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Levels of Macro- and Trace Elements and Select Cytokines in the Semen of Infertile Men

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Abstract
The current study evaluated levels of macro-/trace elements, select cytokines, and sperm quality, in the semen of men with abnormal spermograms. The study population of men with abnormal spermograms was divided into three groups, i.e., oligospermic, asthenozoospermic, and oligoasthenozoospermic. The control group was fertile men with normal semen parameters. Analyses showed that in comparison with that in the semen of the fertile men, levels of calcium, magnesium, and selenium were significantly lower in men with all three groups. Semen levels of zinc were significantly lower in men with asthenospermia as compared with that in control. GGT (gamma-glutamyltranspeptidase) activity in semen was significantly higher in men in any of the three states as compared with that seen in control semen. In contrast, semen ALT (alanine aminotransferase) activity was reduced in men with any of these abnormalities compared with that in the controls. Semen cholesterol levels were significantly lower in men with asthenospermia as compared with control semen. Of all the measured cytokines, only IL-5 levels were reduced in the semen of the men with any of the conditions as compared with control semen. The semen of infertile males is characterized by reduced levels of calcium, magnesium, and trace metals such as zinc and selenium. The study also indicated that measures of cholesterol and of GGT/ALT activities could serve as supplementary parameters indicative of semen quality. Further investigations are needed to clarify the role of the measured parameters in sperm physiology.

Keywords Infertility · Macroelements · Trace metals · Immune response

Introduction

Infertility is a health problem that affects approximately 13–18% of the global population, regardless of race. The increasing trends in reproductive defects are related to environmental, lifestyle, dietary, or occupational factors [1]. Male factor is considered as a cause of about 30–40% cases of infertility [2]. Defective sperm function is the most prevalent cause of male infertility [1], which can be related to the altered composition of semen. Indeed, among others, electrolytes and metals are essential for the viability and function of spermatozoa [3].

The human semen contains high concentrations of calcium and magnesium. Calcium is important for sperm physiology, including motility, metabolism, acrosome reaction, and fertilization. Indeed, sperm motility is dependent on intracellular-free-calcium concentration in the principal piece of the flagellum; however, the quantitative relationship between sperm intracellular calcium levels and sperm motility is still controversial [4]. Magnesium is the second crucial element of cell physiology, being a cofactor in more than 300 enzymatic reactions. Similarly to calcium, magnesium may play a role in spermatogenesis and is necessary for sperm motility. Abnormal levels of these macroelements are believed to negatively affect production, maturation, motility, and fertilizing capacity of the spermatozoa [5].

Human seminal plasma has been shown to contain several trace elements that are believed to play an important role to keep a normal sperm function. In human seminal plasma, zinc

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concentration is higher than in other tissues [6]. Zinc is responsible for DNA transcription and protein synthesis necessary for sperm development. By binding thiol groups in proteins and by occupying binding sites for copper in lipids and DNA, zinc acts as an antioxidant. Zinc is also a cofactor of the copper/zinc isoenzyme of superoxide dismutase [7]. Copper is essential not only for detoxification of free radicals by superoxide dismutase but also for many copper-dependent enzymes involved in biological reactions, such as respiration. On the other hand, copper can be highly toxic due to its ability to generate reactive oxygen species. The role of copper in male fertility is still poorly understood [8]. Other than copper, iron is able to induce oxidative stress by catalyzing the Fenton and Haber-Weiss reactions [9]. Nevertheless, iron is crucial for cyclooxygenases, cytochromes, hydroxylase/oxidase enzymes, ribonucleotide reductase, and catalase [10]. The role of selenium is not completely defined. Selenium is believed to protect against oxidative stress and serves as an essential element for testicular development, spermatogenesis, and spermatozoa motility [7].

Many reports linked semen quality with the immune response. Cytokines regulate a physiologic function of the testes and are involved in the fertilization process. However, proinflammatory cytokines may cause a decrease in semen quality, particularly in males with urogenital tract infections [11]. It has been shown that trace metals have an impact on the immune system function. For instance, zinc is essential for highly proliferating immune cells and influences both innate and acquired immune functions. It is conceivable that an adequate zinc intake could support a Th1-mediated immune response [12]. Thus, it can be speculated that the level of trace minerals in the semen may influence sperm quality also via immunomodulation; however, this hypothesis needs to be verified.

Therefore, we aimed at evaluating the levels of macro- and trace elements, together with selected parameters of the immune response and sperm quality, in the semen of males with abnormal spermogram.

Patients

The experimental set-up has been approved by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/I/13/09).

The examined population included 346 males living in Upper Silesia (Poland) who attended the andrology clinic to diagnose infertility. Spermogram test provided information about the fertile abilities of the study subjects. The exclusion criteria included drug consumption, tobacco smoking, alcohol abuse, and a history of any chronic disease, such as diabetes, coronary artery disease, or malignant neoplasm. The seminal samples were collected by masturbation on the same day in the morning before the first meal after 3 days of sexual abstinence. All of the semen specimens were analyzed according to the WHO standards [12].

The experimental ($n = 243$) group included males with abnormalities in spermogram, which were split in oligospermic (sperm count less than 15×10^6/mL, $n = 152$), asthenozoospermic (less than 40% of progressively moving sperm cells, $n = 142$), and oligoasthenozoospermic (both criteria met, $n = 90$). The control group included 103 fertile males with normal semen parameters according to the WHO criteria [12].

Methods

Sample Preparation

After liquefaction, the semen samples (1.5 mL) were centrifuged at 6000×*g* for 10 min to separate the spermatozoa from the seminal plasma. The seminal plasma was transferred to fresh tubes and stored at − 75 °C until required for biochemical analyses. Additionally, a 10% spermatozoa lysate in bidistilled water was prepared.

Semen Analysis

The semen specimens were analyzed according to WHO standards [13]. The evaluation included assessment of the seminal volume, sperm cell density, total sperm cell count, motility, and supravital eosin staining (for the percentage of live spermatozoa). The sperm morphology was examined after Papanicolaou staining.

Biochemical Analysis

Macro- and Trace Elements Determination

Magnesium was determined in seminal plasma using atomic absorption spectrometer ICE 3300 Thermo Fisher. The control material (TruLab N and TruLab P (DiaSys, Germany)) was determined in each series of measurements after the calibration curve preparation and at the end of the series. A standard curve was drawn using a standard solution of magnesium at a concentration of 1 mg/mL (Merck). Magnesium concentrations were expressed as milligram per deciliter.

The seminal plasma calcium concentration was determined using a biochemical analyzer method based on the reaction with o-cresolphthalein. The color intensity is directly proportional to the calcium concentration. The results were expressed in milligram per deciliter.

The seminal plasma iron concentration was determined photometrically. In this method, ascorbate reduces Fe^{3+} ions to Fe^{2+} ions which react with ferrozine to form a colored

complex. The color intensity is directly proportional to the iron concentration. The results were expressed in microgram per deciliter.

The concentration of zinc in seminal plasma was measured by atomic absorption spectrophotometry using Unicam 929 and 939OZ Atomic Absorption Spectrometers at a wavelength of 213 nm. The calibration curve was prepared according to Merck standards. For the internal control, we used a 0.1 mg/mL zinc solution which is a certified Merck standard control. The data were shown in milligram per liter.

The concentration of copper in seminal plasma was measured by atomic absorption spectrophotometry using Unicam 929 and 939OZ Atomic Absorption Spectrometers at a wavelength of 324.8 nm. The calibration curve was prepared according to Merck standards. For the internal control, we used a 10 mg/L copper solution which is a certified Merck control. The data were shown in microgram per deciliter.

The concentration of selenium in seminal plasma was determined by a flameless method using Unicam 929 and 939OZ spectrophotometers. The calibration curve was prepared using Nycomed® standards. Certified Nycomed® controls (containing 78.0 and 11.4 μg/dm^3 of selenium) were used to perform the internal control. The data were shown in microgram per deciliter.

Determination of Cholesterol

The cholesterol concentration in seminal plasma was measured using a biochemical analyzer. The action of hydrolytic cholesterol esterase on cholesterol ester produces fatty acids and free cholesterol. Hydrogen peroxide reacts with 4-aminophenazone and phenol in the presence of peroxidase, creating a colored compound. The color intensity of the resulting product is proportional to the concentration of cholesterol. Results were presented in milligram per deciliter.

Determination of Enzymatic Activities in Seminal Plasma

Gamma-glutamyltranspeptidase (GGT), alkaline phosphatase (AlP), alanine (ALT), and aspartate (AST) aminotransferase activity were determined using a biochemical analyzer.

GGT converts the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine. The amount of released 5-amino-2-nitrobenzene is proportional to the activity of GGT.

In the presence of magnesium and zinc, p-nitrophenyl phosphate is degraded by the enzyme to phosphate and p-nitrophenol. The amount of released p-nitrophenol is directly proportional to ALP activity and can be measured photometrically. ALT catalyzes the reaction of α-ketoglutarate and L-alanine in both directions. In the initial reaction, the pyruvate and reduced nicotinamide adenine dinucleotide (NADH) were a substrate for lactate dehydrogenase. In the second reaction, NADH is oxidized to NAD. The decrease in the optical density of NADH measured photometrically is directly proportional to the concentration of pyruvate and thus, to ALT.

AST catalyzes the reaction of α-ketoglutarate and L-aspartate in both directions. The resulting oxaloacetate and NADH is then an indicator substrate for malate dehydrogenase. NADH is oxidized to NAD. The decrease in the optical density of NADH measured photometrically is directly proportional to the concentration of oxaloacetate and, thus, to AST.

In all cases, results were presented in international units per liter.

Determination of Cytokines

IL-2, IL-4, IL-5, IL-10, IL-13, and INF-γ were detected in seminal plasma using a Bio-Plex 200 System (Bio-Rad Laboratories Inc., USA) according to the manufacturer's instructions. The results were reported in picogram per milliliter.

Statistical Analysis

Results are reported as mean ± standard deviation (SD) for normal distribution, and median and interquartile range (IQR) for non-normal distribution. Shapiro-Wilk's test was used to verify normality, and Levene's test to verify homogeneity of variances. Statistical comparisons between groups were performed by one-way analysis of variance (ANOVA) and Tukey as post hoc test, or Kruskal-Wallis test and Dunn's as post hoc test (non-parametric). Spearman's coefficient R for non-parametric correlation was calculated. A value of $p < 0.05$ was considered significant. Statistical analysis was performed using Statistica 10.0 PL software.

Results

Control and experimental groups (34 ± 5 years old) were similar as regards age (33 ± 6 vs 34 ± 5 years old). Differences between the control group and experimental groups in semen volume, pH, count, motility, and morphology are presented in Table 1.

Among the macroelements considered, the levels of both magnesium ($F = 5.426$, $p < 0.001$) and calcium ($F = 5.054$, $p < 0.001$) were significantly lower in males presenting with sperm abnormalities as compared with fertile subjects (Fig. 1a, b). In particular, with respect to healthy, the levels of magnesium were lower by 19%, 22%, and 22%, while the levels of calcium by 12%, 15%, and 15%, in those patients with oligospermia, asthenospermia, and oligoasthenospermia, respectively. As regards trace elements, we found significant differences in the seminal plasma levels of selenium ($F = 14.41$, $p < 0.001$), which were lower by 26%, 28%, and 27%

Table 1 Semen quality parameters of the patients included, according to study groups. Data are presented as mean ± standard deviation of the mean; statistical differences were assessed by one-way ANOVA and Tukey as post hoc test

	Controls ($n = 107$)	Oligospermia ($n = 155$)	Asthenospermia ($n = 146$)	Oligoasthenospermia ($n = 91$)	p
Sperm volume (mL)	3.70 ± 1.73	3.58 ± 1.53	3.42 ± 1.63	3.41 ± 1.58	0.472
pH value	7.56 ± 0.08	7.57 ± 0.09	7.57 ± 0.08	7.56 ± 0.09	0.648
Sperm cell count (mln/mL)	79.5 ± 60.1	4.39 ± 4.51***	25.4 ± 42.8***,+++	3.81 ± 4.68***,^^^	< 0.001
Total sperm cell count (mln)	269 ± 189	15.5 ± 19.9***	73.9 ± 122***,+++	12.5 ± 19.8***,^^^	< 0.001
Total motility after 1 h (% motile)	58.1 ± 9.48	32.4 ± 20.6***	22.9 ± 14.0***,+++	18.3 ± 14.1***,+++	< 0.001
Rapid progressive motility (a) after 1 h (%)	26.0 ± 9.96	9.16 ± 7.82***	6.75 ± 5.97***,+	5.14 ± 5.55***,+++	< 0.001
Slow progressive motility (b) after 1 h (%)	18.0 ± 6.81	9.53 ± 8.11***	6.76 ± 7.13***,++	4.77 ± 4.98***,+++	< 0.001
Unprogressive motility after 1 h (%)	10.9 ± 10.7	12.6 ± 17.5	12.9 ± 20.6	11.9 ± 21.5	0.833
Progressive motility (a+b) after 1 h (%)	44.0 ± 9.74	18.7 ± 14.22***	13.5 ± 10.66***,+++	9.9 ± 9.41***,+++	< 0.001
Motile spermatozoa after 24 h (%)	19.0 ± 15.8	3.02 ± 6.67***	2.70 ± 5.41***	1.30 ± 3.73***	< 0.001
Progressive motility after 24 h (%)	6.38 ± 7.79	0.68 ± 2.08***	0.52 ± 1.58***	0.15 ± 0.57***	< 0.001
Morphology (% normal)	51.7 ± 7.98	37.4 ± 20.18***	34.6 ± 20.45***	29.8 ± 20.99***,+	< 0.001
Leucocytes (mln/mL)	0.239 ± 0.191	0.267 ± 0.185	0.202 ± 0.165++	0.268 ± 0.152^	0.005

***$p < 0.001$ vs control

+$p < 0.05$ vs oligospermia

++$p < 0.01$ vs oligospermia

+++$p < 0.001$ vs oligospermia

^^^$p < 0.001$ vs asthenospermia

in oligospermic, asthenospermic, and oligoasthenospermic patients with respect to controls (Fig. 1f).

A significant difference between groups was also reported for the seminal plasma concentration of cholesterol ($F = 4.074$, $p < 0.001$), which was lower by 19% in males with asthenospermia as compared with healthy subjects (Fig. 2a).

When the activities of several enzymes in the seminal plasma were studied, we observed differences between groups in GGT ($F = 7.181$, $p < 0.001$), AST ($F = 44.64$, $p < 0.001$), and ALT ($F = 4.722$, $p = 0.003$). In particular, post hoc analysis showed that the GGT activity was higher in males with oligospermia, asthenospermia, and oligoasthenospermia by 16%, 12%, and 12%, respectively, as compared with the control group (Fig. 2c); by contrast, the AST activity was lower by 54%, 55%, and 45%, respectively (Fig. 2d), and the ALT activity by 27% in oligoasthenospermic males as compared with controls (Fig. 2e).

The seminal plasma level of several interleukins was analyzed; however, significant differences between groups were observed for IL-5 ($F = 23.09$, $p < 0.001$). Post hoc analysis showed that IL-5 concentration was significantly lower in males with oligospermia, asthenospermia, and oligoasthenospermia by 87%, 78%, and 87%, respectively, as compared with the control group (Fig. 3b).

Correlations between semen quality characteristics and the levels of macro- and trace elements, enzymatic activities, and interleukins in all examined population are provided in Table 2. Spearman correlation was positive between the sperm cell count, total sperm cell count, and the concentration of iron, selenium, IL-5, and AST activity. Additionally, motility parameters correlated positively with the concentration of magnesium, calcium, iron, zinc, selenium, cholesterol, IL-5, and AST activity. By contrast, the activity of GGT negatively correlated with the sperm cell count, total sperm cell count, and motility after 24 h.

Discussion

In the present study, we found lower levels of calcium in males with abnormal spermogram, including asthenospermia. Similar results were obtained by Adamopoulos and Deliyiannis [14] and Skandhan et al. [15], who showed lower concentration of calcium in asthenozoospermic and oligozoospermic patients than normozoospermic men. Meseguer et al. [16] reported lower level of intracellular calcium in infertile patients compared with fertile ones, but calcium levels in seminal plasma did not differ significantly. Other studies reported no difference in the seminal plasma concentration of calcium between fertile and infertile/subfertile males [5, 6, 17]. Nevertheless, Schmid et al. [4] concluded that higher sperm calcium may be associated with poorer semen quality.

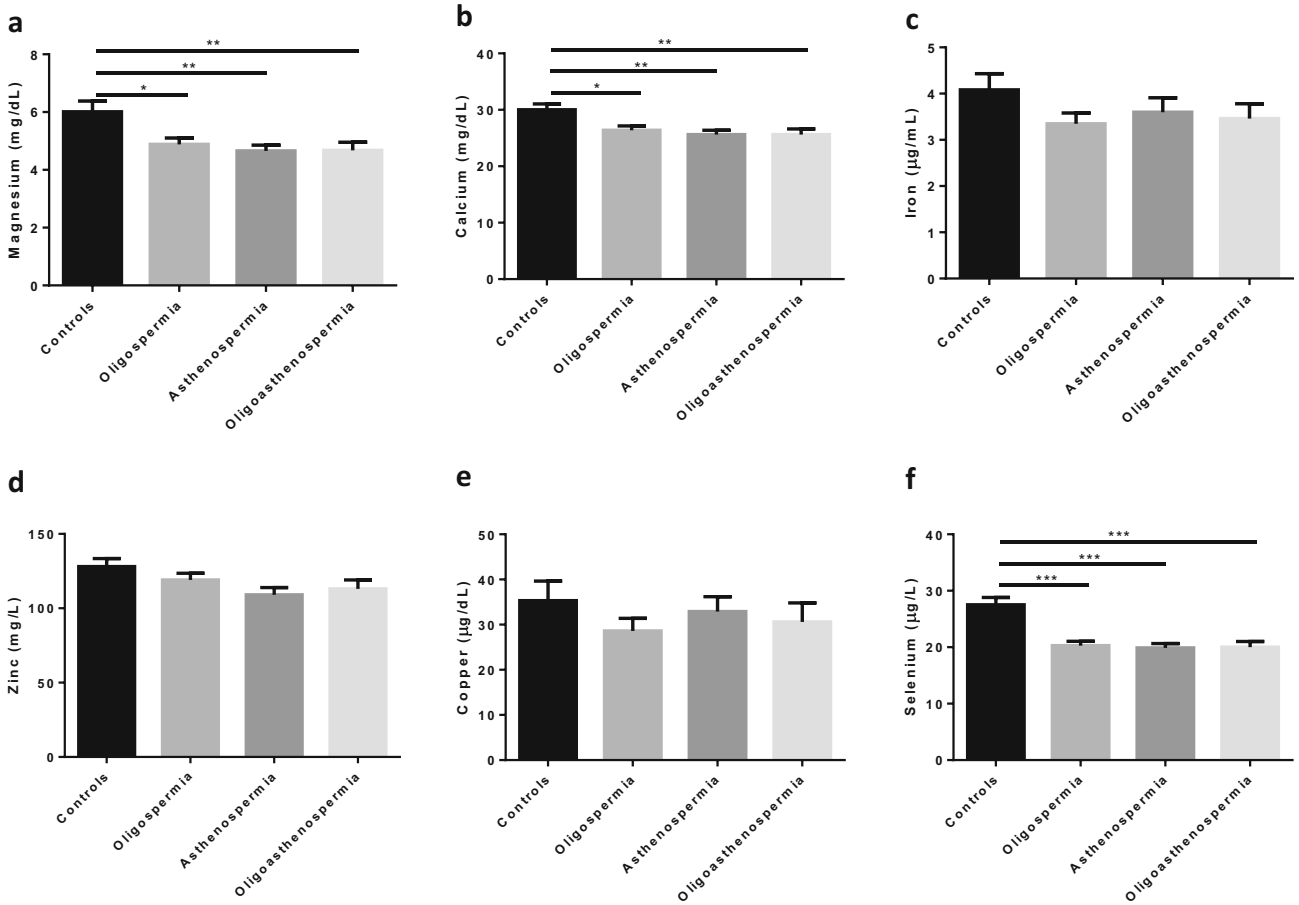

Fig. 1 Seminal plasma concentration of macro- (magnesium and calcium; **a**, **b**) and trace elements (iron, zinc, copper, and selenium; **c**–**f**) in the patients enrolled, according to the study groups. Data are expressed as mean ± standard error. Statistical differences were assessed by one-way ANOVA and Tukey as post hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

The discrepancies between different studies are probably due to the complexity of the regulatory role of calcium. The maintenance of calcium gradient across the plasma membrane is required for calcium homeostasis, crucial for normal cell function [18]. Consistently, it has been shown that calcium influx through the calcium channels of the sperm plasma membrane is an absolute requirement to induce the acrosome reaction [19]. In light of this, it should be concluded that the extra/intracellular calcium ratio should better correlate with semen quality than irrespective measurements of calcium levels in seminal plasma and sperm cells.

In the present study, the pattern of magnesium level in the seminal plasma of studied groups was similar to that of calcium. This observation confirms the postulated crucial role of magnesium for cell metabolism. By contrast, in other studies, magnesium level in the seminal plasma of infertile males did not differ significantly with that of fertile ones [4, 11, 20, 21]. Furthermore, in our previous study, no association was observed between magnesium levels and standard semen parameters in fertile males [22, 23]. As in the case of calcium, the discrepancies between studies are probably due to the complex magnesium metabolism and its interactions with other elements.

The physiological role of calcium and magnesium can be linked with zinc, which regulates the activity of the Ca^{2+}-Mg^{2+}-ATPase responsible for sperm motility (18). The role of zinc in the maintenance of sperm motility is also supported by our results. Indeed, we showed decreased zinc level in males with asthenospermia. The association between zinc level and semen quality has been investigated by many authors. Atig et al. [6] reported decreased levels of zinc in the seminal plasma of males with oligo-, astheno-, and teratospermia. Similarly, Huang et al. [8] reported lower concentration of zinc in oligospermic and asthenospermic males than in normospermic ones. Colagar et al. [5] reported lower zinc levels in infertile males compared with that of fertile subjects. In this study, seminal zinc level was also positively correlated with sperm count and morphology. A positive correlation between zinc level and sperm count was also observed in a study of Camejo et al. [24]. In our previous study on fertile males, we showed a positive correlation between seminal plasma zinc level and motility [25]. However, all these results are not confirmed by other studies which do not show any association between zinc level and parameters of semen quality [2, 26].

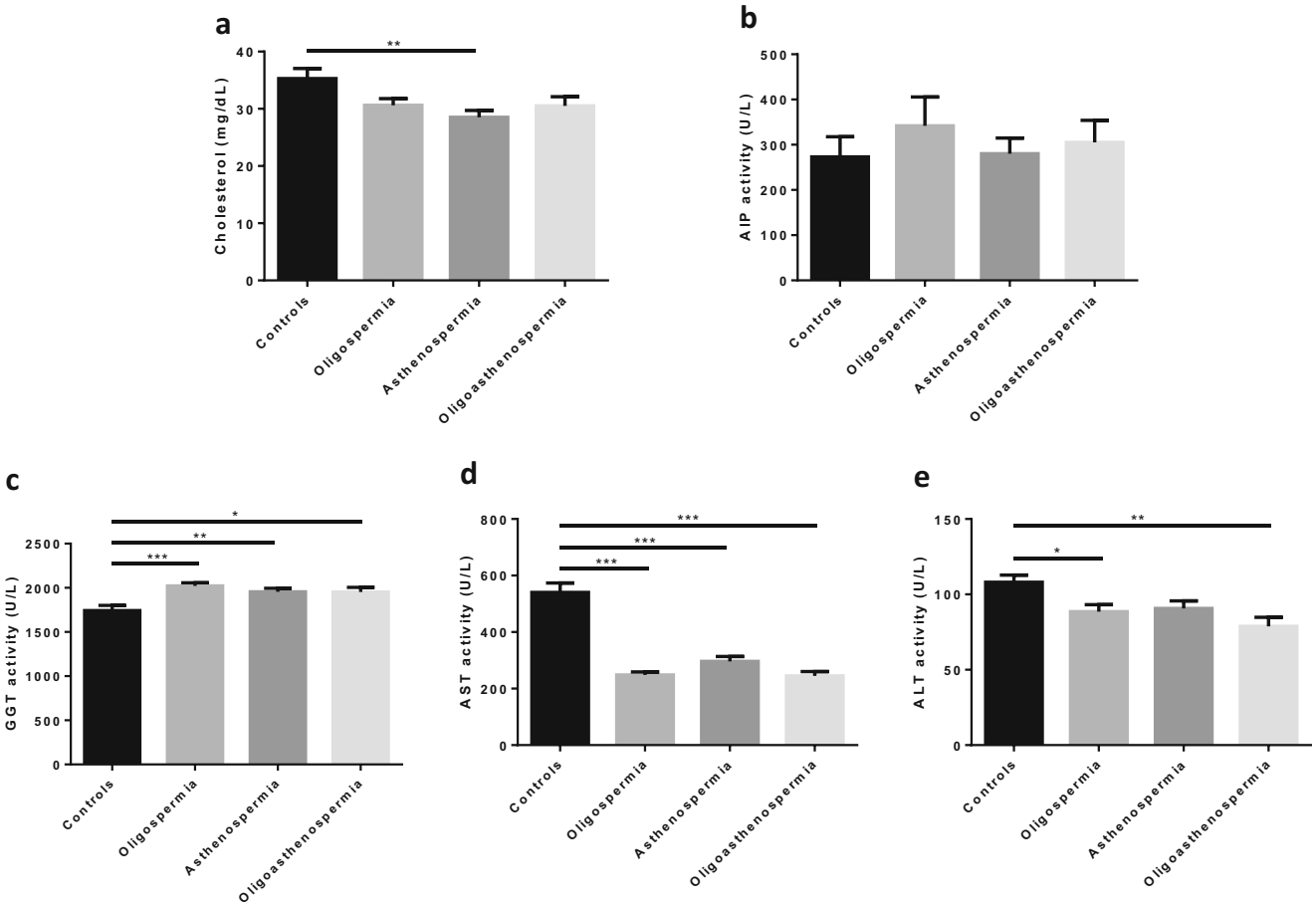

Fig. 2 Seminal plasma cholesterol concentration (**a**) and enzymatic activities (**b**–**e**) in the patients enrolled, according to the study groups. Data are expressed as mean ± standard error. Statistical differences were assessed by one-way ANOVA and Tukey as post hoc test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. AlP, alkaline phosphatase; GGT, gamma-glutamyltranspeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase

In the present study, the levels of IL-5 were lower in the experimental group than in the control group. IL-5 promotes the development of B cells and the production of IgA. Therefore, IL-5 may play a role in the humoral immune defense of the male genital tract. Besides, IL-5 may also play a role in the physiology of the human testis, because its receptors are expressed in the germ line of human testis and in ejaculated sperm [27]. It is not excluded that decreased IL-5 levels observed in the present study are due to the simultaneously decreased level of selenium, because it has been reported that selenium supplementation increases IL-5 expression [28].

Selenium is a trace mineral involved in many physiological functions, including antioxidant defense (29). In the present study, the levels of selenium were significantly lower in males with oligospermia, asthenospermia, and oligoasthenospermia than in the control group. Besides, selenium level correlated positively with sperm cell count, total sperm cell count, and the percentage of motile spermatozoa after 24 h. Consistently, it has been established that a characteristic spermiogram abnormality due to selenium deficiency is oligoasthenospermia. Mice with knock-out of the genes encoding selenoproteins developed infertility related to the reduced numbers of spermatozoa in epididymis and absence of motility of spermatozoa, mainly due to mitochondrial dysfunction and tail bending in a hairpin form [29]. Human studies are generally in concordance with these findings. Ergolu et al. [30] and Türk et al. [26] found lower selenium concentration in oligospermic males than in normospermic ones. Besides, positive correlations between selenium levels and sperm concentration, motility, and normal morphology were shown in these studies. Atig et al. [7] found no differences between the control group and asthenospermic, oligospermic, and teratospermic males in terms of selenium level; however, they reported a positive correlation between seminal selenium level and sperm motility.

The association between seminal copper and iron levels and semen quality seems to be less conclusive than that of zinc and selenium. Both copper and iron are important elements for numerous metalloproteins involved in energy or

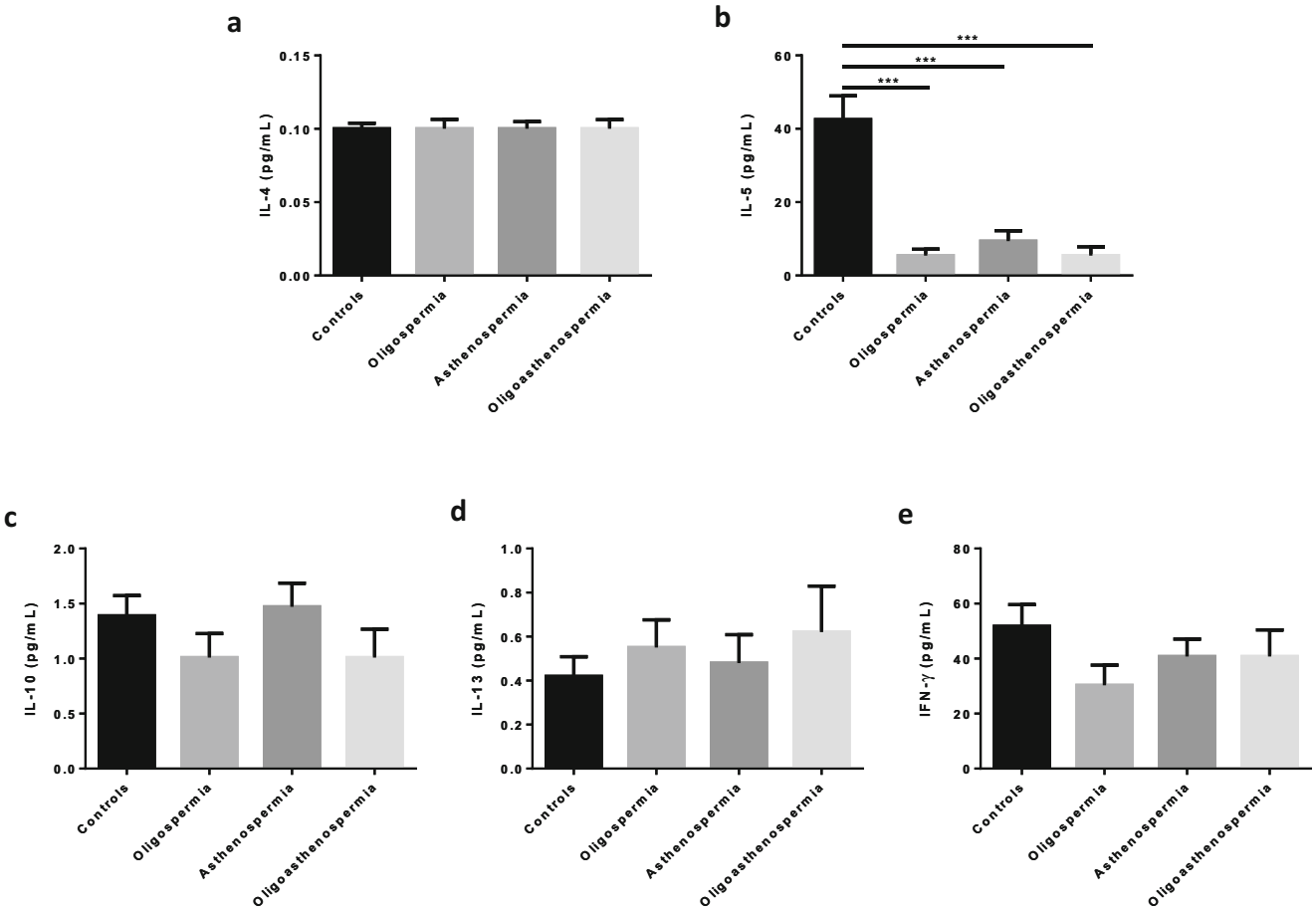

Fig. 3 Seminal plasma interleukins concentration in the patients enrolled, according to the study groups. Data are expressed as mean ± standard error. Statistical differences were assessed by one-way ANOVA and Tukey as post hoc test. ***$p < 0.001$. IL, interleukin; IFN-γ, interferon-γ

antioxidant metabolism. On the other hand, both metals can promote reactive oxygen species formation catalyzing the reaction between the superoxide anion and hydrogen peroxide, producing the hydroxyl radical [8]. In the present study, the levels of copper and iron were not significantly different when the control group was compared with the groups of males with abnormal spermiogram. The results of other studies on this topic are not consistent. In a study by Aydemir et al. [10],

Table 2 Significant ($p < 0.05$) Spearman's rank correlation coefficients between semen quality and study parameters in all the patients enrolled. Boldface, positive correlation; italics, negative correlation; empty cell, lack of correlation

	Mg^{2+} (mg/dL)	Ca^{2+} (mg/dL)	Fe^{2+} (mg/dL)	Zn^{2+} (mg/dL)	Se^{2+} (mg/dL)	Cholesterol (mg/dL)	GGT (U/L)	AST (U/L)	IL-5 (pg/mL)
Sperm cell count (mln/mL)			**0.14**		**0.25**		*− 0.20*	**0.50**	**0.40**
Total sperm cell count (mln)					**0.24**		*− 0.20*	**0.47**	**0.40**
Total motility after 1 h (% motile)				**0.16**				**0.22**	**0.18**
Rapid progressive motility (a) after 1 h (%)				**0.19**				**0.38**	**0.24**
Slow progressive motility (b) after 1 h (%)		**0.14**	**0.21**					**0.17**	**0.17**
Unprogressive motility after 1 h (%)	**0.16**	**0.19**	**0.16**						
Motile spermatozoa after 24 h (%)	**0.19**	**0.20**	**0.15**	**0.15**	**0.20**	**0.25**	*− 0.18*	**0.39**	**0.29**
Progressive motility after 24 h (%)	**0.19**	**0.16**	**0.15**	**0.16**		**0.26**	*− 0.18*	**0.37**	**0.27**
Leucocytes	*− 0.21*			**0.21**					

seminal plasma copper and iron levels were shown to increase in subfertile males compared with the fertile ones. Marzec-Wróblewska et al. [2] and Wong et al. [5] showed no significant differences between iron and copper levels, respectively, in normozoospermic males and males with pathological spermiogram, while Skandhan et al. [3] reported decreased iron levels in males diagnosed with oligoasthenozoospermia and asthenozoospermia. The authors hypothesized that there is a narrow optimal range of iron levels for the maintenance of proper semen quality, and any iron levels outside this range may result in sperm quality impairment. The same hypothesis seems to be applicable for the copper levels.

The lipid content of sperm plasma membranes plays an important role for sperm motility and viability [31]. Besides, the membrane structure of spermatozoa is crucial for the acrosome reaction and sperm-oocyte fusion [32]. Animal studies suggest that cholesterol is an important component of the sperm plasma membrane and plays an important role in promoting sperm membrane permeability and fluidity, which contribute to the maintenance of sperm motility and viability [31]. Results of the present study are in agreement with these findings, because we observed lower cholesterol level in the seminal plasma of males with asthenospermia than in normospermic subjects. Besides, there was a positive correlation between cholesterol level and the percentages of motile and progressive motile spermatozoa after 24 h.

Apart from cholesterol, seminal plasma diagnostics involves evaluation of several enzymes, such as AST, ALT, GGT, and AlP. The activities of AST and ALT are related to the secretory activity of male accessory sex glands. Besides, AST plays an important role in sperm metabolism through its involvement in the vital cellular process. Consistently, a positive correlation between AST activity and sperm concentration, live sperm percent, motility, seminal total protein, semen volume, and fertility rate of semen was reported [33]. On the other hand, the elevated activities of ALT, AST, and AlP in seminal plasma may be interpreted as a result of acrosomal damage and sperm cell disintegration [34, 35]. Results of the present study support the beneficial role of AST and ALT in the maintenance of good semen quality. ALT activity correlated positively with rapid progressive motility after 1 h, and negatively with non-linear progressive and unprogressive motility after 1 h. At the same time, AST correlated positively with sperm count, concentration, motility, and normal morphology. Besides, males with abnormal spermiogram showed decreased activities of AST and ALT. No association between semen quality and AlP activity was observed. Nevertheless, the pattern of GGT activity in males with oligospermia, asthenospermia, and oligoasthenospermia was opposite to that of AST. Furthermore, GGT activity correlated negatively with sperm count, concentration, and motility. Results of other studies are not consistent. GGT has been used as a marker for testicular growth and development. Studies on GGT knock-out mice showed that GGT deficiency results in sexual immaturity and hypoplasia of testes, seminal vesicles, and epididymis [36]. Besides, Pesch et al. [37] showed a positive correlation between GGT and motility, speculating that GGT may play an important role in the protection of spermatozoa from oxidative stress.

Conclusions

Abnormal semen parameters classified as oligospermia, asthenospermia, and oligoasthenospermia are related to low seminal levels of calcium and magnesium as well as trace metals, such as zinc and selenium, without accompanying significant impairment of the immune response balance. The present results encourage to evaluate the possible beneficial influence of macro- and trace elements supplementation on semen quality. Seminal plasma cholesterol concentration and activities of AST, ALT, and GGT could serve as supplementary parameters of semen quality. However, interpretation of their values requires special carefulness, and further investigations are needed for clarifying their role in sperm physiology.

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Compliance with Ethical Standards

The experimental set-up has been approved by the Bioethics Committee of the Medical University of Silesia in Katowice (KNW/0022/KB1/I/13/09).

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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