RESEARCH ARTICLE

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Study on biological and environmental factors for azoospermia

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ABSTRACT
The absence of sperm in the seminal fluid is known as azoospermia. This medical condition is in a direct association with male's infertility. A complete medical history, physical examination, tests of selected hormones and tests for male fertility (including sperm analysis) are used for the diagnosis of azoospermia.
The number of 1314 men, with fertility problems, at age between 20 and 67 years, were included in this investigation. The data obtained in the study showed that azoospermia occurred in 5.25% of the group studied. The Klinefelter syndrome was cytogenetically diagnosed in 2.9% of the male individuals. There was found that 44.3% of males with azoospermia have been smokers, 7.1% of them – addicted to drugs, 17.1% have taken different medicaments, 4.35% – have had radiation therapy, 4.35% have had a diagnosis "varicocele". The volume of the ejaculate was less than 2 ml in 37.68% of the men and more than 7 ml in 2.89% of them.

Keywords: azoospermia, ejaculate, genetics and environment

Introduction

Infertility is one of a major public problems related to the human reproductive health. It was found that the global prevalence of infertility ranges between 3.5% and 16.7% in different nations (Boivin et al., 2007; Suganthi et al., 2013). There are many studies devoted to the hypothesis for the interaction between genetic and environmental factors for infertility in human populations. According to the opinion of some authors, this complex of factors could act at some stages of testicular development and disrupt spermatogenesis (Sharpe & Irvine, 2004; Krausz, 2008).

Infertility is defined as the failure to conceive a child subsequent to 12 months of unprotected intercourse between a couple in reproductive age. It is recognised by the World Health Organisation (WHO) as a public health concern (Boivin et al., 2007). Different factors, such as defects related to genetics, urogenital and reproductive systems, gametogenesis, gamete function, fertilization and embryonic development (Matzuk & Lamb, 2008), could be in relation with infertility. At the same time, numerous lifestyle factors have shown to affect fertility in both sexes (Ferreira et al., 2010).

The absence of sperm in the seminal fluid is known as azoospermia. This medical condition is in a direct association with male's infertility. The problems in the sperm production or the presence of any obstruction in sperm delivery could be the most common reason for azoospermia. Azoospermia is defined as complete lack of sperm in the ejaculate and may be observed in up to 20% of cases with male infertility. There are two types of azoospermia – non-obstructive and obstructive. Three types of reasons (pre-testicular, testicular and post-testicular) for azoospermia could be mentioned and they are in connection with endocrine disorders, primary testicular disorders, spermatogenesis failure, ejaculatory dysfunction or duct obstructions.

The aim of this study was to trace and to characterize different causes leading to or connected with azoospermia, and to find potential relations between biological, genetic and environmental factors connected with this state.

Materials and Methods

This study was done accordingly with ethical principles and norms. All subjects included in the study gave their written informed consent to participate in this investigation.

The study was conducted during the period from February 2014 to August 2015. The number of 1314 men has visited an office of Reproductive health in Plovdiv, sharing reproductive problems and for 69 of them azoospermia was detected.

Before the study planned, the men have been instructed 3 to 5 days before semen collection how to be made ready for

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this procedure. A survey form with specific questions was used for collecting data, concerning the different characteristics included in the study. The questions concerned medical history, genital trauma and infections, sexual transmitted infections, smoking, alcohol and drugs intake. The men included in the study were also asked to respond about possible use of steroids, anabolics, testosterone and job-related risks. Physical examination was performed in order to detect varicocelae and to define the size and the structure of the testicles.

All samples were tested after 15 min liquefaction at 37°C in an incubator. All semen samples were evaluated by light microscope for sperm analysisand Makler's chamber. The samples where spermatozoa were not found were centrifuged in 15 ml sterile conical tubes for 10 min at 1800 g and the pellet was assessed by the spermatozoa presence or absence. The volume of the ejaculate (ml) was one of the main indicators included in the present investigation. Klinefelter syndrome was diagnosed by a routine cytogenetic technique.

All statistical analyses were performed by using SPSS version 19.0k (SPSS Inc., Chicago, IL, USA). Data were presented as mean±standard deviation (SD) or the number of patients (as a percentage of the entire cohort). Since distributions of continuous data were skewed, nonparametric methods were used for the group comparison. Qualitative and quantitative differences between subgroups were analyzed using the χ^2 test, Mann-Whitney U-test and Kruskal Wallis H test, respectively. A result was deemed statistically significant when$p<0.05$.

Results

Data obtained in the present investigation showed that the average age of the studied men with azoospermia was 33.67 ± 7.06. The youngest patient was 20, and the eldest – 67 years old. It was found that the average duration of established infertility was 2.97 ± 2.36 years. The longest period of infertility registered in the study was 10 years. The calculated average volume of ejaculate in the group with azoospermia was 2.62 ± 1.88 ml.

Genetic prerequisites for azoospermia were established in 2.9% of the men, for that chromosome analysis demonstrated the presence of Klinefelter's syndrome.

Generally, 32.9% of the patients with azoospermia, included in the study, had had in the past injuries (traumas) and operations of the external genitalia and pelvis. Looking at subdivisions of this group, the operations in the pelvic area and (or) external genitalia (45.5%) were less than the injuries (54.5%).

Nearly half of the cases with azoospermia (44.3%) were found to be smokers. Concerning intensity of smoking, the survey showed that the men smoking up to 20 cigarettes per day were 48.4%, while the intensive smokers (smoking more than 20 cigarettes per day) were 12.9% (Table 1).

Table 1.*Intensity of smoking in men with azoospermia.*

Intensity of cigarette smoking	Frequency	Valid Percent
up to 10 cigarettes per day	12	38.7
up to 20 cigarettes per day	15	48.4
More than 20 cigarettes per day	4	12.9
Total	31	100.0

Although the patients have been instructed not to use alcohol before the test, 14.5% of them reported usage of alcohol within twenty-four hours before the procedure.

Data of this investigation showed that 7.1% of the men have been using drugs (cocaine and (or) marijuana) and 17.1% of them were taking medicine during the period of the survey.

The distribution of the medication type is presented in Figure 1. Data received in the study showed that 33.3% of the patients were taking hormones, 25% – antihypertensive drugs, 25% – anabolic agents, 8.3% – antihistamines and 8.3% – antidepressants.

For the presence of additional risk factors informed 27.1% of the included in the study men with azoospermia and on Figure 2 their distribution is presented.

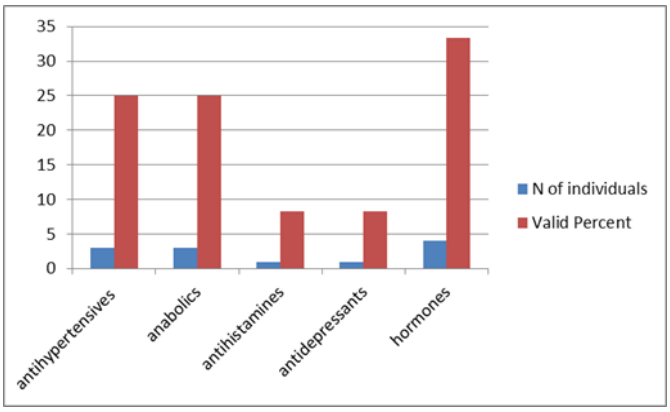

Figure 1. *Type of medicine taken by patients with azoospermia*

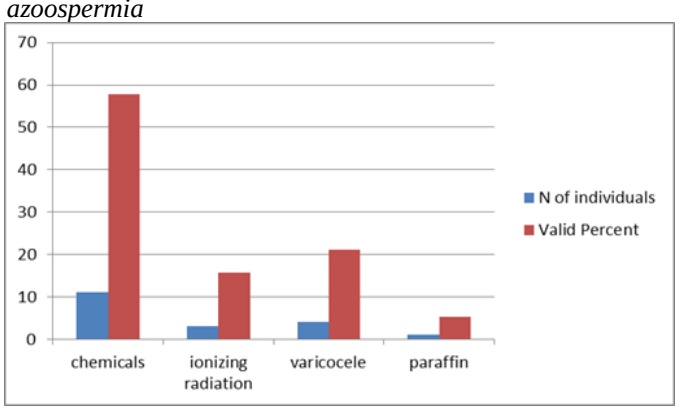

Figure 2. *Distribution of additional risk factors for studied men with azoospermia.*

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In order to study profession as a possible risk factor and its potential relation with azoospermia, distribution of professions for the investigated subjects was considered (Figure 3). In this aspect, data received in our study showed that the highest percentage of men with azoospermia was found among unemployed (28.6). Potential professional risk for azoospermia was found for builders (21.4%), drivers (12.9%), men practicing intellectual work (11.4%).

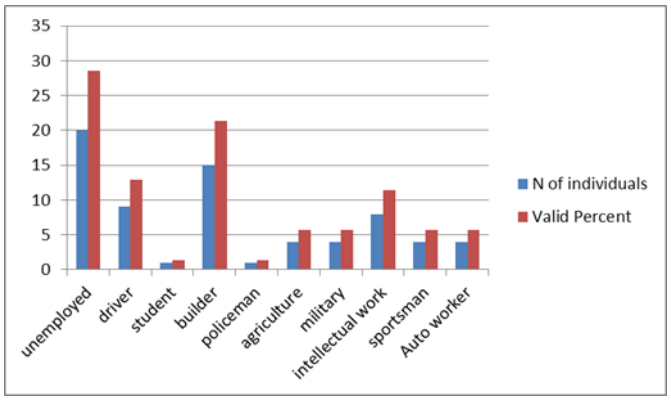

Figure 3. *Distribution of professions for the studied men with azoospermia.*

The results of the present study showed that the volume of the ejaculate was less than 2 ml in 37.68% of the man and more than 7 ml in 2.89% of them.

Data presented in the Tables 2, 3 and 4 showed the relations between volume of ejaculate and other factors studied.

DISCUSSION

Data received in the present study showed some interesting relations and tendencies which should be discussed and considered below.

The common genetic disorders which could be associated with azoospermia are pretesticular, such as Kallmann syndrome, testicular (Klinefelter syndrome and Y chromosome microdeletions) and post-testicular (congenital bilateral absence of the vas deferens – CBAVD) (Gudeloglu& Parekattil, 2013).

Klinefelter syndrome is a chromosomal disorder in which at least one additional X chromosome is observed in the male karyotype. It could be found in human population (including mosaic forms) in a wide spectrum of clinical presentations and lead to spermatogenic and androgenic failure, gynecomastia, and learning difficulties (Lanfranco et al, 2004; Visootsak & Graham, 2006; Oates, 2008).

Table 2.*Klinefelter syndrome and Volume of ejaculate (ml.) found in the men group with azoospermia.*

	Klinefelter syndrome	N	Mean	Std. Deviation	Std. Error Mean
Volume of ejaculate in ml.	Yes	2	2.0000	1.69706	1.20000
	No	67	2.6373	1.89344	0.23132

Table 3.*Relation between volume of ejaculate in ml. and intensity of cigarette smoking by men with azoospermia.*

Intensity of cigarette smoking	N	Mean volume of ejaculate in ml.	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Up to 10 cigarettes per a day	12	2.2083	1.23764	0.35727	1.4220	2.9947	0.10	4.00
Up to 20 cigarettes per a day	15	2.2400	1.48122	0.38245	1.4197	3.0603	0.50	6.20
More than 20 cigarettes per a day	3	2.7333	1.55349	0.89691	1.1258	6.5924	1.00	4.00
Total	30	2.2767	1.35308	0.24704	1.7714	2.7819	0.10	6.20

Table 4.*Relation between volume of ejaculate in ml. and type of drug used by men with azoospermia.*

	Drug type	N	Mean	Std. Deviation	Std. Error Mean
Volume of ejaculate in ml.	marihuana	2	1.2500	1.06066	0.75000
	cocaine	3	3.4333	3.08923	1.78357

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This syndrome has frequencies between 0.1% to 0.2% in the general population and up to 3.1% in the infertile population (Gudeloglu&Parekattil, 2013). In comparison with this, results of our study showed a similar tendency – the Klinefelter's syndrome was established in 2.9% of the men with azoospermia. Our investigation showed that for the patients with a proven Klinefelter syndrome average ejaculate volume was 2.00 ml ± 1.69ml – an average of 0.64 ml. less than for the other patients without commented cytogenetic damage.

According to the World Health organization criteria's, the normal volume of the ejaculate ranges between 2.0 and 6.0 ml. Volume of the ejaculate less than 2 ml shows testicular insufficiency or functional reduction in the sexual glands (37.68% of the patients in our study). Volume of the ejaculate more than 7 ml is in relation with hyperplasia of semen vesicles (2.89% of the patients in our study).

The impact of cigarette smoking on human semen parameters has been studied by different authors. Smoking of cigarettes may be associated with sub-fertility and infertility in males and may result in decreased sperm concentration, lower sperm motility, and a reduced percentage of morphologically normal sperm respectively (Lewin et al., 1991; Sofikitis et al., 1995; Zinaman et al., 2000; Trummer et al., 2002).

Analysis of the data of our study, regarding the relation between the volume of ejaculate and smoking, demonstrated that smokers spend an average of 0.61 ml less ejaculatory volume than non-smokers (u = 1,334, p = 0,187).

The comparison of the established average volume of ejaculate in groups depending on the intensity of smoking showed that increasing the amount of cigarettes smoked per day increased the volume of ejaculate compartments. This is most likely due to the increased production of seminal vesicles and prostate secretions as an expression of the local exudative vascular response which could be a result of intensive smoking. A similar trend was also found for other risk factor – alcohol.

There was not found significant difference in volume of the ejaculate between drug users and non-drug users.

The data from this study indicated also that patients who smoke marijuana emit significantly less volume of ejaculate (1.25 ± 1.06 ml.) in comparison with the average volume of ejaculate of the whole group (2.62 ml.). The average ejaculate volume for the men of "cocaine group" was 3.43 ± 1.09 ml, which was significantly higher than the average volume of the group studied.

In the course of the study it was found that smokers were approximately 3 years younger than nonsmokers. This could be an expression of the trend with increasing the age the individuals to become more responsive to their health.

The results from our study also showed that patients who smoked and had traumas or operations in the pelvic area and genitals were 36.3% more than the patients who did not smoke, and have similar operations.

Additionally, the present investigation show that different hormones, antihypertensive drugs, anabolic agents, antihistamines and antidepressants taken by men with azoospermia, the presence of chemical agents, varicocele, ionizing radiation and injection of liquid paraffin were among the shared risk factors for the studied individuals. Different professions, such as builders, drivers, intellectual workers, agricultural and auto workers, sportsmen, military have also potential professional risk for azoospermia.

The data received in this study showed important dependences and relations between biological (including genetic) and environmental (including social) factors which complexly are in connection with azoospermia and men infertility in general. In order to examine this problem in details, future investigations including a complex approach (combining methods of molecular genetics, psychogenetics and biostatistics) should be conducted.

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References

Boivin J, Bunting L, Collins JA, Nygren KG. 2007. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod., 22:1506-1512.

Ferreira ML, Machado G, Latimer J, Maher C et al. 2010. Factors defining care-seeking in low back pain – a meta-analysis of population based surveys. European Journal of Pain: Ejp, 14 (7): 747.e 1-7.

Gudeloglu A, Parekattil SJ. 2013. Update in the evaluation of the azoospermic male. Clinics (Sao Paulo), 68 (Suppl 1): 27–34.

Krausz C. 2008. Genetic Aspects of Male Infertility. Eur Urol Rev., 3: 93-96.

Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. 2004. Klinefelter's syndrome. Lancet., 364(9430):273–83.

Lewin A, Gonen O, Orvieto R, Schenker JG. 1991. Effect of smoking on concentration, motility and zona-free hamster test on human sperm. Arch. Androl., 27: 51–54.

Matzuk MM, Lamb DJ. 2008. The biology of infertility: research advances and clinical challenges. Nature Medicine, 14: 1197 - 1213

Oates RD. 2008. The genetic basis of male reproductive failure. Urol Clin North Am., 35(2):257, ix–70.

Sharpe RM, Irvine DS. 2004. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? BMJ., 328 (7437):447-51.

Sofikitis N, Miyagawa I, Dimitriadis D, Zavos P, Sikka S, Hellstrom W. 1995. Effects of smoking on testicular function, semen quality and sperm fertilizing capacity. J. Urol.,154: 1030–1034.

Suganthi KS, Parthasarathy M, Rajan KS. 2013. Liquid-layering induced, temperature-dependent thermal conductivity

http://www.jbb.uni-plovdiv.bg
DOI: 10.69085/jbb20162139

enhancement in ZnO–propylene glycol nanofluids. Chem Phys Lett., 561–562:120–124. doi:10.1016/j.cplett.2013. 01.044

Trummer H, Habermann H, Haas J, Pummer K. 2002. The impact of cigarette smoking on human semen parameters and hormones. Hum. Reprod., 17 (6): 1554-1559. doi: 10.1093/humrep/17.6.1554.

Visootsak J, Graham JM Jr. 2006. Klinefelter syndrome and other sex chromosomal aneuploidies. Orphanet J Rare Dis.,1:42. doi: 10.1186/1750-1172-1-42.

World Health Organization. 1997. Towards more objectivity in diagnosis and management of male fertility. Int J Androl., 7: 1-53.

Zinaman MJ, Brown CC, Selevan SG, Clegg ED. 2000. Semen quality and human fertility: a prospective study with healthy couples. J. Androl., 21: 145–153.

http://www.jbb.uni-plovdiv.bg
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