ORIGINAL ARTICLE

ASSESSMENT OF MALE REPRODUCTIVE HEALTH BY CONVENTIONAL METHOD OF SEMEN ANALYSIS

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Background: Data available over the past twenty years reveal that in approximately 30% of cases of infertility, pathology is found in man alone, and in another 20% both man and woman are abnormal. Therefore, the male factor is at least partly responsible in about 50% of infertile couples. The longer a couple remains sub fertile, the worse is their chance for an effective cure. This study was planned to analyse the complete semen picture of infertile men for assigning the specific cause to male infertility related to concentration, motility and morphology and to know the distribution and pattern of male infertility in the various subclasses in Pakistani population. **Methods:** It was a prospective descriptive analytical study conducted at Department of Reproductive Physiology/ Health, Public Health Divisions, National Institute of Health (NIH), Islamabad. One thousand five hundred twenty-one (1,521) infertile male patients, and 97 proven fathers, taken as a control. Conventional semen analysis was performed on all samples. **Results:** Out of 1,521 infertile men, 13.3% were azoospermic, 23.2% oligozoospermic, 0.9% polyzoospermic, 14.5% normozoospermic, 35.2% asthenozoospermic 10.5% oligoasthenozoospermic and 2.4% teratozoospermic. Sperm concentration and active motility of proven fathers, was significantly higher ($p<0.05$) than the normal concentration group. Least liquefaction time was recorded in case of polyzoospermic subjects, and highest for azoospermic cases. Although, the liquefaction time of azoospermic and oligozoospermic subjects varied non-significantly ($p>0.05$) with the proven fathers. Normal forms were significantly higher ($p<0.05$) among the proven fathers and polyzoospermic cases, in comparison with the other groups. Head defects were more in teratozoospermic group, followed by oligoasthenozoospermic and oligozoospermic patients. Neck defects were more profound in oligoasthenozoospermic and oligozoospermic patients, while, tail defect showed significant increase in teratozoospermic and asthenozoospermic cases only. Head and neck defect varied significantly ($p<0.05$) with proven fathers in all groups, while tail defect varied significantly ($p<0.05$) in oligozoospermic, asthenozoospermic and teratozoospermic groups only when compared with proven fathers. **Conclusions:** Complete semen analysis which provides important information about the quality and quantity of the sperm, should be performed before reaching a final conclusion.

Keywords: Semen analysis, male infertility, Pakistani population

INTRODUCTION

Male infertility is a serious problem all over the world as well as in Pakistan. Currently, Pakistan is among the most populous country of the world and has a population growth rate of around 2%, yet it also has high rate of infertility (21.9%); 3.5% primary and 18.4% secondary.[1] The problem is usually treated as a single parameter of sperm defects mostly concentration. The problem does not show a defined clinical syndrome but rather a collection of different conditions exhibiting a variety of etiologies and varying prognosis.[2]

Though the problem is mainly due to defect in sperm, however, other etiological factors responsible for male infertility are absence of testicular tissues, bilateral castration, impaired sperm production and function, AZF gene deletion (y-deletion), hypogonadotropic hypogonadism (cryptorchidism), testicular cancer and varicocele, age >55 years, genitourinary infection, environmental agents like extremes of temperature, irradiation, occupational exposure, drugs, alcohol, and tobacco abuse, nutritional deficiency like trace elements e.g., selenium, Zinc and vitamins, impaired sperm transport as seen in autoimmune infertility, epididymal blockage of vasdeferens, ejaculatory failure, impotence, previous vasectomy and disturbance in sperm oocyte fusion e.g., abnormal egg binding proteins[3] could be the other causes of male infertility, hence it is difficult to declare a person fertile with absolute certainty.

The conventional method of semen analysis examined for all seminal parameters is the best method for determining male infertility because it reports both on quantity and quality of sperm and complete spectrum of sperm defect is displayed and the causative factors for infertility is determined with certainty. The sperm count, motility, morphology and combination of morphology with count and motility needs to be studied because such studies are lacking in Pakistan. Keeping this in mind, the present study was designed to signify the importance of complete semen analysis for knowing the basic cause of infertile in males.

MATERIAL AND METHODS

The present study was carried out in the Department of Reproductive Physiology/Health, Public Health Laboratories Division, NIH, Islamabad, after being reviewed and approved by the intuitional ethical and research committee.

Two thousands (2000) infertile males, who visited Reproductive Physiology/Health, NIH, Islamabad over a period of 5 years (2002–6), were registered for the study along with 97 proven fathers as control, while the rest were unable to fulfil the criteria.

Only primary and secondary infertile males, who did not use any treatment for infertility and had no other relatable cause of male infertility, were included in the study. The subjects, who had undergone pelvic surgery or hernia repair, patients with diabetes mellitus, thyroid disease and subjects who were on drug, e.g., antipsychotic, antihypertensive, neuroleptic, alcohol, nicotine were excluded from the study. Out of 2000 registered patients, 1,521 patients fulfil the criteria and were selected for the study.

The collection and examination of Semen of patients was carried out according to the standardised method of the WHO[4], after taking consent and complete medical history.

The subjects were classified on the basis of concentration, motility and morphology as azoospermic (Az), oligozoospermic (Ol), polyzoospermic (P) and normozoospermic (N), asthenozoospermic (As) and teratozoospermic (T). Men, who had successfully impregnated their wives without any assisted method during the last six months, were placed in the proven fathers' group (F). The data were analysed using SPSS-10.

RESULTS

Out of the 1521 patients, 203 (13.3%) were azoospermic, 353 (23.3%) oligozoospermic, 535 (35.2%) asthenozoospermic, 221 (14.5%) normozoospermic, 13 (0.9%) polyzoospermic, 159 (10.5%) oligoasthenozoospermic and 37 (2.4%) teratozoospermic. (n=97) proven fathers were taken as a control. Age, last emission, volume of ejaculate and *p*H did not appear to be major factors in the differentiation between groups, although significant ($p<0.05$) variation was recorded in some cases. Sperm concentration of proven fathers, was significantly higher ($p<0.05$) than the normal concentration group, as was active motility (Table-2). Least liquefaction time was recorded in case of polyzoospermic subjects, and highest for azoospermic cases. Although, the liquefaction time of azoospermic and oligozoospermic subjects varied non-significantly ($p>0.05$) with the proven fathers. Normal forms were significantly higher ($p<0.05$) among the proven fathers and polyzoospermic cases, in comparison with the other groups.

Results of our study also showed that more head defects were seen in teratozoospermic group, followed by oligoasthenozoospermic and oligozoospermic patients. Neck defects were more profound in oligoasthenozoospermic and oligozoospermic patients, while, tail defect showed significant increase in teratozoospermic and asthenozoospermic cases only. Head and neck defect varied significantly ($p<0.05$) with proven fathers in all groups, while tail defect varied significantly ($p<0.05$) in oligozoospermic, asthenozoospermic and teratozoospermic groups only when compared with proven fathers.

Table-1: Distribution of patients

Groups	N	%age
Az (Azoospermic)	203	13.3%
Ol (Oligozoospermic)	353	23.2%
P (Polyzoospermic)	13	0.9%
N (Normozoospermic)	221	14.5%
As (Asthenozoospermic)	535	35.2%
Oas (Oligoasthenozoospermic)	159	10.5%
T (Teratozoospermic)	37	2.4%
Total	**1521**	**100**
F (Proven Fathers)	**97**	

Classification on the basis of concentration, motility and morphology

Table-2: Seminal parameters in various groups of male infertility

Parameters	GROUPS							
	Az	Ol	As	Oas	T	N	P	F
Age (years)	31.72±0.44a	35.40 ± 0.42b	34.62±0.26bc	34.20±0.34cd	35.57±0.82bcde	33.03±0.39f	32.15±0.85afg	33.23±1.03abcdefg
Liquefaction Time	23.71±1.11a	21.43±0.93ab	18.62±0.64c	22.16±1.79abcd	19.32±1.01bcde	20.31±0.99bcdef	15.00±0.57g	21.46±0.80abdef
Last Emission (days)	9.42±0.49a	6.77±0.11b	8.09±0.14c	6.28±0.11d	5.73±0.33de	7.37±0.22f	9.62±1.48abcfg	7.14± 0.17bfg
Volume (mL)	2.54±0.11a	2.97±0.08b	3.67±0.09c	2.95±0.15bd	2.58±0.14ade	3.67±0.12cf	2.81±0.52abcdefg	3.44±0.76abcdefg
pH	8.07±0.04a	8.13±0.02ab	8.16±0.02bc	8.11±0.04abcd	8.16±0.04abcde	8.54±0.35abcdef	8.35±0.15abcdefg	8.81±5.85abcdefg
Conc. (million/mL)	0.00±0.00a	6.99±0.35b	50.11±2.12c	4.45± 0.42d	5.64±1.15bde	87.49±3.51f	402.23±39.70g	102.12±1.34h
Active motility (%)	0.00±0.00a	29.0±0.96b	17.77±0.49c	14.50±0.86d	17.73±3.22cde	46.61±1.27f	55.92±4.19g	60.01±0.58g
Sluggish motility (%)	0.00±0.00a	15.25±0.51b	11.67±0.29c	2.14±0.56cd	10.73±1.27cde	11.29±0.41cdef	8.85±1.62cdefg	10.94±1.38cdefg
Immotile (%)	0.00±0.00a	55.59±1.07b	70.57±0.61c	73.36±1.18d	71.54±4.08cde	42.10±1.28f	35.23±3.07g	28.85±0.28h
Abnormal Forms (%)	0.00±0.00a	38.54±.22b	36.46±1.00bc	47.79±1.76d	89.84±1.29e	20.92±1.04f	14.46±2.58g	17.46±0.00h
Head Defects (%)	0.00±0.00a	26.42±0.97b	23.40±0.86c	31.69±1.47d	75.43±2.99e	12.93±0.68f	6.31±0.88g	11.32±0.87f
Neck Defects (%)	0.00±0.00a	6.64±0.41b	4.86±0.29c	10.82±0.73d	4.32± 0.89ce	2.90±2.27ef	0.77±0.30g	1.92± 0.23h
Tail Defects (%)	0.00±0.00a	6.60±0.35b	8.89±0.49c	8.49±0.62cd	10.08±1.39cde	5.28±0.53f	7.38±2.51bcdefg	4.24±0.62fg

Means sharing a common letter do not differ significantly, others differ significantly ($p<0.05$)

Az: Azoospermic, Ol: Oligozoospermic, As: Asthenozoospermic, Oas: Oligoasthenozoospermic, T: Teratozoospermic, N: Normozoospermic, P: Polyzoospermic, F: Proven fathers

DISCUSSION

In the present study, data of 1521 male partners of infertile couple were analyzed. The data indicates that 36.5% of subjects with low sperm density were either azoospermic (13.3%) or oligozoospermic (23.2%), whereas in (35.2%) of men, the sperm motility was low, although the sperm concentration was within the normal range (20 million/ml). There were a large number of individuals (14.5%) having normal activity and normal concentration of sperms. The data also indicates the increase of liquefaction time in azoospermic, oligozoospermic and oligoasthenozoospermic groups when compared with proven fathers. Impaired fertility had been reported in the viscous specimen, as the motile sperm can not reach at the site of fertilization[5], the same is true to our study also.

Globally, the male is considered to be a factor in nearly one third couples affected by infertility.[6] It is only in the past few decades that male factor has been recognized as a significant cause of infertility. An important point is that male infertility is not an entity but reflects a variety of different pathogenetic mechanisms.[7] A study on the South African population[8] has indicated the male to be responsible for 70% cases of infertility. The present study has also identified male factor infertility in 74% cases on the basis of concentration, motility and morphology.

In Pakistan, the reported incidence of azoospermia was 12.32%[9] and in another study, the incidence rate was 16 %[10], while in the present study it is 13.3%, which is similar to the earlier studies. Considering the percentage of azoospermia in Pakistan, it is not high as compared to USA and Kenya, which was reported to be 10%[11] and 11.35% respectively[12]. However, when the incidence rate of azoospermia was compared with Turkey and Zimbabwe, it was found to be on lower side.[13]

Comparing the incidence rate of oligozoospermia in our study, it was found to be 23.2%, while in a study conducted earlier; it was found to be 53%, which is in contrast to the present study.[10] This high percentage in the previous studies might be due to a small number of analyzed samples. It has been pointed out that infertility of oligozoospermic men occurs not only, because of the low sperm numbers but also because of the high frequency of defective sperms ZP interaction. More importantly sperms from most of the oligozoospermic men have either low sperm ZP binding or low ZP induced AR, both of which are likely to cause failure of sperms ZP penetration and fertilization.[14]

Regarding the specific causes of affliction, deficiency in the sperm concentration is considered to be the most significant factor. This is supported by the observation that 13.35% (Table-1), of the males examined were azoospermic, while another 23.23% had their concentration less than the WHO's cut-off point. An earlier study[9] compared incidence of azoospermia in men from different countries and reported its incidence among Pakistani males to be 12.32%, which was comparable to that of Nigerian males (12%) and Kenyan males (11.35%). On the other hand its incidence in males from USA and Scotland is lower (10% and 7% respectively). In contrast, the prevalence of azoospermia has been found to be substantially higher in males from Zimbabwe (24.3%) and Turkey (21.5%). Studies on a Mexican population sample showed that its incidence ranges between 4%[15] and 8%[16]. These two studies have also revealed that the incidence of oligozoospermia ranges between 7% and 24%. Bornman *et al*[8], have reported that 9% of the South African males studied had azoospermia.

It has been reported that motility and concentration are better predictor of fertility potentials than sperm morphology assessed both by WHO guidelines as well as by strict criteria, which is an agreement to our study as well, as the mean and standard deviation of motility is higher in the fertile population and significantly lower in the infertile population. Several studies have demonstrated the correlation of motility with the fertilization rate in vivo and in vitro. Krause [17] also found sperm concentration and percentage of motile spermatozoa, a predictor of fertility outcome in vivo. Normal motility is indicative of normal development of spermatozoal axoneme during spermatogenesis in the testis, a normal maturation process in the epididymis, and normal seminal plasma constituents[18-19]. Sperm motility is a critical indicator of semen quality and fertility potential, because it is required for penetration of cervical mucus, transport through the female genital tract, and penetration through the corona radiate and zona pellucida before oocyte fertilization. Isolated asthenozoospermia has been reported in as many as 24% of patients undergoing infertility evaluation and contributed to another 55% patients with other sperm defects such as oligozoospermia and teratozoospermia[19]. In our study population, isolated asthenozoospermia was observed alone in 35.2% while oligoasthenozoospermia in 10.5%.

The incidence of polyzoospermia in Pakistan, as indicated in this study is only 0.85%, while in Mexico is 13%[15], in Germany 1.75%[20], in South Africa 5%[8], 4.2% in the United States [21] and 13.84% in the former East Germany[22]. Two of the thirteen polyzoospermic patients were hypospermic (volume less than 2 ml), but had all other parameters (except count) within the normal range recommended by WHO. The patient who had his semen volume exactly 2.0 ml had an active motility of 35%, but his total motility was 50%. The highest sperm count reported in this study was 660 million/ml, but an earlier study carried out in the United

States[23] has reported counts even up to 1.75 billion/ml. Another group in Pakistan[24], working on *in vitro* fertilization methods, did not come across patients with more than 600 million sperms/ml.

In polyzoospermic men, capacitation slows down, both the induced and spontaneous acrosomal reactions are significantly lowered and even capacitated sperms fail to undergo induced acrosomal reaction.[25] It has also been reported that several degrees of acrosomal membrane alterations are observed in semen of polyzoospermic patients.[26] Functionally defective acrosomes hinder fertilization[27–28], as they are incapable of penetrating the outer investments of the oocyte, which is one of the reasons for reduced fertility[29]. Polyzoospermic men exhibit a considerably decreased sperm acrosin activity[30].

Globozoopsermia is a human infertility syndrome caused by spermatogenesis defects. In patients with teratozoospermia and familial infertility, all the spermatozoa were globozoospermic with no acrosome enzyme activity.[31–32] Literature review showed that familial infertility in brothers with teratozoospermia had acrosomesless round-headed sperm and aberrant mitochondrial sheath.[33]

A familial case of asthenotertozoospermia showed a high percentage of irregular large heads and up to four tails.[34] A patient with disturbed meiosis had an absence of the acrosome in 65% of spermatozoa, large head shape in 32% and two to four tails in 61% of spermatozoa [35], whereas, our study showed total of 37 (4.68%) patients with teratozoospermia. Out of these patients the overall sperms abnormality was 89.8%, in which the head, neck and tail defects were 75.4%, 4.3% and 10.10% respectively. Our results differed from Pieters *et al*[35], who had observed 61% of tail abnormalities, whereas, we found 10% overall tail abnormalities in teratozoospermic cases.

Our results are supported by the several studies in the literature, who had reported that percentage of normal morphology is an essential characteristic for in vivo fecundity and *in vitro* fertilization.[36] There is a continuing debate over the role of normal morphology in male Infertility and its value in the evaluation and management of infertile men[37] Most of these studies indicate that morphology is the best predictor among all of the sperm characteristics. WHO recommends that each laboratory recruit fertile men, in order to investigate and determine the real cut off values for normality in that laboratory, as these are difficult to recruit, therefore only a few laboratories are actually performing this specialised analysis. Sperm defects are the major contributors to complete failure of fertilization in IVF. Most common sperm defects are in oligozoospermia, asthenozoospermia and teratozoospermia, the same has been supported by our findings as well. Although, many new sperm function tests have been developed, but for routine semen analysis, sperm morphology is one of the most useful values for the prediction of sperm function. Since infertility is considered a deficiency, only low sperm counts have been considered to constitute a pathological condition. In a study on prevalence of infertility among Pakistani patients, Khan *et al*[9] have dealt with only azoospermia, oligozoospermia, and normal populations with reference to sperm concentration. No attempt was made in this study to assess polyzoospermia and teratozoospermia. With an increased sperm population per millilitre of ejaculate, and decreased in sperm morphology, the sperms lose their fertilization potential due to acrosomal damage.[35–36] The acrosomes play a very important role in fertilization, as spermatozoa with defective acrosomes are not capable of penetrating the oocyte.[38] Infertility can result even when acrosome-reacted spermatozoa have defective fusing ability.[39] The extremely rare condition of polyzoospermia necessitates patient-specific action, for treating infertility. Sperm motion characteristics are of extreme importance in regard to prediction of chances of fertility.[40]

CONCLUSION

In our study, 36.5% of infertile males were either azoospermic or oligozoospermic, a fair number of asthenozoospermic infertile males (35.2%) prompt us to look for causes of male infertility. Moreover, only 13 out of 1,521 patients examined in this study were found to have their sperm concentrations in excess of 250 million/ml, this condition needs attention and must be taken into consideration for assessment of the male partner of an infertile couple because it drastically reduces the fertility potential. The incidence and characteristics of polyzoospermia need to be investigated further not only in Pakistan but also in other countries of Asia for which scant information is available currently.

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