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Prevalence of Abnormal Semen Parameters among Male Patients Attending the Fertility Center in Khartoum, Sudan

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Abstract:

Background: More than 70 million couples worldwide are affected by infertility. With a male factor infertility account for about half of the cases. Semen analysis is critical for determining male fertility potential.

Objective: To estimate the prevalence of abnormal semen parameters among Sudanese Patients Fertility Center in Khartoum city, Sudan. **Materials and methods:** This is a retrospective cross-sectional study conducted on 2585 males who were referred to the fertility Center in Khartoum, in 2018, to evaluate the semen quality (sperm count, motility, and morphology) according to World Health Organization (WHO) criteria. The descriptive statistics and frequency studies were conducted using the SPSS version (23).

Results: There is a higher prevalence of abnormal semen parameters 86.8 % (sperm count 14.9% - sperm motility 0.7% - morphology 40.7% and 30.5% is a combination of two or more abnormal sperm counts). Sperm motility and morphology and only 13.2% showed normal semen parameters according to WHO criteria.

Conclusion: the finding of this study revealed there is low semen quality. It shows a high prevalence of low sperm count, abnormal motility, and morphology according to the WHO criteria.

Keywords: Prevalence, Semen, Quality, Sudan

Introduction

The WHO defined infertility as the inability to conceive a child after 1 year of regular, unprotected intercourse. Approximately 50% of cases of infertility can be attributed to male factors (1). The defects causing male infertility can be classified as related to the hypothalamus or pituitary gland, the testicles, or defective sperm delivery due to disorders in the penis or related sexual glands (2). Azoospermia and abnormal semen parameters, such as a decrease

in sperm count, are often observed in infertile men. Azoospermia can be classified as obstructive azoospermia (OA) or non-obstructive azoospermia (NOA) (3). The causes of OA can be further divided into congenital and acquired closure of the ejaculatory tract, which can occur anywhere along the spermatic cord and epididymis (3). The most common cause of OA is vasectomy (3). However, it may also be caused by the epididymis, vas deferens, or ejaculatory duct pathology relating to genitourinary

infection, and iatrogenic injury during scrotal or inguinal surgical procedures, and congenital anomalies such as congenital bilateral agenesis of the vas deferens (CBAVD) (4). Historic data showed that the bulk of young men in the 1940s had sperm counts far above 40 million per ml with averages higher than 100 million per ml. A semen sample should ideally contain more than 40 million sperm per ml to be considered normal (5). World Health Organization guidelines suggest that the cut-off value for a normal semen sample should be 20 million sperm per ejaculate, with 50% motility and 60% normal morphology. These indicate that if the concentration is less than 20 million sperm per milliliter of ejaculate, fertility may be impaired; Notwithstanding, if the sperm show adequate forward motility concentrations as low as 5 to 10 million can produce a pregnancy (6). Based on data available in the literature on sperm count, only a small proportion of males will have sperm values that satisfy these ideal figures in today's Western industrialized countries. Not only are sperm counts decreasing, but the proportion of sperm with abnormal morphology and reduced motility is also increasing. For example, the proportion of sperm with abnormal morphology increased (from 26% to 45%) and sperm motility decreased (7, 8). In Oslo, Norway, the proportion of abnormal sperm rose from 40% to 59% between 1966 and 1986 (9). A Belgian study also found that the proportion of sperm with normal morphology decreased from 39.2% in the period 1977-1980 to 26.6% in 1990-1995 and their mean percentage motility decreased from 52.7 to 31.7% (10). Some studies have suggested that the semen quality of sperm of young men in Northern Europe is declining (11). Other reports have confirmed the presence of extraordinarily poor semen quality among otherwise healthy young men in the general population (12). Carlsen and colleagues first raised the possibility of a substantial fall in male fertility levels in 1992. They reported that sperm concentration in healthy men appeared to have dropped from 113 million/ml in 1940 to 66 million/ml in 1990 (13). In a more

extensive re-analysis of the Carlsen data, Swan SH confirmed a significant mean sperm count decline of 1.5% per year in the USA between 1938 and 1988, and of 3.1% per year in Europe between 1971 and 1990 (14). According to National Center for Health Statistics, the absolute number of impaired fecundities increased by about 2.7 million, from 4.56 million in 1982 to 7.26 million in 2002, then fell slightly to 6.71 million in 2006–2010 (15). So, this study aimed to estimate the prevalence of abnormal semen fluid during the year 2018 in Khartoum state.

Materials and Methods

This was a retrospective cross-sectional study conducted in Dr. Elsir Abo-Elhassan Fertility Center in Khartoum state. This study included 2585 men attending the center for evaluation during the year 2018.

Sample collection and processing

Before ejaculate collection, the specimen container was kept at ambient temperature, between 20c° and 37c° to avoid large changes in temperature that may affect the spermatozoa. An individual under evaluation was given clear written and spoken instructions concerning the collection of the semen sample. We confirmed that the ejaculate was completely collected, and the man did not report any loss of any fraction of the sample it was collected after a minimum of 2 days and a maximum of 7 days of ejaculate abstinence.

Ethical consideration:

The study was approved by the scientific committee of the Faculty of Medical Laboratory Science, Al-Neelain University, this is according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Methods

Semen analyses were performed according to the world health organization recommendation (WHO, sixth edition) (16). The semen samples were assessed for sperm motility, sperm count, and morphology. Before the analysis, the semen samples were kept in an incubator at a temperature of 37c°.

analysis was started as soon as the samples had liquefied beginning with a simple inspection soon after liquefaction, preferably at 30 minutes, but no longer than 1 hour after ejaculation, to prevent dehydration or changes in temperature from affecting semen quality.

Sperm motility

10 μ l of well-mixed semen was placed on a clean glass slide and covered with a coverslip. The preparation was immediately examined at a final magnification of $\times 400$. The microscope field was classified as per the world health organization guidelines (16) as active (progressively motile), slowly progressive, sluggish (non-progressively motile), and non-motile (immotile). Calculate the proportions (%) of the four categories of motility. The sum of the four grades should be 100.

Sperm count

Directly after the ejaculate has been properly mixed. place a 10 μ l well-mixed aliquot onto a clean microscope slide that preferably is prewarmed to 37 $^{\circ}$ in an incubator. take care to avoid the formation and trapping of air bubbles between the coverslip and the slide. place a 22 mm \times 22 mm coverslip by dropping it horizontally over the drop. the weight of the coverslip spreads the sample. assess the freshly made wet preparation as soon as. the dilution of the ejaculate required to allow sperm concentration to be measured accurately is estimated from the number of spermatozoa observed in an entire high magnification microscopic field (wet preparation). The fixative for diluting ejaculate aliquots is an aqueous solution containing 0.595 M sodium bicarbonate and approximately 0.14 M formalin .an exact volume of 10 μ l of liquefied semen was withdrawn from the well-mixed semen sample with a positive displacement pipette and added to the diluent in an Eppendorf (test) tube with a tight lid. the tube containing the diluted sample was mixed for at least 10 seconds (on a vortex mixer) immediately before filling the Neubauer counting chamber. one chamber consists of the 25 large squares (five rows of five large squares each). the large squares are also

known as the counting chambers. each large square is surrounded by triple lines and contains 16 small squares (four rows of four small squares each). each counting chambers measures 1mm \times 1mm and has a depth of 0.1 mm. the volume of one counting chamber is 0.1 μ l. counting was performed with a 20 \times objective (phase contrast) all spermatozoa within the central counting chamber were counted as well as those with their heads between the two inner lines but not those with heads that lie between the outer two lines of the large square being assessed. Spermatozoa with most of their head lying on the central line were counted only if that line is the bottom or left-hand line of the large square but not if it is the top or right hand of the square (16).

Sperm morphology

Preparation of two or more ejaculate smears should be made from the fresh semen sample in case there are problems with staining, or one slide is broken. Mix the semen sample well, remove an aliquot immediately, allowing no time for the spermatozoa to settle out of suspension, remix the semen sample before removing replicate aliquots (the second smear is for use as a reserve should there be staining issues). Clean both surfaces of the frosted slides either by rubbing vigorously with lint-free tissue paper or by wiping them off with ethanol. Label the frosted portion with identifying information and apply a 5-10 μ l aliquot of semen depending on sperm concentration to the end of the slide. Use a second slide to pull the drop of semen along the surface of the slide. Allow the slide to dry in the air and then fixed using ethanol and stained by the use of Papanicolaou staining, which gives the best overall visibility of all regions of the human spermatozoon. With this staining method in bright field optics, the head is stained pale blue in the chromosomal region and dark blue in the post chromosomal region. The midpiece is stained red and the tail is stained blue or reddish. Excess residual cytoplasm usually located behind the head and around the midpiece is usually stained green. If colored reddish it can indicate other abnormalities.

Slides can be viewed unmounted or mounted (without or with a coverslip attached). Mounting the slide permits long-term storage. Evaluate at least 200 spermatozoa, to achieve an acceptably low sampling error. Tally the number of normal spermatozoa and abnormalities in the four regions with the aid of a laboratory counter. Calculate the proportion of typical forms and the proportions of abnormalities in the different regions and report the percentages of typical forms to the nearest whole number.

Results

This study included 2585 males who attended the fertility center for the investigation of infertility causes. Semen analysis was performed on them according to WHO guidelines (16). The age ranged from 19 to 68 years with a mean of 40 years. Table 1 shows, the descriptive analysis of the main parameters, the range of sperm count was 0-200 (10^6) with a mean \pm SD of (50.3 ± 34.7). The means

percentage of abnormal sperm morphology was 97.0 ± 7.8 . Table 2 shows, the prevalence of abnormal semen analysis, about 40.7 % ($n=1051$) of the included patients were with Teratozoospermia, while 7.7 % ($n=200$) were with Azoospermia. The activity of sperms was classified into rapid progression, sluggish, and immotile. Of the included patients, the mean count of the immotile sperms was ($56.3 \pm 12.9 \times 10^6$), and ($8.9 \pm 6.3 \times 10^6$) was sluggish movement, while the count of rapid progression was ($34.4 \pm 13.4 \times 10^6$), as shown in table 3. Table 4 shows the frequency of abnormality in morphology according to age group, with the highest frequency at age of 40-49 years with an abnormality of 97.2 %, the frequency was 863 patients. The sperm count according to the age group is shown in table 5, the lowest sperm count was observed in the age group of 19-29 years with a mean \pm SD count of 45.6 ± 34.6 ($\times 10^6$).

Table1: Descriptive analysis of main semen parameters

Parameter	Range (mean \pm SD)
Age (Years)	19-68 (40 ± 28)
Duration of infertility (Years)	1-7 (5.2 ± 1.5)
Sperm count ($\times 10^6$)	0-200 (50.3 ± 34.7)
Volume (ml)	1.5-4.5 (3.2 ± 1.2)
Abnormal morphology (%)	0-100 (97.0 ± 7.8)

Table (2): Frequency of abnormal semen analysis

Comments	Frequency	Percent
Normozoospermia	342	13.2
Asthenozoospermia	13	0.5
Asthenoteratozoospermia	356	13.8
Azoospermia	200	7.7
Necrozoospermia	5	0.2
Necroteratozoospermia	7	0.31
Oligo asthenoteratozoospermia	134	5.2
Oligo asthenozoospermia	183	7.1
Oligo necroteratozoospermia	1	0.04
Oligo necrozoospermia	1	0.04
Oligo teratozoospermia	103	4.0
Oligozoospermia	187	7.2
Teratozoospermia	1053	40.7

Table (3): Descriptive statistics of the mean and standard deviation of sperm activity

Parameter	Count x 10 ⁶ /ml±SD
Rapid progression	34.4 ± 13.4
Sluggish	8.9 ± 6.3
Immotile	56.3 ± 12.9

Table (4): Frequency of abnormal morphology according to age groups

Age group	Frequency	% Of abnormal morphology
19-29	161	97.1
30-39	785	96.7
40-49	863	97.2
50-59	210	97.1
60-69	27	97.5

Table 5: Mean of sperm count according to age groups

Age group	Sperm count X10 ⁶ /ml±SD	Frequency
19-29	45.6±34.6	179
30-39	49.2±33.8	850
40-49	52.2±34.9	942
50-59	50.7±36.7	222
60-69	49.7±39.3	30

Discussion

To provide an insight into the prevalence of abnormal semen parameters in Sudan, this study was conducted. Moreover, there are very few studies in this area as far as infertility is now becoming a health problem. In the present study the sperm count, sperm motility, and morphology percent were tested in 2585 men the age range was 19-68 years with a mean of 40 years. The duration of infertility is from 1 to 7 years. The results showed that 342 men had normal semen quality with 13.2% and 2243 men had abnormal semen analysis with 86.8%. The results indicated that amongst men with abnormal semen analysis results, 14.9% showed low sperm count. The lowest sperm count was observed in the age group of 19-29 years with a mean±SD count of ((45.6±34.6) X 10⁶). In comparison with the previous study, Jajoo S et al (17), reported that 25% of their patients were with low sperm count. Abdalla et al (18) did a study on Sudanese patients and reported a percent of 44% of the included patients were with low sperm count. About 0.7% with abnormal sperm motility and the mean count of the immotile sperm was ((56.3 ± 12.9) X 10⁶) and 40.7% with abnormal sperm morphology. The highest frequency at age of 40-49 years with an abnormality of 97.2 %, the frequency was 863 patients, the remaining abnormal semen analysis result percent 30.5% is a combination of two or more abnormal

sperm count, sperm motility, and morphology. The present study agrees with the study done by the National Center for Health Statistics, in that there is a higher prevalence of abnormal semen parameters (15). The findings of the previous study revealed that the absolute numbers of impaired fecundity increased by about 2.7 million, from 4.56 million in 1982 to 7.26 million in 2002, then fell slightly to 6.71 million in 2006–2010 (15). The results of the current study showed a lower percentage of oligozoospermic cases (7.2 %) and asthenozoospermic cases (0.5 %) compared to other studies like Samal et al (19), Kalavathi et al (20) and Renuka et al (21). They reported oligozoospermia of about 29.13%, 24.8%, and 32.1%, respectively. Asthenozoospermia was 1.45% in the study of Samal et al (22), 1.2% in a study of Kalavathi et al (20), and 23.2% in Renuka et al study (21). In the current study, abnormal semen parameters were studied with age, it found that most of the included individuals with abnormal sperm morphology were in the range age of 60 to 69. This is comparable to Jajoo S et al (17) and Renuka et al (21) studies. Historic data showed that the bulk of young men in 1940 had sperm counts far above 40 million per ml. A semen sample should ideally contain more than 40 million sperm per ml to be considered normal (23). In 1999 world health organization (WHO) guidelines suggest that the cut-off value for a normal

semen sample should be 20 million sperm per ejaculate with 50% motility and 60% normal morphology (24). Limitations of the study: Environmental factors such as heat, chemicals, and lifestyle including diet, frequency of intercourse, smoking, and alcohol are known to have adverse effects on sperm parameters. Other causes of semen abnormalities are stress (emotional and physical), insomnia, tight brief, and hot tubs were not included in the study.

Conclusion

The result of this study revealed that only a small proportion of males will have sperm values that satisfy these ideal figures in today's western industrialized countries and confirmed the presence of extraordinarily poor semen quality and raised the possibility of a substantial fall in male fertility levels.

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