Original Article

Human Reproduction

Functional Status of Sperm in Teratozoospermic Infertile Males of Mysore Population, South Karnataka, India: an Oxidative Stress and Hormonal Profile Approach

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ABSTRACT [ENGLISH/ANGLAIS]

This original work evaluates the biochemical parameters in the teratozoospermic and associated teratozoospermic infertile males in Mysore population of South Karnataka, India. This study mainly focused on the evaluation of oxidative stress parameters along with the routine semen and hormonal analysis which helps to understand functional status of sperm, inturn indicating the impact on normal spermatogenesis. Total of 130 subjects were accessed and classified into different infertile condition based on sperm count, motility and morphology. Among them, 13 subjects were teratozoospermic, 8 subjects were oligoteratozoospermic, and 7 subjects were oligoasthenoteratozoospermia. Other infertility conditions were excluded from the study. Semen analysis is done according to WHO protocol. Hormones like follicle stimulating hormone, leutinizing hormone and testosterone levels are measured by using ELISA kit. The levels of antioxidant enzyme like super oxide dismutase and the amount of lipid peroxidation are also observed. Our study shows significant values for different semen parameters. Sperm function tests such as acrosome intactness test and hypo osmotic swelling test show decreased mean value of all infertile conditions. The mean value of serum follicle stimulating hormone, leutinizing hormone and testosterone showed variation in teratozoospermic condition whereas in oligoteratozoospermic and oligoasthenoteratozoospermic conditions leutinizing and testosterone levels are upregulated. Teratozoospermic and oligoteratozoospermic conditions showed increased value of lipid peroxidation and decreased superoxide dismutase value. On the contradictory oligoasthenoteratozoospermic condition showed increased value of superoxide dismutase and lipid peroxidation.

Keywords: Teratozoospermia, hormones, antioxidant, sperm function test, infertility

RÉSUMÉ [FRANÇAIS/FRENCH]

Ce travail original évalue les paramètres biochimiques dans le teratozoospermic et associés teratozoospermic mâles stériles dans la population Mysore du Sud Karnataka, en Inde. Cette étude a principalement porté sur l'évaluation des paramètres du stress oxydatif avec le sperme de routine et l'analyse hormonal qui aide à comprendre l'état fonctionnel des spermatozoïdes, à leur tour en indiquant l'impact sur la spermatogenèse normale. Total de 130 sujets ont été consultées et classés en condition stérile différente basée sur la numération des spermatozoïdes, la motilité et la morphologie. Parmi eux, 13 sujets ont été teratozoospermic, 8 sujets ont été oligoteratozoospermic, et 7 sujets ont été oligoasthenoteratozoospermia. Autres conditions d'infertilité ont été exclus de l'étude. L'analyse du sperme est effectué selon le protocole OMS. Les hormones comme l'hormone folliculo-stimulante, hormone lutéinisante et de testostérone sont mesurés en utilisant un kit ELISA. Les niveaux d'enzymes antioxydantes comme la superoxyde dismutase et le montant de la peroxydation lipidique sont également observées. Notre étude montre des valeurs significatives pour les paramètres du sperme différents. Tests de la fonction du sperme tels que le test intégrité acrosomique et hypo montrent essai de gonflement osmotique diminution de la valeur moyenne de toutes les conditions stériles. La valeur moyenne de l'hormone folliculo-stimulante du sérum, l'hormone lutéinisante et de testostérone ont montré une variation dans un état teratozoospermic alors que dans les conditions et oligoteratozoospermic oligoasthenoteratozoospermic lutéinisante et de testostérone sont régulés à la hausse. Conditions Teratozoospermic et oligoteratozoospermic a montré une valeur accrue de la peroxydation lipidique et une diminution de la valeur la superoxyde dismutase. A la condition contradictoires oligoasthenoteratozoospermic ont montré une augmentation de valeur de la superoxyde dismutase et de la peroxydation des lipides

Mots-clés: Tératozoospermie, hormones, antioxydants, tester la fonction des spermatozoïdes, l'infertilité

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INTRODUCTION

Infertility is defined as the inability to conceive after one year of regular unprotected sexual intercourse. It affects approximately 15% of couples of reproductive age. Globally 60–80 million couples suffer from infertility every year and 15–20 million are in India alone [1]. Male related factor is solely responsible in about 50% of cases of infertility [2]. Complete semen analysis is one of the most valuable tests that plays critical role in andrology and in demystifying male infertility. Men with a defect in sperm maturation tend to have problems with sperm morphology and may then be at risk for failure of oocyte fertilization [3]. Sperm morphology is assessed routinely as part of standard laboratory analysis in the diagnosis of male infertility. It has been reported that physical sperm aberrations may occur during either production of sperm or during the storage of sperms in the epididymis [4]. Male fertility depends upon an intact hypothalamopituitary-testicular axis to initiate and maintain quantitative and qualitative normal spermatogenesis, hormone profile of infertile subject has to be studied [5]. Even though oxygen is essential to sustain life, the breakdown products of oxygen such as reactive oxygen species (ROS) can be detrimental to cell function and survival [6]. As human semen is known to contain different types of cells, such as mature and immature spermatozoa, leukocytes, and epithelial cells cell types such as leukocytes and spermatozoa have been shown to be the two main sources of ROS [7]. Spermatozoa are rich in mitochondria because they need a constant supply of energy for their motility. Unfortunately, when spermatozoa contain dysfunctional mitochondria, increased production of ROS occurs, affecting mitochondrial function [8]. The imbalance between ROS and antioxidant levels, may arise either from increased exposure to radicals/ oxidants or may be a result of decreased antioxidant [9]. Hence an attempt was made to evaluate the variation in reproductive hormones and production of superoxide dismutase (SOD) an antioxidant enzyme against ROS and its impact on functional status of the sperms in teratozoospermic and associated teratozoospermic conditions.

MATERIALS AND METHODS

A total of 130 subjects were recruited for the present study from different parts of Mysore, Karnataka, and were classified into different infertile conditions of which thirteen subjects were teratozoospermic, eight were oligoteratozoooospermic (OT), seven were oligoasthenoteratozoospermic (OAT). Exclusion criteria include azoospermia, asthenospermia, oligospermia, oligoasthenospermia and aspermic conditions. Normal healthy donors with proven fertility were used as control population (n=20). Institutional ethical clearance was approved from University ethical clearance committee and respective hospitals. Written informed consent letter was obtained from subjects and control group before include them in the study.

Semen collection and semen analysis

The semen samples were collected from the infertile subjects as well as the control group through masturbation after 3-5 days of ejaculatory abstinence. The samples were collected in a sterile plastic container in a room specially provided for this purpose by following the WHO protocol [1]. The collected semen samples were allowed to liquefy at 37°C for 30 minutes and analyzed within 1 hour of collection. Physical examination such as liquefaction time, color, odor, and pH were recorded after 30 minutes. Basic microscopic examination was carried out to record the count, vitality, density, morphology and motility of the sperm according to WHO protocol [1].

Hormone assay

Hormonal levels were determined using ELISA kits, with microwell plate reader (Biotek ELx 800), FSH and LH were measured using ERBA ELISA kit (Germany) and testosterone levels were measured by using DRG ELISA kit (Germany).

Superoxide dismutase (SOD) activity

Liquefied semen samples were centrifuged at 3000 rpm for 10 min to collect the seminal plasma with out disturbing the pellet. SOD activity was estimated by the protocol of Das and coworkers [10] SOD scavenges superoxide radicals that are produced by photo-reduction of riboflavin. These superoxide radicals are then allowed to react with hydroxylamine hydrochloride to produce nitrite. The nitrite in turn reacts with sulphanilic acid to produce a diazonium compound, which subsequently reacts with napthylamine to produce a red azo compound and the absorbance reading was measured at 543 nm.

Lipid peroxidation

Liquefied semen samples were subjected for centrifugation at 3000 rpm for 10 min to collect the seminal plasma without disturbing the sperm pellet for

measurement of lipid peroxidation (LP). LP was estimated by measuring the levels of MDA [11]. The assay is based on the reaction of Thiobarbituric acid (TBA) with malnoaldehyde (MDA), one of the aldehyde products of lipid peroxidation. The amount of MDA-TBA by-product produced in this reaction was measured at 532 nm.

Sperm Function test

Functional capacity of the sperms was examined by sperm function tests through hypo-osmotic swelling test (HOS) [12], Acrosome intactness test (AIT) [13], and Nuclear chromatin decondensation test (NCD) [13]. Values were recorded and further statistical analyses (Paired t-test) were carried out using Minitab version 15 statistical software.

RESULTS

The control individuals had mean sperm count above 20 million per ml and progressive motility of sperm > 50 according to WHO guidelines [1], shown in the table 1. There was significant ($p = 0.003$) decrease in the sperm count in the OT and OAT ($p=0.002$) and OAT condition showed significant variation ($p=0.001$) in the motility compared to control population. Semen volume was normal in all the conditions but, sperm vitality was found to be decreased in OT (42.5±20) and OAT (43.8±21.5).

Table 2 shows the comparison of sperm function test in different teratozoospermic conditions. Teratozoospermic cases showed decreased mean value (42.5±18.0) for the acrosome intactness test (AIT) and Hypo osmotic swelling test (HOS) (49.6±21.2) but normal range was observed for Nuclear Chromatin Decondensation test (NCD) (65±16.8). In Oligoteratozoospermia response was similar for HOS and AIT but, slight decrease found in the mean value of NCD (56.8±27). But OAT cases showed decreased mean values for all sperm function tests.

The values of total serum testosterone and the gonadotropins (FSH and LH) are depicted in table 3. The mean value of serum follicle stimulating hormone (FSH), leutinizinf hormones (LH) and testosterone didn't show any much variation in teratozoospermic condition but, in OT and OAT conditions LH and testosterone levels were found to be increased (Table 3). Figure 1 shows the level of antioxidant SOD and LP in different conditions. Decrease in the SOD mean value and increased LP mean value was evident in teratozoospermic cases comparing to control group. Similarly OT condition showed decreased SOD and increased LP mean value but in case of OAT conditions, level of SOD and LP were in normal range when compared to the control. Figure 2 shows immature and abnormal sperm morphology in different teratozoospermic infertile cases.

DISCUSSION

Teratozoospermic semen samples are characterized by a higher content of morphologically abnormal and immature spermatozoa. Immature spermatozoa retain cytoplasmic residues in the mid-piece, which contains enzyme G6PD that generates NADPH, which in turn stimulates ROS production [14]. In presence of cytoplasmic residues ROS levels are expected to rise at a faster pace and in greater intensity but, spermatozoa possess primarily enzymatic antioxidants, where SOD being the most predominant suppressor of ROS in seminal plasma [15, 16]. Many studies indicate that abnormal sperm carry a much higher probability of ROS production than motile sperm suspensions even from fertile individuals [18] and study by Moosani et al. [17] showed deficiency of SOD for endogenous reasons, among teratozoospermic males. The present study accords with previous studies on teratozoospermic conditions, wherein decreased SOD enzyme level and increase in lipid peroxidation was evident. Peroxidation damage to cell membrane could be due to hyper activation of ROS, in turn decreases the membrane integrity which was evident through HOS test. Membrane defects in the spermatozoa resulting defective membrane functions in the embryo leading to miscarriages because the normal spermatozoan membrane is the prerequisite for the specialized cell-to-cell communications and cell-to-cell binding. Present study shows the decreases HOS response for all teratozoospermic conditions compared to control group.

Nuclear chromatin of the spermatozoan is in a highly condensed state prior to fertilization. In-vivo decondensation occurs in the ooplasm and is essential for successful fertilization and to the formation of male pronucleus which fuses with the egg nucleus leading to the formation of the zygote [19]. In our study less mean value was recorded among OT and OAT conditions for nuclear chromatin decondensation test with increase in the LP value and decreased SOD activity. As sperm DNA integrity is a keystone of reproductive success, DNA-damage in sperm can form pronuclei at fertilization but may not possible of normal embryo development [20].

DNA damage in our study could be due to increased LP and decreased SOD activity.

As spermatogenesis is initiated and regulated by the quantitative and qualitative production of spermatozoa generally requires prime regulator hormones. FSH acts directly on the seminiferous tubules, whereas luteinizing

TABLE 1

Table 1 shows the Spermeogram of teratozoospermic and associated teratozoospermic conditions with different semen parameters

Conditions	Sperm count	Volume	pH	Vitality	Motility
Teratozoospermia	52 ± 20 (t = -0.54, *p* = 0.600)	2.1 ± 0.9 (*p* > 0.05)	8.2 ± 0.9 (*p* > 0.05)	68.2 ± 16 (t = -0.78, *p* = 0.452)	58.4 ± 17 (t = 0.91, *p* = 0.380)
Oligoteratozoospermia	11.8 ± 5.1 * (t = -4.91, *p* = 0.003)	2.5 ± 0.8 (*p* > 0.05)	8.1 ± 0.2 (*p* > 0.05)	49.1 ± 21 * (t= -3.13 *p* = 0.020)	42.5 ± 20 (t = -0.79, p = 0.461)
Oligoasthenoteratozoospermia	8.5±5.7 * (t= -4.79, p = 0.002)	2.3 ± 0.7 (*p* > 0.05)	8.2 ± 0.2 (*p* > 0.05)	43.8 ± 21.5 * (t = -4.52, p = 0.003)	17.25 ± 11 * (t =-7.76, *p* = .000)
Control	68.4 ± 44.4	2.3 ± 1.1	8.0 ± 0.2	74.5 ± 10.7	59.1 ± 23.7

Data are shown as Mean ±SD. Information regarding significance test are written on brackets

TABLE 2

Table 2 shows the Response of sperm function tests in different teratozoospermic conditions.

Conditions	NCD	HOS	AIT
Teratozoospermia	65±16.8	49.6±21.2	42.5±18.0
Oligoteratozoospermia	56.8±27	42.1±20.3	47±22.1
Oligoasthenoterato-zoospermia	40.3±2.2	45±25.4	41.7±15.4
Control	62.9±18	63±18.2	63.7±15.9

Data are shown as Mean ±SD. NCD=Nuclear decondensation test, HOS= Hypoosmatic swelling test, AIT= Acrosome intactness test

TABLE 3

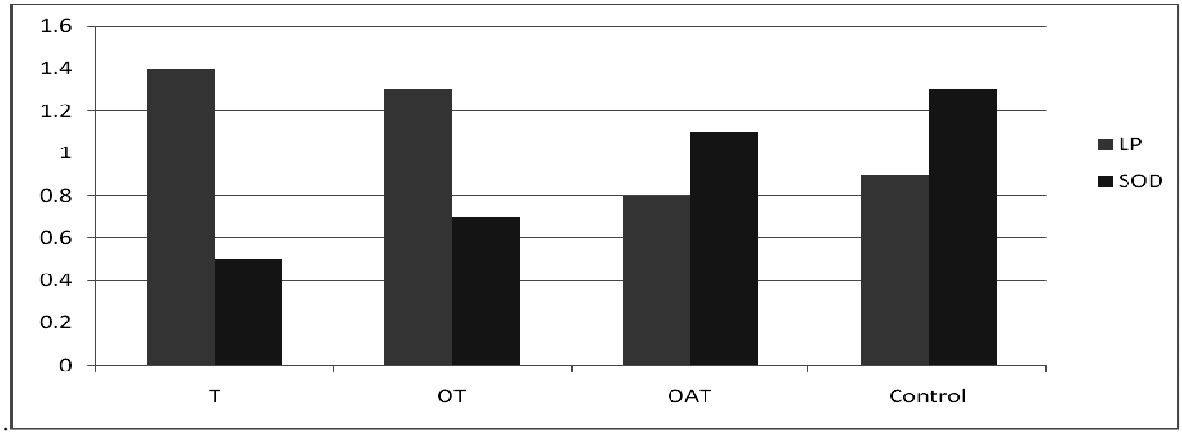
Table 3 shows the hormonal levels with respect to infertility conditions.

Conditions	N=45	FSH	LH	Testos-terone
Teratozoospermia	13	1.7 ±0.6	5.6 ±2.3	6.4 ±2.9
Oligoteratozoo-spermia	7	3.4 ±0.6	7.9 ±1.9	9.5 ±4.2
Oligoastheno-teratozoospermia	8	3.02±0.6	7.3 ±4.6	7.8 ±4.5
Control	17	5.9±2.7	3.4±1.5	4.5±1.2

Data are shown as Mean ±SD. FSH = Follicle stimulating hormone, LH = Leutinizing hormone

FIGURE 1

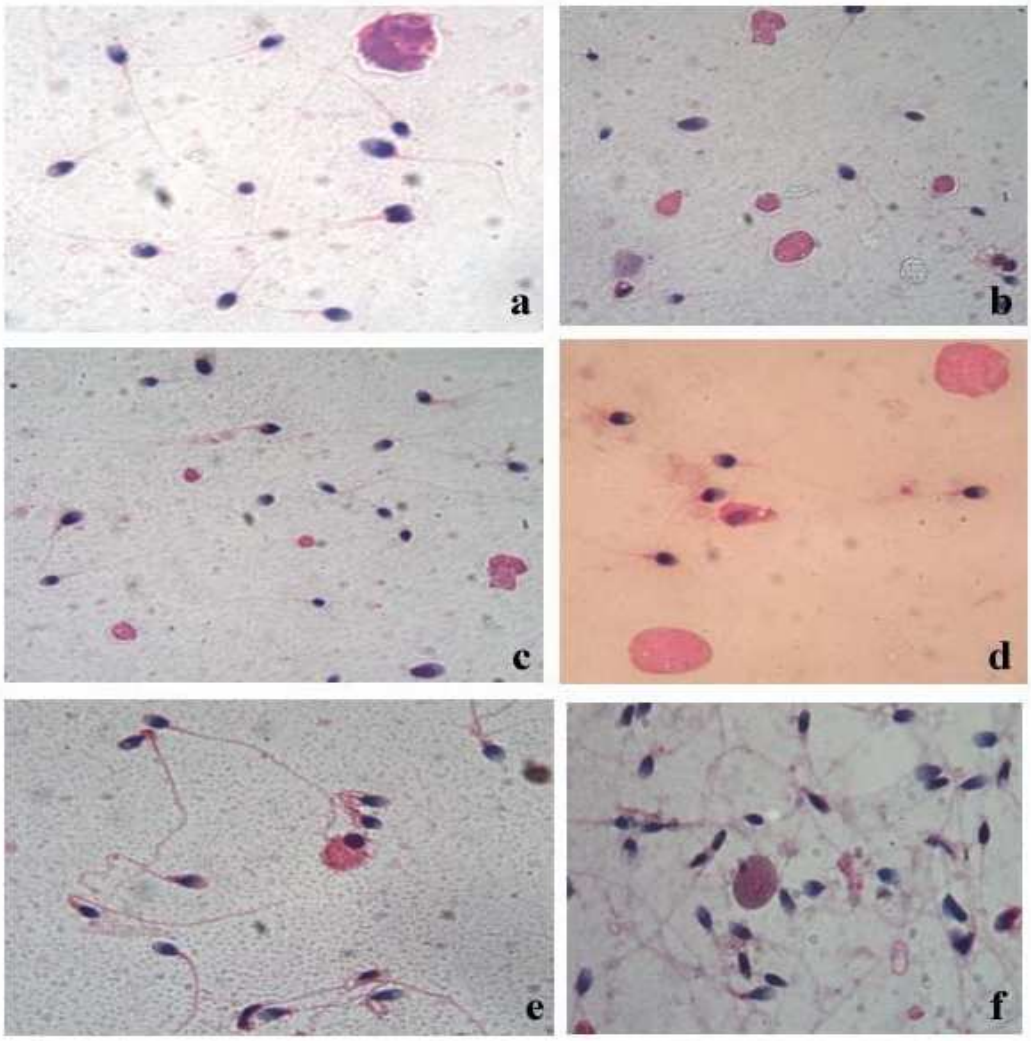
Figure 1 shows the Level of lipid peroxidase and superoxide dismutase with respect to different condition



T = Teratozoospermia, OT = Oligoteratozoospermia, OAT = Oligoasthenoteratozoospermia, LP = Lipidpreoxidase, SOD = Super-oxidedysmutase

FIGURE 2

Figure 2 shows abnormal and immature sperm Morphology in different teratozoospermic infertile cases



hormone stimulates spermatogenesis indirectly via testosterone [21]. Many studies also suggested that low levels of FSH were also associated with male infertility, as FSH is necessary for sperm maturation [22]. In our study there was a slight decrease in the mean value for FSH was observed in case of teratozoospermic condition. Increase in the LH and testosterone levels in OT and OAT condition was observed compare to normal indicating it could be due to partial androgen resistance and hyper gonadotropic gonadism.

CONCLUSION

The present study highlights quantitative and qualitative variation in the semen among teratozoospermic individuals. Further, the results give an insight to the biochemical changes that could bring alteration in the normal spermatogenesis leading to the morphological and functional abnormalities and further emphasize the role of increased oxidative stress in poor reproductive health. Thus, evaluation of oxidative stress parameters along with routine semen analysis helps in precise diagnosis and increases the chances of improving the fertility status of the individual or which helps in enhancing the assisted reproductive technique (ART).

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CONFLICT OF INTEREST: Nil