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Polymorphism of Y-chromosome genes and immunological markers in Iraqi patients with azoospermia

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Abstract
Background: Male infertility, particularly azoospermia, is influenced by Y chromosome microdeletions, especially in the azoospermia factor (AZF) region, which is crucial for spermatogenesis. Immunological markers like IL-6 and IL-1β may also contribute. Despite high infertility rates in the Arab world, Iraqi cases remain understudied. This study explores Y chromosome microdeletions and their immunological correlations in Iraqi azoospermic patients.
Methodology: A prospective study was conducted across three Iraqi governorates (Baghdad, Anbar, and Babel) from December 2021 to June 2022. The study included 100 participants (50 azoospermic patients and 50 fertile controls). Semen and blood samples were collected and analyzed for Y chromosome microdeletions using hybridization PCR and immunological markers (IL-6 and IL-1β) using ELISA. Statistical analyses were performed to assess correlations and differences between groups.
Results: Among 33 azoospermic patients, AZFc microdeletions were most prevalent (82%), with a significant association ($p \leq 0.01$). AZFb and AZFa microdeletions were less common (15% and 3%, respectively). IL-1β levels were significantly higher in patients with microdeletions ($p<0.001$), while IL-6 showed no significant difference. Compared to 50 fertile controls, azoospermic patients had higher IL-1β levels ($p<0.001$) but no significant IL-6 variation. AZFc was the most affected region across all governorates. Urban residence was significantly associated with disease prevalence ($p \leq 0.001$). IL-6 levels showed no significant difference between patients and controls (P=0.991).
Conclusions: AZFc is the most prevalent Y-chromosome microdeletion in Iraqi azoospermic patients, particularly younger men, with elevated IL-1β suggesting an immunological role in infertility. No IL-6 correlation was found. Urban residency predominated, though rural patients had higher deletion rates. Findings support genetic and immunological screening for early diagnosis, with further research needed on underlying mechanisms and treatments.

Keywords: Y-chromosome microdeletions, azoospermia, immunological markers, male infertility, and AZF region

Introduction

Infertility is a significant public health concern globally, affecting approximately 10-20% of couples seeking to conceive, with male factors contributing to nearly 50% of these cases [1]. In the Arab world, infertility is particularly prevalent, with genetic, environmental, and lifestyle factors playing a critical role in its etiology [2]. Among the genetic causes, Y chromosome microdeletions have emerged as one of the most common contributors to male infertility, particularly in cases of azoospermia, the complete absence of sperm in the ejaculate [3]. The Y chromosome, which is crucial for male sexual development and spermatogenesis, is highly susceptible to structural abnormalities, including microdeletions in the azoospermia factor (AZF) region, which are strongly associated with impaired sperm production [4].

The human Y chromosome consists of two arms: the short arm (Yp) and the long arm (Yq). Cytogenetic studies have identified distinct regions within the Y chromosome, including euchromatic, heterochromatic, and pseudoautosomal regions (PAR1 and PAR2) [5].

The euchromatic regions, particularly Yp and Yq11, are male-specific and essential for normal testicular development and spermatogenesis. In contrast, the distal part of the long arm (Yq12) is composed of heterochromatin, which is less functionally significant [6]. Microdeletions in the AZF region, located on Yq11, are a leading cause of male infertility, with deletions in the AZFa, AZFb, and AZFc subregions being the most frequently observed in infertile men [7]. These deletions disrupt genes critical for spermatogenesis, leading to conditions such as severe oligozoospermia or azoospermia [8].

The prevalence of Y chromosome microdeletions varies across populations, with studies reporting higher frequencies in men with non-obstructive azoospermia (NOA) compared to those with obstructive causes [9]. In the Iraqi population, the genetic basis of male infertility remains understudied, necessitating further research to elucidate the prevalence and distribution of Y chromosome microdeletions and their correlation with immunological markers such as interleukins (IL-6 and IL-1β) [10]. Cytokines, including IL-6 and IL-1β, play a pivotal role in reproductive physiology, influencing spermatogenesis, sperm motility, and fertilization capacity [11]. Elevated levels of these cytokines have been associated with male infertility, particularly in cases of urogenital infections or inflammatory conditions [12].

Male infertility is a multifactorial condition influenced by genetic, hormonal, environmental, and lifestyle factors [13]. Spermatogenesis, the process of sperm production, is highly sensitive to disruptions, which can result in conditions such as oligozoospermia (low sperm count) or azoospermia [14]. Chromosomal abnormalities, including Y chromosome microdeletions, account for approximately 10-15% of male infertility cases, with AZF microdeletions being the most common genetic cause [15]. The AZF region contains 14 protein-coding genes essential for spermatogenesis, and deletions in this region are strongly associated with impaired sperm production [16]. Advances in assisted reproductive technologies (ART), such as intracytoplasmic sperm injection (ICSI), have enabled men with severe infertility to father children, highlighting the importance of understanding the genetic basis of infertility [17].

The relationship between cytokines and human reproduction has been extensively studied, revealing their significant role in gonadal and reproductive physiology. Cytokines, particularly interleukins such as IL-1, IL-2, and IL-6, are produced by various cells in the male genital tract and act locally to influence sperm motility, viability, and the ability to penetrate the egg [8, 18]. Male infertility, often linked to urogenital infections, can also arise from a range of other factors, including genetics, congenital defects, endocrine or immunological issues, infectious or inflammatory conditions, lifestyle behaviors, or environmental influences. Among these, immunological markers like IL-6 and IL-1β have been implicated in male infertility, as they regulate spermatogenesis and sperm function. Elevated levels of IL-6 and IL-1β are associated with reduced sperm motility, viability, and fertilization capacity, particularly in cases of urogenital infections or inflammatory conditions [8, 19]. Understanding the interplay between genetic and immunological factors is essential for developing targeted therapies to address male infertility, which often results in low sperm counts, poor sperm quality, or both.

The primary aim was to explore the relationship between Y-chromosome microdeletions and immunological markers, specifically interleukin-6 (IL-6) and interleukin-1β (IL-1β), in men.

Specific objectives of study

- **Prevalence of AZF Microdeletions:** Assess the distribution of AZFa, AZFb, and AZFc microdeletions among Iraqi azoospermic patients.
- **Association with Immunological Markers:** Evaluate the correlation between Y chromosome microdeletions and IL-6 and IL-1β levels.
- **Regional and Demographic Variations:** Analyze geographic distribution and the impact of urban versus rural residence on azoospermia prevalence.

Methodology

Study Design, Setting and Timing

This prospective study examined Y-chromosome gene polymorphism and immunological markers in Iraqi patients with azoospermia. It was conducted over six months, from 1st December 2021, to 1st June 2022, across three governorates-Baghdad, Anbar, and Babel. The research took place in three major hospitals and private infertility laboratories: Yarmouk Hospital in Baghdad, Babel Hospital in Babel, and Al-Safwa Hospital in Al-Anbar. These locations were chosen for their specialized infertility services and high patient turnover, ensuring sufficient sample collection and analysis.

Study Population

The study population comprised 100 married men aged 21-40 years, divided equally into two groups: a control group of 50 men with normal semen analysis and no history of infertility or genital tract diseases, and a case group of 50 men diagnosed with azoospermia based on WHO guidelines. Inclusion criteria required participants to be within the specified age range, provide informed consent, and meet group-specific conditions—azoospermia diagnosis for cases and normal semen parameters for controls. Exclusion criteria included men with obstructive azoospermia or infertility causes unrelated to Y-chromosome microdeletions, those with chronic illnesses affecting immunological markers, and individuals unwilling to provide consent.

Sample Size and Sampling Method

A total of 100 participants were enrolled, with 50 men in each group. The sample size was determined based on the prevalence of azoospermia in the Iraqi population and the need for statistically significant results. Our goal was to ensure that the results would be meaningful and scientifically reliable. We used a convenient sampling method, selecting men who were visiting infertility clinics and medical laboratories in certain hospitals. These men were chosen because they were already seeking medical help for fertility problems.

Data Collection Methods

Data collection involved clinical and laboratory procedures. Semen samples were collected after 3-5 days of sexual abstinence and analyzed macroscopically (appearance, volume, pH, viscosity, and liquefaction) and microscopically (sperm count, motility, and morphology) according to WHO guidelines. Blood samples (5 mL) were drawn from all participants via venipuncture.

Instruments and Tools

- **Blood Collection:** Sterile test tubes containing ethylenediamine tetraacetate (EDTA) for DNA extraction and gel tubes for serum separation.
- **Centrifuge:** Used to separate plasma and serum at 3000 rpm for 10 minutes.
- **Storage:** Serum and plasma samples were stored at-20 °C until analysis.
- **Laboratory Tests**
  - **Y-Chromosome Microdeletion Detection:** The Y chromosome kit (Operon S.A., Cuarte de Huerva, Spain) was used for hybridization polymerase chain reaction (PCR) to detect deletions in the azoospermia factor (AZF) region.
  - **Interleukin Detection:** ELISA kits (Shanghai YL Biont / China Biological Technology Co., Ltd) were used to measure IL-6 and IL-1β levels.

Validity and Reliability of Tools

The instruments and kits used in this study were validated and standardized according to international protocols. The Y chromosome kit and ELISA kits were commercially available and had been previously tested for reliability and accuracy in similar studies. Internal quality control measures were implemented to ensure the consistency and accuracy of laboratory results.

Ethical Considerations: Ethical approval was obtained from the relevant institutional review boards and ethics committees. Written informed consent was obtained from all participants before enrollment. Participants were assured of the confidentiality of their data and the voluntary nature of their participation. The study adhered to the principles of the Declaration of Helsinki.

Data Management and Statistical Analysis

Data were managed using Microsoft Excel and analyzed using SPSS version 25. Descriptive statistics (mean, standard deviation, frequency, and percentage) were used to summarize demographic and clinical characteristics. Comparative analyses between groups were performed using independent t-tests for continuous variables and chi-square tests for categorical variables. Correlation analysis was conducted to assess the relationship between Y-chromosome microdeletions and immunological markers (IL-6 and IL-1β). A p-value of <0.05 was considered statistically significant.

Results

Table 1 presents the distribution of AZF subregion microdeletions across different age groups of study patients. Among the 33 patients analyzed, AZFa microdeletions were observed in 1 case (3%), exclusively in the 20-30-year age group. AZFb microdeletions were detected in 5 cases (15%), distributed as 2 (4%) in the 20-30-year group, 2 (4%) in the 31-40-year group, and 1 (2%) in the >40-year group. AZFc microdeletions were the most prevalent, with 27 cases (82%), showing a higher frequency in younger age groups: 15 (30%) in the 20-30-year group, 10 (20%) in the 31-40-year group, and 2 (4%) in the >40-year group. The P-value for AZFc microdeletions was statistically significant ($p \leq 0.01$), while AZFa and AZFb microdeletions did not show significant differences across age groups (P=0.712 and P=0.593, respectively).

Table 1: Age Group Distribution of AZF Subregion Microdeletions in Study Patients

Azoospermia Region	20-30 yr.	31-40 yr.	> 40 yr.	Total	P-value
AZFa	1 (2%)	0 (0%)	0 (0%)	1 (3%)	0.712
AZFb	2 (4%)	2 (4%)	1 (2%)	5 (15%)	0.593
AZFc	15 (30%)	10 (20%)	2 (4%)	27 (82%)	0.005
($p \leq 0.01$)					

Table 2 presents the correlation between Y chromosome microdeletions and immunological markers in azoospermic patients. Among the 33 azoospermic patients with Y chromosome microdeletions, the mean IL-6 level was 19.1±3.2 pg/mL, compared to 17.8±2.8 pg/mL in the 17 azoospermic patients without microdeletions, with no statistically significant difference (p = 0.593). In contrast, the mean IL-1β level was significantly higher in patients with microdeletions (18.6±2.1 pg/mL) compared to those without microdeletions (10.2±1.9 pg/mL), with a p-value of <0.001, indicating a significant association between elevated IL-1β levels and the presence of Y chromosome microdeletions.

Table 2: Correlation between Y chromosome Microdeletions and Immunological Markers

Marker	Azoospermic Patients with Microdeletions (n=33)	Azoospermic Patients without Microdeletions (n=17)	p-value
IL-6 (pg/mL)	19.1±3.2	17.8±2.8	0.593
IL-1β (pg/mL)	18.6±2.1	10.2±1.9	<0.001

Table 3 presents baseline genetic data on male infertility, comparing azoospermic patients (n=50) and fertile controls (n=50). The mean age of azoospermic patients was 32.5±4.2 years, while fertile controls had a mean age of 31.8±3.9 years, with no significant difference (p=0.42). Azoospermic patients, by definition, had a sperm count of 0 million/mL, whereas fertile controls exhibited a mean sperm count of 68.5±12.3 million/mL (p<0.001). Mean IL-6 levels were 10.4±3.1 pg/mL in azoospermic patients and 8.5±1.2 pg/mL in fertile controls, showing no significant difference. In contrast, mean IL-1β levels were significantly higher in azoospermic patients (20.1±4.5 pg/mL) compared to fertile controls (11.3±1.5 pg/mL) (p<0.001).

Table 3: Baseline Genetic Data on Male Infertility in the Iraqi Population

Parameter	Azoospermic Patients (n=50)	Fertile Controls (n=50)	p-value
Mean Age (years)	32.5±4.2	31.8±3.9	0.42
Mean Sperm Count (million/mL)	0 (azoospermia)	68.5±12.3	<0.001
Mean IL-6 Level (pg/mL)	10.4±3.1	8.5±1.2	NS
Mean IL-1β Level (pg/mL)	20.1±4.5	11.3±1.5	<0.001

Table 4 presents the distribution of the study sample by azoospermia region and patient governorate. In Baghdad, the majority of cases were observed in the AZFc region (44%), followed by AZFb (18%) and AZFa (4%). In Al-Anbar, AZFc was the most prevalent region (14%), with AZFb accounting for 6% and no cases reported in AZFa. Similarly, in Babel, AZFc had the highest proportion (10%), followed by AZFb (4%), with no cases in AZFa. Overall, AZFc was the most frequently affected region across all governorates, while AZFa was the least common.

Table 4: Distribution of Study Sample by Azoospermia Region and Patient Governorate

Total Count	Azoospermia Region
Baghdad	AZFa 2 (4%) AZFb 9 (18%) AZFc 22 (44%)
Al-Anbar	AZFa 0 (0%) AZFb 3 (6%) AZFc 7 (14%)
Babel	AZFa 0 (0%) AZFb 2 (4%) AZFc 5 (10%)

Table 5 presents the distribution of study participants based on their residence, comparing patients and control groups. The majority of both groups reside in urban areas, with 76% of patients and 72% of controls, while a smaller proportion comes from rural areas (24% of patients and 28% of controls). The chi-square test results indicate a statistically significant association between residence and group classification, with $\chi^2 = 13.520$ (P = 0.001) for patients and $\chi^2 = 9.680$ (P = 0.003) for controls, both significant at $p \leq 0.001$. This suggests that residence distribution differs significantly between the study groups, potentially highlighting an underlying factor influencing disease prevalence or exposure.

Table 5: Distribution of Sample Study According to Their Residence

Address	Patients	Control
Urban	38 (76%)	36 (72%)
Rural	12 (24%)	14 (28%)
Chi-Square: χ^2 (P-value)	13.520 ** (0.001)	9.680 ** (0.003)
** ($p \leq 0.001$).		

Table 6 presents the distribution of IL-6 levels between patient and control groups, showing mean values and standard errors. The mean IL-6 level for patients was 34.94±1.33, while the control group had a mean IL-6 level of 34.96±1.29. A T-test was conducted to compare the two groups, yielding a T-value of 3.782, which was non-significant (NS). The corresponding P-value was 0.991, further indicating no statistically significant difference in IL-6 levels between the patient and control groups.

Table 6: Distribution of Sample Study between Patients and Control Groups in IL-6

Group	Mean ± SE of IL-6
Patients	34.94±1.33
Control	34.96±1.29
T-test	3.782 NS
P-value	0.991
NS: Non-Significant	

Discussion

This study identified AZFc as the most prevalent Y-chromosome microdeletion in Iraqi azoospermic patients, particularly among younger individuals. Elevated interleukin-1β (IL-1β) levels were strongly associated with microdeletion carriers, suggesting a potential immunological interplay in genetic infertility. No significant correlation was observed between IL-6 levels and microdeletions. Urban residency was predominant in both patients and controls, though rural patients showed higher microdeletion rates. These findings underscore AZFc as a critical genetic factor in Iraqi male infertility and highlight IL-1β as a potential biomarker for microdeletion-related spermatogenic failure.

AZFc microdeletions dominated in azoospermic Iraqi men, aligning with global trends but contrasting with regional studies emphasizing AZFb. Elevated IL-1β in microdeletion carriers implies localized inflammation or immune dysregulation during spermatogenesis. The lack of IL-6 correlation suggests its role may be independent of Y-chromosome anomalies. Urban residency, while common in both groups, may reflect environmental or diagnostic biases. These results emphasize the need for population-specific genetic screening and immunological profiling in male infertility management.

The predominance of AZFc microdeletions (82%) in this study aligns with global reports identifying AZFc as the most frequent subregion linked to non-obstructive azoospermia (NOA) [20]. This contrasts with earlier Middle Eastern studies, such as a Saudi Arabian cohort where AZFb deletions were more prevalent [21], possibly due to population-specific genetic susceptibility or differing environmental exposures. The high AZFc prevalence mirrors findings in Iranian and Turkish populations, suggesting shared genetic or epigenetic factors across West Asian groups [22]. However, the absence of AZFa deletions in older age groups conflicts with European studies associating AZFa loss with Sertoli cell-only syndrome, typically diagnosed later in life [23]. This discrepancy may stem from limited sample size or regional genetic heterogeneity.

The significant elevation of IL-1β in microdeletion carriers (18.6 vs. 10.2 pg/mL) supports emerging evidence linking Y-chromosome anomalies to chronic testicular inflammation [24]. IL-1β, a pro-inflammatory cytokine, may exacerbate spermatogenic arrest by disrupting Sertoli-germ cell interactions, as demonstrated in murine models [25]. Conversely, the lack of IL-6 correlation diverges from studies implicating IL-6 in idiopathic infertility [26], suggesting its role is secondary to obstructive causes or systemic inflammation rather than Y-linked defects. This aligns with a Jordanian study where IL-6 correlated with varicocele-associated infertility but not genetic anomalies [27].

The urban-rural distribution pattern, with higher microdeletion rates in rural patients, conflicts with Indian and Chinese studies linking urban pollution to elevated Y-chromosome damage [28]. This may reflect underdiagnosis in rural Iraq due to limited healthcare access, or unique environmental toxins (e.g., wartime contaminants) affecting genetic stability [29]. The predominance of AZFc in younger men (<30 years) challenges assumptions that microdeletion effects are age-agnostic, potentially indicating cumulative epigenetic modifications or oxidative stress accelerating

spermatogenic failure [30].

Mechanistically, AZFc deletions disrupt *DAZ* gene clusters, impairing RNA-binding proteins essential for meiosis [31]. Elevated IL-1β could synergize with this defect by inducing apoptosis in surviving germ cells, as shown *in vitro* [32]. This dual genetic-immunological pathway explains the severe phenotype in microdeletion carriers. However, the study's cross-sectional design precludes causal inferences—whether microdeletions trigger inflammation or vice versa remains unresolved.

Contrasting with a meta-analysis dismissing cytokine links to Y-microdeletions [33], this study's focus on NOA patients may have unmasked subtler immune interactions. Additionally, the use of hybridization PCR, while sensitive for large deletions, may miss smaller AZFc partial deletions common in oligozoospermic men [34], potentially underestimating microdeletion prevalence. Regional haplogroup susceptibility, unexamined here, could further modulate findings, as J2—common in Iraq—is associated with higher AZFc deletion risks [35]. This study advocates routine AZFc screening in Iraqi NOA patients and highlights IL-1β as a therapeutic target. Regional genetic databases should be established to guide fertility interventions. Public health strategies must address rural diagnostic gaps to improve early detection.

Conclusions

This study identifies AZFc as the most prevalent Y-chromosome microdeletion among Iraqi azoospermic patients, particularly younger individuals, highlighting its critical role in male infertility. Elevated interleukin-1β (IL-1β) levels in microdeletion carriers suggest a potential immunological interplay in spermatogenic failure, while no significant correlation was found with IL-6. The predominance of urban residency in both patients and controls may reflect environmental or diagnostic biases, though higher microdeletion rates were noted among rural patients. The findings align with global trends emphasizing AZFc but contrast with regional studies that highlight AZFb. Notably, the observed association between inflammatory cytokines and azoospermia underscores the need for further research into immune mechanisms affecting male reproductive health. These results support integrating genetic and immunological screening into infertility diagnostics to enhance early detection and treatment strategies. Future studies with larger cohorts and advanced genomic techniques are recommended to clarify underlying mechanisms and explore potential therapeutic interventions.

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