records
Hypertension	8 (2.8%)	Systolic BP $\geq$140 mmHg or diastolic BP $\geq$90 mmHg on two occasions, or on antihypertensive therapy
Diabetes mellitus	5 (1.7%)	Fasting plasma glucose $\geq$126 mg/dL, HbA1c $\geq$6.5%, or prior physician diagnosis
Obesity	4 (1.4%)	BMI $\geq$30 kg/m^2
Osteoporosis	3 (1.0%)	T-score ≤ -2.5 measured by dual-energy X-ray absorptiometry (DEXA) scan
Breast cancer	2 (0.7%)	Histopathology confirmation and clinical oncology records
Ischemic heart	1 (0.3%)	History of myocardial infarction or angina, confirmed by ECG or imaging
Gastric ulcers	1 (0.3%)	Endoscopic confirmation with biopsy
Total	36 (12.4%)	

BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; ECG, electrocardiogram.

Qiagen, Hilden, Germany), following recommended procedures. DNA concentration and purity were measured using a NanoDrop Lite Plus spectrophotometer (Catalog No. NDLPLUSGL; Thermo Fisher Scientific, Waltham, MA, USA) by assessing A260/A280 and A260/A230 ratios, and the DNA was stored at –20 °C for subsequent use. Testing included karyotyping and *FMR1* PM analysis [15], as well as direct sequencing of established POI-associated genes such as *BMP15*, *NOBOX*, and *GDF9* [16] for all participants. *FOXL2* sequencing was performed selectively in women presenting with features suggestive of both POI and BPES, in whom no variants were identified in the other established POI-associated genes. Segregation analysis was performed in available family members to assess inheritance patterns of identified variants. *In silico* prediction tools were used to assess the potential impact of identified variants, and each variant was classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines [17]. Autoimmune evaluation (21OH-Abs) was carried out selectively in women without an identified genetic or iatrogenic cause, and in those presenting with symptoms suggestive of autoimmune involvement.

2.4 Classification of Cases

Cases were categorized according to the underlying cause, in line with ESHRE 2024 guidelines: iatrogenic POI (history of chemotherapy, radiotherapy, or ovarian surgery), genetic POI (chromosomal abnormalities, *FMR1* PM alleles, or pathogenic/likely pathogenic variants), autoimmune POI (positive 21OH-Abs or abnormal TSH levels), and idiopathic POI (no identifiable cause after a comprehensive diagnostic workup).

2.5 Data Analysis

Data was analyzed using IBM SPSS Statistics, Version 30.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± SD (standard deviation) or median, and categorical variables as frequencies and percentages. Associations between clinical presentation groups and diagnostic outcomes (confirmed vs. non-confirmed) were assessed using two-tailed Chi-square or Fisher's exact tests, applied as appropriate. The null hypothesis assumed no association between clinical presentation and diagnosis. A p-value < 0.05 was considered statistically significant, indicating an association between clinical presentation and diagnostic outcome.

3. Results

3.1 Study Cohort

A total of 345 women under 40 years presenting with amenorrhea or menstrual irregularities and/or estrogen-deficiency symptoms were initially recruited. Women who were pregnant, currently using hormonal therapy, or had not provided consent for the diagnostic workup were excluded. After these exclusions, 290 women were included in the final analysis. The mean age at presentation was 33 ± 4.5 years, with a median symptom duration of 6 months.

3.2 Clinical Features

Most women presented with amenorrhea (80%, n = 232) and oligomenorrhea (20%, n = 58), with a mean duration of amenorrhea of 6 ± 2 months. The mean age at symptom onset was 31.6 ± 3.8 years, and the mean age at menarche was 13 ± 1 years. The mean BMI of the participants was 24.5 ± 3.4 kg/m^2. Reproductive history revealed secondary infertility in 72.8% (n = 211) and primary infertility in 2.4% (n = 7). Miscarriage history was documented in 5.9% women (n = 17). A family history of infertility or early menopause was documented in 9.3% (n = 27).

Women exhibited a wide spectrum of clinical features, including manifestations of estrogen deficiency, neuropsychiatric symptoms, and musculoskeletal, neurological, and cardiovascular complaints. The most prevalent features were hot flushes (75.9%), depression (72.4%), high stress levels (65.5%), mood changes (62.1%), vaginal dryness or dyspareunia (55.2%), and night sweats (54.5%) (Table 1). Coexisting comorbidities were observed in 12.4% of cases, with migraine being the most common (4.1%) (Table 2).

3.3 Etiological Clues

Clinical evaluation revealed iatrogenic factors (previous ovarian surgery, chemotherapy, or radiotherapy) in 7.2% (n = 21), features suggestive of a genetic etiology in 16.6% (n = 48), and coexisting autoimmune disorders in 14.5% (n = 42) (Table 3).

3.4 Hormonal and Genetic Findings

All included women had elevated FSH (>25 IU/L) confirmed on two occasions, with a mean FSH of 30 ± 12 IU/L and mean estradiol of 25 ± 10 pg/mL. Among the 290 women with POI, 21 had iatrogenic causes due to ovarian surgery, chemotherapy, or radiotherapy, all of whom had confirmed diagnoses (100%). Among women without iatrogenic causes, pathogenic genetic variants were detected in two groups. In 48 women with Turner syndrome stigmata, BPES, a family history of early menopause or infertility, or early age at onset, 8 had confirmed diagnoses, including karyotypic abnormalities consistent with Turner syndrome (2 cases), *FMR1* PM alleles with 50–65 CGG repeats (5 cases), and a heterozygous *FOXL2* variant [c.223C>T, p.(Leu75Phe)] identified in one woman with both POI and BPES. The same *FOXL2* variant was present in her father and brother, who exhibited BPES features. Based on ACMG criteria (PM1, PM2, PP1, PP3), the variant is classified as a variant of uncertain significance (VUS). Among 179 women with POI features only 3 rare genetic variants were detected, including *FMR1* PM alleles with 50, 57 CGG repeats in 2 cases and a homozygous *GDF9* variant [c.604C>T, p.(Gln202*)] identified in one woman with POI. This *GDF9* variant segregated in an autosomal recessive manner, with both parents being heterozygous carriers. Based on ACMG criteria (PVS1, PM3, PP3), the variant is classified as likely pathogenic. Autoimmune evaluation confirmed the diagnosis in 19 women with autoimmune disease, while no confirmed cases were observed in women with features suggestive of a genetic etiology or POI features only. Statistically significant differences in the proportion of confirmed diagnoses were observed among most clinical presentation groups, except for women with features suggestive of a genetic etiology ($p \approx 0.8500$). Overall, the proportion of confirmed diagnoses differed significantly across the underlying etiological categories, with highly significant associations observed for iatrogenic ($p < 0.0001$), autoimmune ($p < 0.0001$), and idiopathic/other genetic cases ($p < 0.0001$) (Table 3).

3.5 Etiological Classification

Confirmed causes of POI were identified in a subset of women: iatrogenic 7.2%, genetic 3.8%, and autoimmune 6.6%. The remaining women without a definitive cause, or with suggestive genetic or autoimmune features, were classified as idiopathic POI, accounting for 82.4% of the cohort (Table 4).

4. Discussion

POI is a heterogeneous reproductive disorder, characterized by diverse etiologies and a wide range of clinical manifestations [18], yet in many cases the underlying cause remains unidentified despite advances in understanding. Studies from high-income countries have clarified the genetic, autoimmune, and iatrogenic factors associated with

Table 3. Underlying causes of POI in 290 women, determined by clinical features and diagnostic investigations.

Clinical presentation (n, %)	Investigations confirmed diagnosis	Confirmed diagnosis (n, %) (N = 51)	Non-confirmed diagnosis (n, %) (N = 239)	Statistical test (*p*-value)	Underlying etiology of POI
History of ovarian surgery, chemotherapy, or radiotherapy (21, 7.2%)	Confirmation from medical/surgical history and records	21 (100.0%)	0 (0.0%)	<**0.0001***	**Iatrogenic**
Turner syndrome stigmata, family history of infertility/early menopause, blepharophimosis, early age at onset (48, 16.6%)	Genetic testing: *FMR1* PM analysis, *FOXL2*, *BMP15*, *NOBOX*, and *GDF9* genes sequencing	8 (16.7%)	40 (83.3%)	≈0.8500	**Genetic** (Turner syndrome, *FMR1* PM alleles, one *FOXL2* variant)
Coexisting autoimmune disorders: thyroid dysfunction, systemic lupus erythematosus, rheumatoid arthritis, autoimmune haemolytic anaemia, vitiligo, Addison's disease (42, 14.5%)	Autoimmune serological testing for 21OH-Abs and serum TSH levels	19 (45.2%)	23 (54.8%)	<**0.0001***	**Autoimmune** (Positive 21OH-Abs and/or abnormal TSH levels)
POI features without additional conditions (179, 61.7%)	Genetic and autoimmune serological testing	3 (1.7%)	176 (98.3%)	<**0.0001***	**Idiopathic** (no definitive cause identified)/Genetic (*FMR1* PM alleles in 2 cases, a *GDF9* variant in 1 case)

Note: Values are presented as frequency (n) and percentage (%). Statistical significance was assessed using Chi-square or Fisher's exact tests. An asterisk (*) indicates a statistically significant *p*-value ($p < 0.05$). Bold = significant values and underlying POI etiology. PM, premutation; 21OH-Abs, 21-hydroxylase autoantibodies; TSH, thyroid-stimulating hormone.

Table 4. Distribution of underlying causes of POI among women (N = 290).

Underlying etiology	Number of cases (n)	Percentage (%)
Iatrogenic (surgery, chemotherapy, radiotherapy)	21	7.2
Genetic (Turner syndrome, *FMR1* PM alleles, one *FOXL2* variant, one *GDF9* variant)	11	3.8
Autoimmune (thyroid dysfunction, lupus, rheumatoid arthritis, Addison's disease, etc.)	19	6.6
Idiopathic (no definitive cause identified)	239	82.4
Total	290	100.0

Note: Values represent frequency (n) and percentage (%) of participants in each etiological category.

POI [7,19,20], but large gaps remain in low- and middle-income regions. In Pakistan, only a single study by Izhar *et al.* [12] has explored POI, focusing on poor ovarian response criteria to detect occult POI in women with infertility and oligomenorrhea. More recently, Kazi *et al.* [13] assessed the diagnostic and management challenges of POI in women attending a tertiary hospital in Lahore, highlighting the association of infertility, hot flashes, mood swings, vaginal dryness, and insomnia with disturbed hormonal and ovarian reserve markers. However, these studies relied mainly on clinical symptoms and surrogate biochemical markers, without integrating broader clinical, hormonal, and genetic investigations. To our knowledge, the present study is the first in Pakistan to comprehensively evaluate POI by integrating detailed clinical assessment, hormonal profiling, and genetic testing according to ESHRE diagnostic criteria.

Consistent with ESHRE recommendations, all women of reproductive age (under 40 years) in this study fulfilled the diagnostic criteria for POI, presenting with at least four months of oligo/amenorrhea, repeated serum FSH levels >25 IU/L, and low estradiol levels of approximately 50 pg/mL. Although POI can occur across a broad age range, from early adolescence to 40 years [21], the age of onset and age at diagnosis remain key factors for timely recognition and management. In the current cohort, the mean age at symptom onset was 31 years, and the mean age at clinical presentation was 33 years, indicating an approximate delay of 24 months. This delay is shorter than the 48-month median time to diagnosis reported by Minis *et al.* [22], but comparable to findings from Sun *et al.* [23], who reported an average interval of approximately 28 months between symptom onset and POI diagnosis, and to Bakhsh [24], who observed diagnostic delays exceeding 18 months in a substantial proportion of patients. Such delays are often related to variability and inconsistency in presenting features, as highlighted by Kapoor [25]. In Pakistan, additional factors such as limited awareness of POI, sociocultural barriers affecting care-seeking, and variability in access to specialized diagnostic services may further contribute to delayed diagnosis.

The clinical spectrum observed was broad, with vasomotor symptoms such as hot flushes, vaginal dryness or dyspareunia, and night sweats, alongside mood changes and psychological disturbances including depression and stress, being highly prevalent. Comparable clinical presentations have been observed in studies of other populations, where vasomotor and neuropsychiatric symptoms dominate the clinical profile [2–4]. The high prevalence of depression (72.4%) and stress (65.5%) in our cohort is consistent with previous reports of psychological disturbances in women with POI [26], highlighting the substantial psychosocial impact of POI, which may be further aggravated by cultural stigma surrounding infertility in South Asian societies.

The association between POI and long-term comorbidities, such as osteoporosis, metabolic syndrome, and cardiovascular disease, is well established [8,9]. Comorbidities of POI were documented in 12.4% of cases, with migraine being the most common (4.1%). Although the prevalence of metabolic and cardiovascular comorbidities in our cohort was lower than reported in other studies [27,28], this is not solely explained by age, as participants in these studies were also predominantly below 40 years. In contrast, women aged ≥45 years with a prior diagnosis of POI have been reported to exhibit a higher prevalence of cardiometabolic comorbidities [29,30], highlighting the cumulative risk associated with longer duration of estrogen deficiency. These findings emphasize that age and duration of POI are important factors influencing the manifestation of long-term systemic comorbidities.

The etiological distribution of POI in our cohort illustrates its complexity. Iatrogenic causes accounted for 7.2% of cases, consistent with a study reporting 6–47% [31], and aligning with another study reporting a ~8% cumulative risk of POI by age 40 in female cancer survivors [32]. The highest risk was observed after alkylating agents and ovarian radiotherapy [31], influenced by age at exposure, treatment dose, and baseline ovarian reserve [2]. In our cohort, the difference in confirmed iatrogenic diagnoses was highly significant ($p < 0.0001$), highlighting that this subgroup represents a clearly distinguishable and reliably diagnosed etiology. Genetic causes were confirmed in 3.8%, collectively from women with features suggestive of a genetic etiology (16.6%) and those presenting with POI features only (61.7%), which is markedly lower than the 20–25% reported in a previous study [6]. *FMR1* PM alleles were detected in seven cases, Turner syndrome in two, one *FOXL2* variant [c.223C>T, p.(Leu75Phe)] previously reported in POI with BPES [5] in one case, and a *GDF9* variant [c.604C>T, p.(Gln202*)] previously re-

ported in POI [7] in sisters. The frequency of confirmed cases was not statistically significant among women with suggestive genetic features ($p \approx 0.8500$), whereas the difference was highly significant among those with POI features only ($p < 0.0001$), indicating a substantial burden of undiagnosed genetic predisposition in the idiopathic group and emphasizing the limitations of conventional diagnostic approaches. This underscores the importance of advanced genetic testing, particularly whole-exome sequencing (WES), in women with idiopathic POI or subtle clinical indicators such as Turner stigmata, BPES, family history of infertility/early menopause, or early disease onset. Screening for 21OH-Abs and TSH confirmed an association of POI with autoimmune disease in 19 women (6.6%), with no cases detected in women with features suggestive of a genetic etiology or POI features only. This difference was highly significant ($p < 0.0001$), indicating that autoimmune testing effectively identified women with POI associated with autoimmune disease and highlighting its utility in detecting cases with an underlying autoimmune etiology. However, a causal relationship could not be established, as autoimmune disorders are common and autoantibody positivity may not always reflect a direct cause of ovarian insufficiency [2].

Limitations

Despite providing a comprehensive evaluation of POI in women across multiple centers in Pakistan, this study has several limitations. First, the cohort included only Pakistani women, which may limit generalizability to other populations. Second, although our study aimed to identify underlying etiologies and potential undetected genetic predispositions in POI using ESHRE-guided evaluation, no control group was included, limiting comparative analyses. Third, in Pakistan, genetic testing is generally not performed for POI cases, and due to high costs, our study focused on a limited gene panel, which may underestimate rare or novel variants and highlights the need for broader genetic evaluation. Fourth, autoimmune assessment relied on specific serological markers, which may not capture all relevant mechanisms. Finally, the cross-sectional design precludes assessment of long-term outcomes, including the progression of comorbidities and reproductive or metabolic consequences. These limitations underscore the need for larger, multi-center, longitudinal studies with broader genetic and immunological testing to fully characterize POI.

5. Conclusions

In conclusion, this study provides the first comprehensive evaluation of POI in Pakistan, integrating clinical, hormonal, and genetic assessments in line with ESHRE guidelines. The findings highlight delayed diagnosis, a high psychological burden, and underrecognized comorbidities in affected women. Iatrogenic causes were clearly identifiable and statistically significant, while genetic causes were confirmed in a small proportion, with many women showing subtle features or idiopathic presentation, indicating a substantial burden of undiagnosed genetic predisposition. Autoimmune contributors were confirmed in a subset of women, with testing proving effective in identifying true cases. Overall, the high proportion of idiopathic cases underscores the need for broader genetic evaluation, including WES, and earlier diagnostic approaches to improve recognition and management of POI, particularly in resource-limited settings.

Availability of Data and Materials

All data reported in this study are included within the manuscript.

Author Contributions

SS contributed to the conceptualization and study design, genetic investigations, data validation and interpretation, manuscript drafting, and final approval. HT performed data collection, analysis, and laboratory work. UNT and MJ were responsible for participant recruitment and clinical evaluation, including investigations and interpretation. ZZ contributed to the clinical evaluation and interpretation of participants presenting with acute or overlapping symptoms such as palpitations, dizziness, and headaches. SAK performed psychiatric and psychological evaluations and their interpretation. MY contributed to data collection, analysis, laboratory work, and oversight of commercial hormonal and genetic testing. All authors contributed to critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Kohat University of Science and Technology (KUST), Kohat, Pakistan (REF:/KUST/Ethical Committee/837). Written informed consent was obtained from all participants prior to inclusion in the study.

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT-3.5 to check spelling and grammar. After using this tool, the authors reviewed and edited all content as needed and take full responsibility for the content of the publication.

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