



## RESEARCH ARTICLE

## Assessment of Seminal Plasma and Serum Chitotriosidase-1 as Biomarkers for Silent Inflammation in Infertile Iraqi Males: A Comparative Study among Normozoospermic, Asthenospermia, and Oligozoospermic Groups

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ARTICLE INFO	ABSTRACT
Received: May 28, 2024	<p>The aim of this study was to evaluate the concentrations and activities of cHit1 in the seminal plasma and serum of Normozoospermic, Asthenospermia, and Oligozoospermic groups in relation of serum IL-1b. 90 males aged between 20-45 were included in a cross-sectional study conducted at the infertility unit of Al-Batool Teaching Hospital from February 2022 to February 2023. All participants were diagnosed with infertility by specialists at the hospital. The subjects were categorized into three groups: the Normozoospermic Group (G1), the Asthenospermia Group (G2), and the Oligozoospermic Group (G3). Measurements Interleukin1Beta (IL1B), and Chitinase1 (CHIT1) by Enzyme-linked immunosorbent assay (ELISA) technique. Significantly higher levels of serum IL1B, Serum Ch1t1 and SF Ch1t1 and were found in G2 group compared to G3 and G1 groups. In the G2 group, a moderate positive correlation was observed between Serum Ch1t1 and SF Ch1t1, which was statistically significant. However, a weak positive correlation between Serum Ch1t1 and IL1B was found to be not statistically significant. The correlation between SF Ch1t1 and IL1B was deemed negligible due to the lack of a significant correlation. In the Oligozoospermic group, a weakly negative correlation was noted between Serum Ch1t1 and SF Ch1t1, which was not statistically significant. Regarding IL1B, Serum Ch1t1 showed a weak, non-significant positive correlation, while SF Ch1t1 exhibited a strong, statistically significant negative correlation with IL1B. Finally, in the correlation analysis among Serum Ch1t1 and SF Ch1t1, in the three study groups (G1, G2, and G3), a moderate negative correlation was observed but was statistically insignificant in G1. In G2, a significant positive correlation was found between Serum Serum Ch1t1 and SF Ch1t1. The average negative correlation demonstrated between iL-1β concentrations and cHit1 concentrations seem to confirm the hypothesis about the role of cHit1 in the manifestation of inflammation at a very early stage.</p>
Accepted: Jun 16, 2024	
<p><b>Keywords</b></p> <p>Human Seminal Plasma chitotriosidase-1</p> <p>Human Serum Chitotriosidase-1 Inflammation</p> <p>Serum IL-1B</p>	
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### INTRODUCTION

Fertility is the ability of the individual to reproduce through normal sexual act. Normal fertility requires the production of enough healthy sperm, a problem with this step cause infertility[1].The World Health Organization (WHO) has classified infertility as a major worldwide public health issue

since it is a common and severe health issue, after a year of unprotected sexual activity, infertility is defined as the inability to achieve a viable pregnancy [2,3]. It is estimated that over 186 million people worldwide are affected by this condition, abnormal sperm morphology (teratospermia), low sperm numbers (oligospermia), low sperm motility (asthenospermia), and an absence of spermatozoa (azoospermia) are all clinical manifestations of male infertility [4,5].

Obstruction, testicular failure, hormone issues, drug and alcohol use, cryptorchidism, sperm agglutination, low semen volume, idiopathic infertility, ejaculatory dysfunction or erectile, varicocele, abnormal viscosity, high thickness of sperm, endocrine trouble, environmental causes, and genetic causes have all been identified as potential contributors to infertility. An examination of the relationship between total antioxidant status (tas), which reflects the capacity to maintain the oxidative-antioxidant balance in semen, and non-standard inflammatory mediator, chitotriosidase-1 (also called chitinase 1, cHit1) Macrophages and neutrophils express the mammalian chitinase cHit1, which is a member of the family 18 of glycoside hydrolases [6]. It helps the body's natural defenses fight against chitin-coated viruses and bacteria. The cHit1 activity is employed in the diagnosis and follow up of gaucher disease as a measure of macrophage activation. The activity of this enzyme has been shown to significantly rise in different inflammatory disorders. Research also shows that cHit1 has a synergistic impact with some proteases, like MMP-9, but not with LE [7].

However, the function of cHit1 in the underlying pathomechanisms of these disorders remains poorly understood, Chitinolytic activity may or may not be essential for this enzyme to carry out its biological functions because there are no known endogenous substrates for it in humans [8]. cHit1's involvement in macrophage activation and differentiation has consequences for other immune cell types. Although this may point to a role for cHit1 in triggering an inflammatory response, data on the enzyme's involvement in the inflammation that contributes to male infertility is still lacking [9]. Several evidences indicates that cytokines are involved in male infertility; they are secreted by different parts of the male genetic tract and may exert effects on the steroidogenesis, spermatogenesis and sperm function [10].

As a pro-inflammatory cytokine that is essential to the immune response, interleukin-1 beta (IL1B) has been found to be elevated in the semen of infertile men, this finding raises the possibility that IL1B contributes to the pathophysiology of male infertility by changing the quality and function of sperm [11]. Several studies were carried out on association of infection and inflammation with male infertility, which revealed great variations in the prevalence of genital infection in different parts of the world [12].

The aim of this study was to evaluate the concentrations and activities of cHit1 in the seminal plasma and serum of Normozoospermic, Asthenospermia, and Oligozoospermic groups in relation of serum IL-1B.

## **RESEARCH METHODOLOGY**

### **Study population**

Ninety males aged between twenty and forty-five were included in a cross-sectional study conducted at the infertility unit of Al-Batool Teaching Hospital from February 2022 to February 2023. All participants were diagnosed with infertility by specialists at the hospital. The subjects were categorized into three groups: The Normozoospermic Group (G1), the Asthenospermia Group (G2), and the Oligozoospermic Group (G3). Blood samples were collected, stored in a blood bank, and preserved for subsequent analysis.

## Blood sampling

All blood samples were collected from Diyala province. Five ml of blood samples were taken from patients who attended to the Al-Batool teaching hospital, blood sample were centrifuged for 10 minutes at 3000 rpm and the upper part of centrifuged sample in tube (serum) was transferred in to the tube (0.5 ml micro centrifuge tube) for freezing. Serum were divided into small aliquots for measuring the levels of (ChiT1, and IL1B) by Elisa.

## Seminal fluid samples

Preparation of seminal fluid samples in accordance with the guidelines outlined by the World Health Organization, (1999).

Measurements Interleukin1Beta (IL1B), and Chitinase1 (CHIT1) by Enzyme-linked immunosorbent assay (ELISA)

The Biochemical parameters in this study—IL1b, and CHIT1 Elisa kits—are based on the sandwich principle according to the manufacturer (Cloud-Clone Corp/USA/Cat No.SEA563Hu, SEJ374Hu,SEA181Hu).

## Statistical analysis

The data input and preparation, including organizing and cleaning the data for analysis, were conducted using Microsoft Excel. Subsequently, a One-way ANOVA followed by a multiple comparisons test was carried out utilizing GraphPad Prism version 19.5.1 for Windows, developed by GraphPad Software in San Diego, California, USA. Additionally, MedCalc(R) Statistical Software version 20.215 was employed for the statistical analysis.

## RESULTS

In table (1), and figure (1), the results showed, the Seminal Fluid Volume (SF Vol) revealed mean values of  $2.550 \pm 0.1701$ ,  $2.083 \pm 0.1337$ , and  $2.033 \pm 0.1809$  for study groups respectively. Although differences were observed, the effect size indicated by the  $R^2$  value of 0.07 and a borderline  $P = 0.05$  suggest that these differences are not substantial.

For Sperm Count, however, a striking disparity among the groups was evident. The means were  $71.833 \pm 1.8489$ ,  $39.333 \pm 2.3700$ , and  $11.000 \pm 0.7350$  for study groups, respectively. The  $R^2$  value of 0.87 and a  $P = 0.001$  indicate not only a statistically significant difference but also a large effect size, denoting a considerable variation in sperm count among these groups.

Motility categories, designated as A%, B%, C%, and D%, also showed substantive differences among the groups. For category A%, the mean values were  $21.833 \pm 0.9123$ ,  $6.500 \pm 1.0491$ , and  $2.000 \pm 0.9160$  for study groups, respectively. Category B% displayed similar disparities with means of  $33.833 \pm 0.5715$ ,  $18.000 \pm 1.0057$ , and  $8.833 \pm 1.6380$ . Both A% and B% had  $R^2$  values of 0.73 and p-values less than 0.001, signifying statistical significance and substantial effect sizes. Category C%, while also statistically significant with  $P = 0.01$ , showed a modest  $R^2$  value of 0.10, implying a smaller effect size. Category D% exhibited an  $R^2$  value of 0.77 and a p-value less than 0.001, indicating significant differences with a large effect size.

Finally, the percentages of morphologically Normal and Abnormal sperms were  $70.000 \pm 0.8970$  and  $30.333 \pm 0.8949$  for G1,  $40.833 \pm 2.5380$  and  $59.167 \pm 2.5380$  for G2, and  $23.833 \pm 3.5934$  and  $76.167 \pm 3.5934$  for G3, respectively. These differences were statistically significant with  $P = 0.001$  and  $R^2$  values of 0.65, highlighting a sizable effect size.

**Table 1: Semen profile for study groups**

SFA	Study groups				
	G1	G2	G3	ANOVA	
	Mean± SE	Mean± SE	Mean± SE	R <sup>2</sup>	P-value
SF Vol ml	2.550± 0.1701	2.083± 0.1337	2.033± 0.1809	0.07	0.05
Sperm count	71.833± 1.8489	39.333± 2.3700	11.000± 0.7350	0.87	< 0.001
A%	21.833± 0.9123	6.500± 1.0491	2.000± 0.9160	0.73	< 0.001
B%	33.833± 0.5715	18.000± 1.0057	8.833± 1.6380	0.73	< 0.001
C%	10.000± 0.0000	10.833± 0.3460	9.333± 0.4632	0.10	0.01
D%	34.333± 1.0095	64.667± 1.9613	79.833± 2.5312	0.77	< 0.001
Normal%	70.000± 0.8970	40.833± 2.5380	23.833± 3.5934	0.65	< 0.001
Abnormal%	30.333± 0.8949	59.167± 2.5380	76.167± 3.5934	0.65	< 0.001

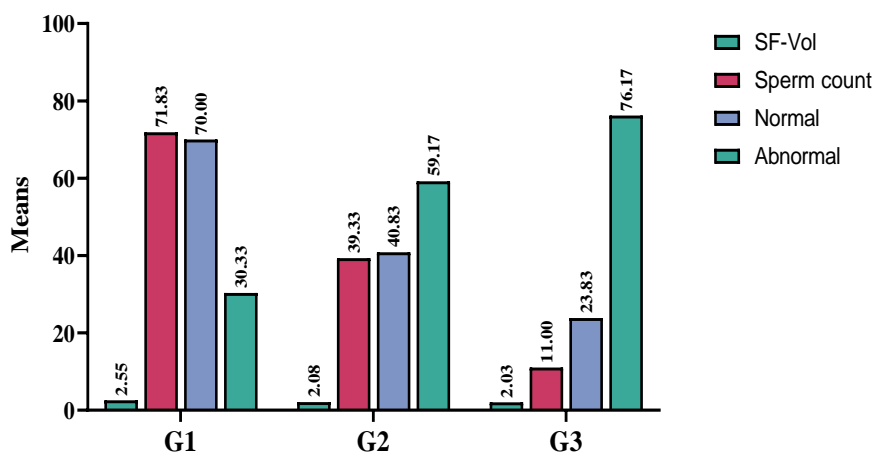
**Figure 1: Means of Semen profile by the study groups**

Table 2, and figure2, shows the serum levels of IL1B was significantly higher (P=0.001) in G2 group 420.75± 24.74 compared to G3, and G1 groups 234.85± 10.14, and 106.09± 3.61 respectively.

**Table 2: The Mean ± SE of IL1B for study groups**

Parameter	Mean ± SE			P-value
	G1	G2	G3	
Serum IL1 B (pg/ml)	106.09± 3.61	420.75± 24.74	234.85± 10.14	<0.001

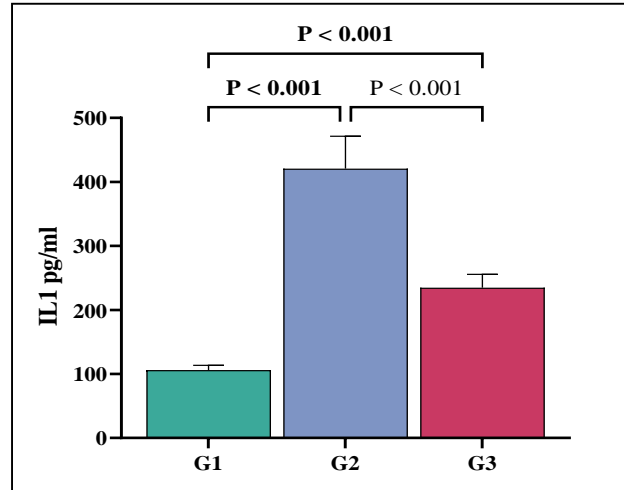


Figure 2: Means of IL1B by study groups

For the serum Ch1t1 levels, the mean concentrations were  $4.09 \pm 0.17$ ,  $16.22 \pm 0.58$ , and  $10.87 \pm 0.62$  for study groups, respectively. Seminal fluid plasma Ch1t1 levels also displayed pronounced disparities among the groups with mean concentrations of  $5.62 \pm 0.19$ ,  $15.96 \pm 0.29$ , and  $12.46 \pm 0.18$  for study groups, respectively. As seen Table 3 and Figure3.

Table 3: The Mean  $\pm$  SE of CHIT1for study groups

Parameter	Mean $\pm$ SE			P-value
	G1	G2	G3	
SerumCh1t1(ng/ml)	$4.09 \pm 0.17$	$16.22 \pm 0.58$	$10.87 \pm 0.62$	< .001
SF_Ch1t1 (ng/ml)	$5.62 \pm 0.19$	$15.96 \pm 0.29$	$12.46 \pm 0.18$	< .001

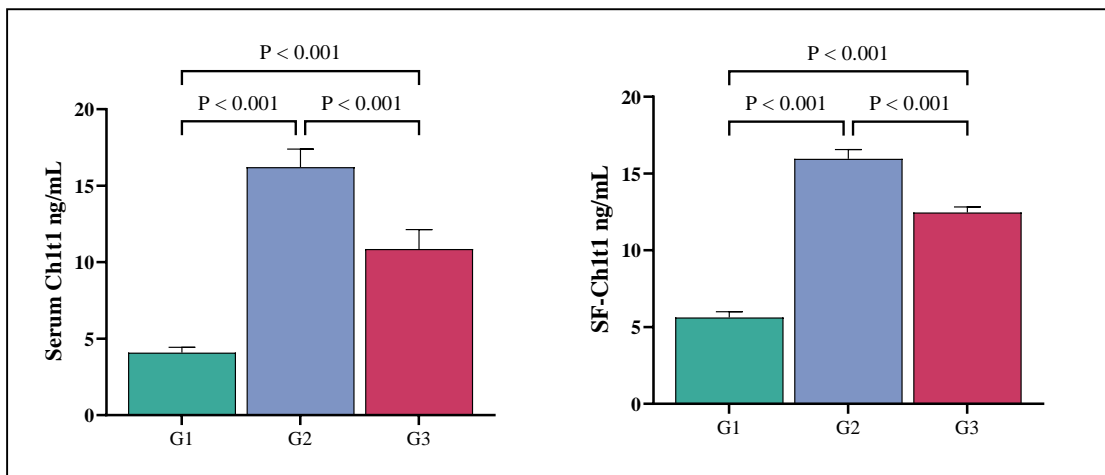


Figure 3: Means of Ch1t1 by study groups

Using Pearson correlation analysis, the G2 (Asthenospermia) group's serum and SF\_Ch1t1 were correlated with IL1b. For the correlation between Serum Ch1t1 and SF Ch1t1, a moderate positive correlation was observed, with a correlation coefficient  $P = 0.47$ ,  $r = 0.47$ . The 95% confidence interval ranged from 0.13 to 0.71, and the p-value was 0.009, indicating that this correlation is statistically significant. When correlating Serum Ch1t1 with IL1b, a weak positive correlation was observed ( $P = 0.18$ ,  $r = 0.18$ ) with a wide 95% confidence interval of -0.19 to 0.51. The p-value of 0.346 indicates that this correlation is not statistically significant. The correlation between SF Ch1t1 and IL1b was essentially negligible ( $P = -0.01$ ,  $r = -0.01$ ) with a 95% confidence interval of -0.37 to 0.35 and a p-value of 0.966, further confirming the lack of a significant correlation.

**Table 4: Correlations between Serum and SF Ch1t1, and biochemical parameters in G2 group**

Parameters	SerumCh1t1			SF_Ch1t1		
	<i>r</i>	95.00% CI	<i>p</i>	<i>r</i>	95.00% CI	<i>p</i>
<b>SF_Ch1t1</b>	.47	[.13, .71]	.009			
<b>IL1b</b>	.18	[-.19, .51]	.346	-.01	[-.37, .35]	.966

The oligospermia cohort, also comprising 30 subjects, correlation analyses were conducted to explore the relationships between Serum Ch1t1 and SF Ch1t1, along with IL1b. For the correlation between Serum Ch1t1 and SF Ch1t1, a moderate positive correlation was observed, with a correlation coefficient  $P = 0.47$ ,  $r = 0.47$ . The 95% confidence interval ranged from 0.13 to 0.71, and the p-value was 0.009, indicating that this correlation is statistically significant. When correlating Serum Ch1t1 with IL1, a weak positive correlation was observed ( $P = 0.18$ ,  $r = 0.18$ ) with a wide 95% confidence interval of -0.19 to 0.51. The p-value of 0.346 indicates that this correlation is not statistically significant. The correlation between SF Ch1t1 and IL1b was essentially negligible ( $P = -0.01$ ,  $r = -0.01$ ) with a 95% confidence interval of -0.37 to 0.35 and a p-value of 0.966, further confirming the lack of a significant correlation.

**Table 5: Correlations between Serum and SF Ch1t1, and biochemical parameters in G2 group**

Parameters	SerumCh1t1			SF_Ch1t1		
	<i>r</i>	95.00% CI	<i>p</i>	<i>R</i>	95.00% CI	<i>p</i>
<b>SF_Ch1t1</b>	.47	[.13, .71]	.009			
<b>IL1b</b>	.18	[-.19, .51]	.346	-.01	[-.37, .35]	.966

The statement highlights a significant and substantial difference in sperm count among the Normozoospermic Group (G1), Asthenospermia Group (G2), and Oligozoospermic Group (G3), the statement mentions a statistically significant difference in sperm count among the groups, this indicates that the observed variation in sperm count is unlikely to have occurred by chance alone.

## DISCUSSION

The observed variation is substantial and not merely a result of the sample size, a considerable difference in sperm count among the groups has important clinical implications, sperm count is a crucial factor in male fertility, and significant variations may affect the likelihood of successful conception, the study may explore potential causes or factors contributing to the observed differences in sperm count [13].

Factors such as lifestyle, environmental exposures, and genetic predispositions could be the cause of such variation among the different sperm health conditions, there are several reasons for a significant difference in sperm count among the groups included in the study [14]. These factors may result from complex interactions between genetic, environmental, and health-related elements, variations in levels of hormones involved in sperm production, such as testosterone, may contribute to this difference, differences in genetic makeup could affect sperm count, genetics play a crucial role in determining reproductive characteristics [15].

Exposure to harmful environmental factors, such as pollutants or temperature changes, might impact sperm production, certain health issues like reproductive system infections or problems with reproductive glands can influence sperm count, lifestyle elements like diet, exercise, smoking, and alcohol consumption can play a role in fertility, stress and psychological pressures may have an effect on reproductive function, age can be a significant factor, as the likelihood of a decrease in sperm count tends to increase with advancing age as shown by Said *et al.* [16] and Tas *et al.* [17].

For Seminal Fluid Volume, although there are differences, p-value suggest that these differences as seen in table (1)  $p \leq 0.05$ , there can be several reasons for differences in semen volume among different groups, including disruptions in hormone levels, particularly those involved in sperm production, can affect semen volume, imbalances in hormones like testosterone may influence semen production, variations in genes among individuals can contribute to differences in semen volume, genetic factors play a role in the physical characteristics of semen [18].

Exposure to specific environmental factors may influence natural sperm production and, consequently, semen volume. This could include exposure to harmful chemicals or elevated temperatures, as shown by Murgia *et al.* [19]. The provided text describes significant differences in motility categories (A%, B%, C%, and D%) among the study groups, namely the Normozoospermic Group (G1), Asthenospermia Group (G2), and Oligozoospermic Group (G3). The statistically significant differences in Category A% suggest that there is a significant variation in the percentage of sperm showing progressive motility among the different groups ( $P < 0.001$ ). This is a crucial parameter for male fertility, as sperm with progressive motility have a higher chance of reaching and fertilizing the egg [20].

Similar to Category A%, the significant differences ( $P < 0.001$ ) with a substantial effect size in Category B% indicate notable disparities in the percentage of sperm showing non-progressive motility. Non-progressive motility may still allow sperm to move but with less efficiency compared to progressive motility [21]. Although statistically significant ( $P < 0.01$ ), the smaller effect size in Category C% suggests a less substantial impact compared to Categories A% and B%. This indicates that the percentage of sperm with local motility varies among the groups but to a lesser degree. Local motility may have limited functional relevance for fertilization compared to progressive motility [22]. The significant differences ( $P < 0.001$ ) with a large effect size in Category D% highlight considerable variations in the percentage of immotile sperm among the study groups. Immotile sperm have reduced or no movement, which can significantly impact fertility [23].

The findings have clinical relevance, as sperm motility is a critical factor in male fertility, understanding the specific patterns of motility among different sperm health conditions can guide clinicians in diagnosing and addressing fertility issues in couples [24], as found by Pereira *et al.* [25]. With advancing age, there can be an effect on sperm motility, aging is often associated with a decline in overall body functions. The provided information indicates significant differences in the percentages of morphologically normal and abnormal sperm among the three groups (G1, G2, and

G3), these morphological differences may have implications for fertility and reproductive health in each group, this agree with Zanetti *et al.* [26].

Interleukin-1 Beta (IL-1 $\beta$ ) play a crucial role in inflammatory responses and immune system regulation, and possibly related to male reproductive health [27]. The comparison of IL-1 $\beta$  levels across different groups (Normozoospermic, Asthenospermia, and Oligozoospermic) suggests that there may be a link between the cytokine levels and the health of sperm, as shown by ÖRDEK *et al.* [28]. Elevated levels of IL-1 $\beta$  can be indicative of an inflammatory response. In the context of male reproductive health, this might suggest inflammation or immune system activation in the reproductive system, potentially impacting sperm quality or function [29].

Immune factors can influence sperm health, and imbalances in cytokines like IL-1 $\beta$  might contribute to fertility issues. Inflammation in the male reproductive tract can have various implications for sperm production and function, chronic inflammation may contribute to oxidative stress, which can, in turn, negatively impact sperm quality and motility, additionally, inflammation can affect the normal functioning of the male reproductive organs, potentially leading to conditions such as asthenospermia (reduced sperm motility) in this case, this agree with Papadimas *et al.* [29] and Rozwadowska *et al.* [30]. Asthenospermia refers to reduced sperm motility, and it appears that there may be a link between elevated IL-1 $\beta$  levels and this particular sperm parameter [31]. The immune system, including cytokines like IL-1 $\beta$ , can influence various physiological processes, and alterations in their levels may have implications for reproductive health, the immune system plays a crucial role in maintaining homeostasis within the body, including the reproductive system [32].

Inflammation, which is often signaled by the presence of cytokines like IL-1 $\beta$ , can influence various physiological processes, in the context of male reproductive health, inflammation within the reproductive organs may adversely affect sperm production, maturation, and motility, as found by Acharyya *et al.* [33]. While, Eggert-Kruse *et al.* [34] showed, the high level of IL-1 $\beta$  not associated with clinically relevant parameters of semen quality, including sperm fertilizing capacity.

The comparison between the Asthenospermia Group (G2) and both the Oligozoospermic Group (G3) and Normozoospermic Group (G1) suggests that the observed elevation in IL-1 $\beta$  levels is specific to the Asthenospermia Group, this could be indicative of a distinct immunological or inflammatory profile associated with asthenospermia compared to other sperm abnormalities (oligozoospermia) or normal sperm parameters (normozoospermia), as found by Rozwadowska *et al.*[30]. Chitinase 1 (CHIT1) is an enzyme that belongs to the chitinase family, Chitinases are enzymes that break down chitin, a complex polysaccharide found in the exoskeletons of insects, fungi, and the cell walls of some bacteria, CHIT1, specifically, is an enzyme that is produced by various cells, including macrophages and neutrophils Kumar *et al.* [35]. CHIT1 is expressed in various tissues, but its levels are particularly high in the lungs and macrophages, the expression of CHIT1 is often associated with immune responses. It is produced by immune cells in response to chitin-containing pathogens, and its enzymatic activity can contribute to the breakdown of these pathogens [36]. Changes in CHIT1 levels or activity have been linked to certain diseases and conditions. For example, elevated CHIT1 levels have been reported in conditions associated with increased macrophage activity, such as atherosclerosis and certain respiratory diseases [37].

CHIT1 is also produced in human skin, while research suggests that CHIT1 acting a role in immune response and inflammation, it is not the primary focus in fertility-related studies, the study by Kuzgunbay *et al.* [38], the authors examined the levels of chitotriosidase-1 in the serum of varicocele patients to study the underlying causes of varicocele. They found that while the average levels of



chitotriosidase were higher in oligozoospermic patients compared to normozoospermic patients, this difference was not statistically significant [39]. This study may be considered one of the first study in Iraq may be the second study in the world to find a relationship between this enzyme and infertility in men, and there is no research to support these results, just only study conducted by Kratz *et al.* [9] The study demonstrated significant differences in CHIT1 concentrations in seminal plasma between the normozoospermic and oligozoospermic groups, which aligns with the findings of this study. Inflammation in the male reproductive system can cause oligozoospermia [39].

The correlation between serum Ch1t1 levels and asthenospermia is not well-established, asthenospermia, which is a condition characterized by reduced sperm motility, may have various causes including hormonal imbalances, genetic factors, infections, and lifestyle factors, while Ch1t1 has been studied in relation to male fertility and sperm quality, more research is needed to determine if there is a direct correlation between serum Ch1t1 levels and asthenospermia. It is possible that elevated levels of Ch1t1 in the serum could be indicative of certain underlying conditions that may contribute to asthenospermia, but further studies are needed to confirm this relationship. The relationship between Serum Ch1t1 levels and IL1 levels in asthenospermia is an interesting area of study. A weak positive correlation between Serum Ch1t1 and IL1 levels in asthenospermia could suggest that there may be some interaction between sperm function and inflammation in individuals with this condition. It is possible that elevated IL-1 levels could influence Ch1t1 levels and vice versa, potentially affecting sperm motility and quality.

The reasons for this negative correlation are not clear and further research is needed to understand the underlying mechanisms. It is possible that factors such as inflammation, hormonal imbalances, or genetic factors may contribute to this relationship between Serum Ch1t1 and SF Ch1t1 levels in individuals with oligospermia. The presence of a weak positive correlation between Serum Ch1t1 and IL-1 $\beta$  in the oligospermia group is an interesting finding. Ch1t1 is a protein involved in sperm maturation and function, while IL-1 $\beta$  is a pro-inflammatory cytokine that plays a role in immune responses and inflammation. The positive correlation between Serum Ch1t1 and IL-1 $\beta$  levels may suggest a potential interaction between sperm function and inflammation in individuals with oligospermia. Elevated IL-1 $\beta$  levels could potentially influence Ch1t1 levels and vice versa, impacting sperm quality and fertility outcomes.

On the other hand, the strong, statistically significant negative correlation between SF Ch1t1 and IL-1 $\beta$  in the oligospermia group is also intriguing. This finding suggests an inverse relationship between Ch1t1 levels in seminal fluid and IL-1 $\beta$  levels, indicating that there may be a regulatory mechanism at play between sperm maturation/function and inflammation in the seminal fluid of individuals with oligospermia. This negative correlation could imply that higher levels of IL-1 $\beta$  are associated with lower levels of Ch1t1 in the seminal fluid of individuals with oligospermia, potentially affecting sperm quality and fertility outcomes. Chitinase 1, on the other hand, has been shown to be involved in the regulation of inflammation and tissue remodeling. In the context of infertility, chitinase 1 may play a role in modulating the immune response in the reproductive tract, potentially affecting fertility [40].

## CONCLUSION

It is possible that elevated IL-1 levels could influence Ch1t1 levels and vice versa, potentially affecting sperm motility and quality this can be utilized to monitor the progression of "silent" subclinical inflammation linked to oligozoospermia. Nevertheless, additional research is necessary on a more extensive cohort of patients.

## Acknowledgement

The Department of Biochemistry College of Medicine, University of Baghdad/Iraq and Department of Infertility , Al-Batool Teaching Hospital, Ministry of Health, Iraq, provided assistance in collecting samples and analyzing them, for which the authors are grateful.

**Ethical approval:** The Iraqi Ministry of Health-Environment, Public Health Directorate's ethical commission gave its clearance for this study (no. 2024-0070).

**Finance:** No funding.

**Conflicts of interest:** No conflicts of interest.

**Protection of persons and animals:** The Institutional Scientific Committee at the University of Baghdad approved this study according to the Declaration of Helsinki for Human Studies 1975, and all were followed for the care and use.

**Declaration:** We confirm that the manuscript “**Assessment of Seminal Plasma and Serum Chitotriosidase-1 as Biomarkers for Silent Inflammation in Infertile Iraqi Males: A Comparative Study among Normozoospermic, Asthenospermia, and Oligozoospermic Groups**” has been read and approved by all the named authors, and they have contributed significantly to the paper. This is also to declare that the paper has yet to be published earlier in whole or as part or been sent to some other journal for consideration for publication. The paper is also accessible from any plagiarism/self-plagiarism. There are no conflicts of interest associated with this paper. The authors would be fully responsible if the paper is found to violate any copyright law in the future.

**Author contributions:** Ali Abdul Aziz , Hedef El-Yaseen, and Hussain Kadhem : Conception, design, performed statistical analysis, explanation; Collecting data, achieved laboratory investigations. All the authors agreed on the final form of the article before the publication and expressed their consent to be responsible for all parts of the work.

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