Relationship Between Testosterone Plasma Concentration and Semen Parameters in the Guys in the Aral Sea Ecological Disaster Region

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Abstract: This paper was aimed to study of the sperm quality and assess the possible relationship between the spermogram parameters and the plasma total testosterone concentration in the local guys in the Nukus city (Uzbekistan). Methods. 302 medical profiles, which included data from the analysis of ejaculate and total testosterone plasma level in apparently local healthy subjects in the Aral Sea ecological disaster were analyzed. According to the World Health Organization guidelines, study participants into «group A» and «group B» (spermogram parameters less and more than the 25th centile respectively) were divided. Total testosterone plasma concentration in subjects from these groups was compared. The proportion of included patients of both groups also was calculated. Outcomes. The proportion and total testosterone level of patients included in «group A» was lower than the proportion of study participants in «group B». The results of the study exposed the problem of fertility disorders in men living in this South Aral Sea region. Disruption of testosterone function is probably a consequence of the antiandrogenic effect of environmental pollutants. The data obtained can be useful in the complex monitoring of the state of men's health among residents of environmentally problematic regions.

1 INTRODUCTION

Southern Kazakhstan, western Uzbekistan and northern Turkmenistan, named the Aral Sea region, is infamous because of the ecological disaster that has existed there since the middle of the 20th century (Krivonogov, 2014; Opp, 2017; Nazhmetdinova, 2017). The Aral Sea basin became the major cotton producer for the Soviet Union (Opp, 2017). This was the reason for the almost complete disappearance of the Aral Sea. Between 1918 and 1960, water withdrawals for irrigating cotton fields increased by about 40%, from 1960 to 2008 by more than 200% (Opp, 2017). Thus, by 2008 almost 90% of the lake's water volume and 74.3% of its former surface area were lost (Opp, 2017). Salting out of heavy metals to the bottom of the former Aral Sea, environmental pollution by products of the metallurgical industry led to the eolian process of the spread of micro-particles with precipitated compounds of lead, chromium, cadmium, mercury and etc. throughout the Aral Sea region (Rzymski, 2019).The use of pesticides in

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agriculture is the cause of the accumulation of organochlorine compounds in the environment due to the disturbance of their elimination under these environmental conditions (Bapayeva, 2018).Organochlorine pesticides and heavy metals enter the human organism with food and water, where they have an endocrine-disrupting chemicals (EDC) effect (Gore, 2015). They interfering with all physiological processes of action and regulation of sex steroids and they are the cause unsatisfactory sperm quality in in men living, for example, in different regions of China (Ren, 2020; Zeng, 2022) and Russia (Abou, 2020; Williams, 2022).

The scientific literature accumulates information on the impact of environmental pollutants on the reproductive health of the population of different countries. Recent studies involving volunteers from Russia and China report a direct effect of organochlorine pesticides found in plasma on spermatogenesis (Abou, 2020; Williams, 2022), genetic polymorphism of enzymes contained in spermatozoa (Miao, 2022), and hormonal profile (Lin, 2021). These studies were carried out with the involvement of a contingent living in ecologically unfavorable areas where the state of the environment is not recognized as catastrophic (Abou, 2020; Williams, 2022). Due to the catastrophic environmental conditions of the Aral Sea region and the design of this work, the results obtained can be considered unique. Present outcomes were obtained during examination of men living under conditions of long-term exposure to pollutants potentiated by climatic factors. Previous studies related to the reproductive health of the inhabitants of the Aral Sea region are extremely limited and were carried out on the inhabitants of Kazakhstan, located more than 1000 km from the epicenter of the Aral Sea catastrophe (Kislitskaya, 2015; Kultanov, 2016).

On this basis the paper was aimed to study of the sperm quality and assessment the possible relationship between the spermogram parameters and the plasma total testosterone concentration in guys living in the Nukus city (Uzbekistan) – the epicenter of the Aral Sea environmental disaster.

2 METHODS

Following the study design, we determined sperogram parameters and plasma testosterone concentrations in volunteers living in the Aral ecological disaster region. Using methods of mathematical statistics, the level of testosterone was compared in volunteers from group «A» consisted of patients with low and «B» – with average high values of spermogram parameters. A correlation between the values of the hormone and the spermogram parameters was also calculated.

2.1 Patient Selection Procedure

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the local ethics committee of the St. Petersburg State Pediatric Medical University (protocol no. 17/3, 10 May 2018). The research was included patients (guys) aged 23-30 years who lived in Nukus city or in the suburbs from birth, students or office workers who do not practice heavy physical work or are employed in hazardous manufacturing establishment with normal body mass index. Anamnesis for all persons did not contain information about inflammatory diseases, including sexually transmitted diseases and genital trauma, varicocele, vas deferens strictures, infectious diseases, including mumps and COVID-19. Smokers, alcohol and drug addicts were excluded from the study. If necessary, all persons by a urologist to clarify the diagnosis were examined.

2.2 Research Limitations

Thus, we received 439 anonymous patient profiles, which included data on health status, lifestyle, spermogram parameters and total testosterone plasma level. 341 profiles of study participants who had no pathology and adhered to a healthy lifestyle were selected. Another 33 patients were excluded due to aspermia, which may indicate a deletion of the Y chromosome, and 6 volunteers due to the inflammatory ejaculate – yellow color and high white blood cell count on microcopy. Thus, the final homogeneous sample consisted of 302 profiles of apparently healthy subjects.

2.3 Semen Collection and Spermogram Parameters Analysis

Collection of semen samples, analysis of spermogram parameters, and assessment of sperm quality were carried out according to the World Health Organization (WHO) guidelines (Campbell, 2021). At the request of the attending physician, patients have excluded alcohol, overheating, including fever, medication, physiotherapy, X-ray diagnostics, and prostate massage, physical and emotional stress for ten days before ejaculate examination. Semen samples were collected by masturbation in a separate equipped laboratory room in a special non-toxic plastic container after 2-7 days of abstinence. The resulting material was placed in a thermostat and kept at a temperature of 37^0 until liquefaction. The analysis of spermogram parameters was carried out in all subjects within one hour after collection. As additional criteria for inclusion of patients in the study, the milky-white color of the sperm and its pH, which in healthy people should not be lower than 7.2, were evaluated. Semen volume was measured with a pipette, then a smear was made, which was microscopically counting sperm concentration (10^6 per ejaculate), total motility (%), vitality (%). Sperm quality was assessed according to the centile distribution of spermogram parameters (Campbell, 2021). In accordance with the methodology proposed by WHO expert's reference intervals 25^{th}-75^{th} centile for semen volume: 2.3-4.2 ml, for sperm concentration: $36\text{-}110\times10^6$, for total motility: 55-73%, for vitality: 69-88% (Campbell, 2021). Thus, the volunteers were divided into two groups: «A» consisted from patients with low (less than 25^{th} centile), and «B» – with average high (more than 25^{th} centile) values of spermogram parameters.

2.4 Blood Sample Total Testosterone Assessment

During the cross-sectional study, blood samples were collected from 302 subjects selected by simple random sampling. Venous blood samples were taken on an empty stomach until 10 am before ejaculate collection on the same day. Blood samples were centrifuged at 1000 rpm for 10 min to obtain the serum. Total testosterone concentrations in serum were measured by the direct solid-phase chemiluminescent enzyme immunoassay («sandwich» method) with commercial test kits (MR-96A Mindray microplate reader, Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China). In accordance with the technical specifications of the device, the reference value of total testosterone plasma concentrations for the assay was 9.03 nmol/l to 38.19 nmol/l, which corresponds to the values accepted in the previous studies (Qin, 2012; Mezzullo, 2020). Values of total testosterone plasma concentrations below 9.03 nmol/l were not found in patients who participated in the present study.

2.5 Statistical Analysis

The comparison of total testosterone plasma concentrations, spermogram parameters between patients from «group A» and «group B» was statistically measured using Mann–Whitney U test. Data were presented as mean valued of total testosterone, spermogram parameters and lower or upper limits of 95% confidence intervals (95% CI). The Spearman's rank correlation coefficient (Spearman's ρ) and its 95% CI (Kelley, 2019; Pugovkin, 2021; Lytaev, 2021) between total testosterone values and semen volume, sperm concentration, total motility and vitality were calculated in all patients, regardless of the comparison group. At a value of equal to 0, the statistical relationship was considered absent; from 0.01 to 0.29 (from -0.01 to -0.29) – weak direct (reverse); from 0.3 to 0.69 (from -0.3 to -0.69) – the average direct (reverse); from 0.7 to 0.99 (from -0.7 to -0.99) – strong direct (reverse); 1 (-1) – full forward (reverse). In addition, the proportion and their 95% confidence intervals of patients included in «group A» and «group B» were calculated. The results were considered to be significant at $p<0{,}001$. Estimations were carried out using statistical programmer (version 2.17, Norway, Oslo, 2012) and algorithm of statistical data processing StatXact-8 with Cytel Studio software shell version 8.0.0.

3 RESULTS AND DISCUSSION

It was found that the proportion of patients from group A was higher than the proportion of study participants from group B (table 1).

Table 1: The proportion and their research patients.

Group	Proportion
A	0.54 (0.47; 0.60)
B	0.46 (0.40; 0.52)

Data analysis revealed statistically significantly lower total testosterone values, sperm concentration, total motility and vitality in patients from «group A» compared to «group B»

The study found an average positive correlation of total testosterone plasma level and sperm concentration, total motility and vitality (table 2). The values of total testosterone and semen volume did not correlate (table 3).

Table 2: The mean and of total testosterone plasma level and spermogramm parameters in patients from «group A» compared to «group B».

Parameters/Groups	A	B	*p*
Total testosterone, nmol/l	15.10 (14.30; 15.91)	19.47 (18.86; 20.10)	9.95 $\times10^{-13}$ ***
Semen volume, ml	2.56 (2.46; 2.67)	2.75 (2.65; 2.84)	0.02 154
Sperm concentration, 10^6 per ejaculate	26.36 (25.16; 27.56)	72.09 (70.06; 74.13)	3.36 1×10^{-6} ***
Total motility, %	29.50 (27.23; 31.38)	63.09 (61.80; 64.37)	6.22 $\times10^{-49}$ ***
Vitality, %	49.28 (46.66; 51.89)	81.66 (80.59; 81.62)	3.16 2×10^{-50} ***

Note. P < 0.001

Table 3. The Spearman's ρ and of total testosterone plasma level and spermogram parameters in all patients, regardless of the comparison group.

	Semen volume	Sperm concentration	Total motility	Vitality
Total testosterone	0.11 (-0.0008; 0.15)	0.45 (0.35; 0.47)	0.41 (0.31; 0.43)	0.39 (0.28; 0.41)
p-values	0.06761	2.00×10^{-16} ***	1.58×10^{-13} ***	2.93×10^{-12} ***

Recent studies involving volunteers from Russia and China report a direct effect of organochlorine pesticides found in plasma on spermatogenesis (Abou, 2020; Williams, 2022), genetic polymorphism of enzymes contained in spermatozoa (Miao, 2022), and hormonal profile (Lin, 2021). These studies were carried out with the involvement of a contingent living in ecologically unfavorable areas where the state of the environment is not recognized as catastrophic (Abou, 2020; Williams, 2022). Due to the harsh catastrophic environmental conditions of the Aral Sea region and the design of this work, the results obtained can be considered unique. They were obtained during examination of men living under conditions of long-term exposure to pollutants potentiated by climatic factors. Studies of reproductive health among the inhabitants of the Aral Sea region are extremely limited and were performed on the inhabitants of Kazakhstan, located more than 1000 km from the epicenter of the Aral Sea catastrophe. The research outcomes are not only consistent with the data presented earlier in the analysis of sperm from local men of the Aral Sea region of Kazakhstan, but also complement them (Kislitskaya, 2015; Kultanov, 2016). Scientific articles are focused on the possible involvement of environmental pollutants in disrupting the integrity of the hereditary material of spermatozoa (Kislitskaya, 2015; Kultanov, 2016), which was also shown early in other studies (Pilsner, 2018; Williams, 2022). Thus, these works do not take into account the EDC effect of heavy metals and organochlorine pesticides, which disrupts the action of sex steroids (Zeng, 2022; Ren, 2020; Williams, 2022) and their mechanisms of regulation of their activity (Williams, 2018; Abou, 2020). At the same time, in our work and in other papers (Keskin, 2015; Yerkudov, 2020; Suslov, 2022), an association of quantitative characteristics of spermogram parameters and testosterone plasma level were reported. In the present investigation, the proportion of healthy men with reduced values of spermogram parameters was 54% (table 1) and the concentration of testosterone in this group was significantly reduced compared to study participants with satisfactory sperm quality (table 2). Thus, the results of the present work suggest an anti-androgenic effect of environmental chemical pollutants on the role of anti-androgenic EDC substances (Gore, 2015; Kislitskaya, 2015; Williams, 2022).

4 CONCLUSION AND FUTURE RESEARCH

The present outcomes exposed the problem of fertility disorders from local men of the Aral Sea ecological disaster region. More than half of the practically healthy participants in the study had a reduced sperm concentration, total motility and vitality. Their unsatisfactory sperm quality was combined with a reduced testosterone plasma level. Disruption of testosterone function is probably a consequence of the anti-androgenic effect of EDC in the environment of the South Aral See region, found in previous studies. The data obtained can be useful in the complex monitoring of the men's health state of among residents of environmentally problematic regions.

The analysis of the data obtained opens up the possibility for expanding the tasks of future research in the development of the designated project. In particular, it is planned to compare the parameters of the spermogram in men living in the Aral Sea region and in the city of Tashkent, which is located at the maximum distance from the epicenter of the Aral ecological catastrophe. Sperm quality assessment will be complemented by a comparison of the hormonal profile in subjects living in both geographic areas.

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