Al-Kufa University Journal for Biology / VOL.8/ NO.3/ Year: 2016
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

The study of Oxidative stress, Sperm chromatin abnormality and seminal parameters in different groups for infertile patients.

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Cited by thesis

Abstract :

The study was carried out between January 2014 and October 2014 including one hundred and fifty five infertile men who attended the Fertility center in AL-Sadr Medical City. The Objective of this study was to study of oxidative stress, sperm chromatin abnormality and semen quality including (semen volume, Liquefaction time, sperm concentration, motility, and normal morphology. The results of the study showed that there was a significant increase ($p<0.05$) in the semen volume, sperm concentration, progressive motility and normal morphology for unexplained compared to five abnormal semen groups. Also it was a significantly decrease ($p<0.05$) in liquefaction time, leukocyte concentration, MDA concentration and sperm chromatin abnormality for unexplained compared with abnormal semen groups. This study supports the conclusion that oxidative stress in seminal plasma is the main causes of sperm chromatin defect with defect of semen parameters and related with male infertility.

Introduction

Generally, worldwide it is estimated that one in seven couples have problems in conceiving [1]. A proximately 15% of couples are considered infertile , with a proximately 35% due to female factors alone, 30% due to male factors alone , 20% due to a combination of female and male factors and 15% unexplained[2]. Areduction in the male infertility maybe due to congenital or acquired conditions such as urogenital abnormalities , varicocele , infection of the genital tract , genetic abnormalities , endocrine disturbances , cancer systemic disease , testicular failure , immunologic problems and exposure to gonado toxic factors.[3].

The common causes of male infertility is related to disorders in sperm production an its imporovement is synonymous with better treatment outcomes . Although , the etiology of infertility is not clear in most cases[4]. The gradual increase in the role of male factors in determining couple infertility , occurring over the last few years , has led to increased attention being focused on the physio-pathological mechanisms of male infertility and its treatment , including also the use of assisted reproductive techniques (ART)[5].

The abnormality of sperm chromatin is essential for fertilizing capacity of spermatozoa and embryonic development [6]. The proportion of sperm with abnormal chromatin condensation in the ejaculate could prognostics factor in essessing the chances of fertilization and pregnancy[7].

The nuclear compaction is involved in the protection of paternal genome during the transit of spermatozoa through the male and female genital tracts and during its interaction with oocyte . Abnormalities in chromatin condensation can cause nuclear damages as DNA denaturation or fragmentation often associated with male infertility [8]. Many analytical techniques have been proposed to assess nuclear compaction in ejaculated sperm . Some

cytochemical or fluorecent dyes were used : acidic aniline blue to detect excessive presence of histones[9] and toluidine blue staining used for assessing sperm chromatin structure and packaging[10].

The compact structure of sperm nuclear chromatin is important for protection of genetic integrity during transport of the paternal genome through the male and female reproductive tracts.[11]. On the other hand increasing attention has been recently paid to sperm chromatin structure as one of the parameters determining male fertility [12].

The second most common proponent of DNA damage is oxidative stress which occurs when there is an excess production of reactive oxygen species (ROS) . The pathogenic effects of ROS occure when they are produced in excess of antioxidant capabilities of the male reproductive tract or seminal plasma .

Pathological levels can damage DNA and adversely affect fertilizing potential and pregnancy rates[13]. Small amount of ROS are necessary for spermatozoa to acquire fertilizing capabilities . Low levels of ROS have been shown to be essential for stabilization of the mitochondrial capsule in the mid – piece , acrosome reaction , hyperactivation , motility , oocyte fusion and capacitation [14].

Furthermore , ROS play an important physiological role , modulating gene and protein activities vital for sperm proliferation , differentiation and fertilizing function . In the semen of fertile men , the amount of ROS generation is controlled by seminal antioxidants[15].

Material and Methods

1)Semen samples

The study was carried out in the fertility center in Al-Sadr medical city. Semen samples were obtained from one handred and fifty five infertile men were divided to six sub groups including: unexplained infertility (n = 30), oligozoospermia (n = 30), asthenozoospermia (n = 30), teratozoospermia (n = 25), oligoasthenozoospermia (n = 20) and asthenoteratozoospermia (n = 20). Semen sample were collected by masturbation into wide – mouth containers after 3-5 days of sexual abstincence in room near the laboratory, immediately placed in an incubator at 37C° till complete liquefaction. After liquefaction time, the liquefied semen was then carefully mixed for few second and the semen was analyzed by macroscopic and microscopic examination using standardization WHO [16].

1) Macroscopic examination

1- Appearance

Specimen with homogenous, opalescent, and grayish – white in color was considered normal. Any other appearance was considered abnormal. Specimen tinged with red suggests the presence of fresh blood, while a brownish specimen may indicate the presence of old blood. Greenish specimens may be caused by infection. A white – yellow color may result from urine contamination or prolonged abstinence.

2- Volume

Normal ejaculate volume is between 2-6 mal. The volume was measured by using gradated centrifuge clinder with a conical base. The semen sample was considered hypovolumic when the volume less than 1.5 ml.

3- Liquefaction time

The semen sample was evaluated within 1 hour of collection and after the coagulum, or clot, has liquefied. Normal liquefaction time was ranged between 30 minutes at 37C° or within 1 hour at room temperature (25C°).

4- Viscosity

The viscosity of semen specimens was estimated by using pasture pipette. A normal sample leaves pipette as small discrete drop. A specimen with abnormal viscosity the drop will form a thread more than 2cm long. If drops were not formed or the semen cannot be easily drawn up into a pipette.

2) Microscope examination

1- Sperm concentration

A drop of 10µl spermatozoa suspension was placed on a microscopic slide and covered with a cover slip (22x22)mm. Concentration of spermatozoa (sperm/million) was calculated from the mean number of spermatozoa in four high power microscopic fields under magnification of (400x). This number was multiplied by a factor of one million.

2- Sperm morphology

The percentage of morphologically normal sperms was performed by using the same prepared slides for sperm motility. A least 100 spermatozoa were calculated by dividing the mean number of normal spermatozoa in four high power microscopic fields under magnification of (400x) on the number of sperm concentration.

3- Motility

The motility should be determined immediately to prevent the temperature change effect. It can be done at room tempearature. It is graded a, b, c, or d.

The normal sperm progressive motility was counted either by taking the percentage of forward progressive motile spermatozoa (grades a + b) which should be ≥ 50% of the total the total sperm count, or the percentage of rapid progressive motile spermatozoa alone (grade a) which should be ≥ 25% of the total sperm count. Semen sample with less than normal progressive motility percentage considered as an asthenozoospermic.

4- Seminal Leukocyte quantification

To distinquish the WB.Cs from immature sperm, round cells. Were assessed with peroxidase staining (End +2 test)[17]. peroxidas- positive Leukocytes staining brown were counted under bright-field microscopy and results reported as $\times 10^6$ WBC/ml semen.

5- Measurement of MDA

MDA levels were estimated by using thiobarbituric acid[18]. To a test tube containing 0.15ml of semen sample, 1ml of 17.5% trichloroacetic acid (TCA) and 1ml of 0.6% thiobarbituric acid (TBA) were added. The mixture was vortexed and boiled in a water bath for 15 minutes, then allowed to cool. One milliliter of TCA (70%) was added with mixing and the mixture was left to stand at room temperature for 20 min. The tube was centrifuged at 2000rpm for 15 minutes and the supernatant absorbance of these was read on a spectrophotometer at 532nm.

6- Acidic Aniline Blue staning

Slide are prepared by smearing 5µl of semen sample. The slides are airderied and fixed for 30 minutes in 3% glutaradehyde in phosphate - buffered saline (PBS). The smear is dried and stained for 5 minutes in 5% aqueous aniline blue solution (pH 3.5). Sperm heads containing immature nuclear chromatin stain blue, and those with mature nuclei do not take up the stain. The percentage of spermatozoa stained with aniline is determined by counting 200 spermatozoa per slide under bright field light microscopy[19].

7- Statistical Analysis :-

It done by mean of SPSS (Statistical package for Social Science; Version 20.0). To compare the parameters, a nova test was used. The differences between the values were considered statistically significant at ($p<0.05$)

Results:

Table (1) shows the result of seminal fluid analysis for all infertile groups including: unexplained infertility, oligozoospermia, asthenozoospermia, teratozoospermic, oligoasthenozoospermia and asthenoteratozoospermia.

It was clear that semen volume a significant ($P < 0.05$) difference among all the above infertile groups, whereas the results was recorded the high value with unexplained (3.8±0.59) but the less value of semen volume was appeared with asthenoteratozoospermia (2.9±0.89) . Also there was a significant ($P < 0.05$) deference in the Liquefaction time were found in infertile groups, but the decline value was showed with unexplained (26.4±7.05). The higher value in Liquefaction time was appeared with asthenoteratozoospermia(43.9±6.61).

There was a significant ($P < 0.05$) difference in the mean of sperm concentration was showed among the infertile groups, therefore, increase value was observed with unexplained (69.1±6.46) while the reduce value was appeared with both oligozoospermia (16.7±1.51) and oligoasthenozoospermia(15.3±2.55) whereas, no significant ($P < 0.05$) difference between two groups.

On the other hand, the percentage of progressive motility (A+B) was showed a significant ($P < 0.05$) difference among the infertile groups, the higher value was recorded with unexplained (65.1±5.99) while the less value was found with oligoasthenozoospermia (26.7±7.81) and then followed asthenozoospermia (38.73±3.9) and asthenoteratozoospermia (32.3±6.18). No significant ($P < 0.05$) difference was observed between oligozoospermia and teratozoospermia.

According to the percentage of normal morphology, there was a significant ($P < 0.05$) difference among the infertile groups, while the increase value was observed with unexplained (58.5±7.76) but the reduce value was showed with asthenoteratozoospermia (22.4±3.29) and teratozoospermia (24±3.75). Moreover, there was a significant ($P < 0.05$) difference in the leukocyte concentration among the infertile groups. The higher value was found with asthenoteratozoospermia (4.37±1.29) then decline value has been recoded with unexplained (1.66±0.76). No significant ($P > 0.05$) difference was appears between oligozoospermia and asthenozoospermia.

Recording to the results of MDA concentration was showed a significant ($P < 0.05$) difference among the infertile groups. The reduce value was noticed with unexplained (4.7±0.44) but the higher value was showed with asthenoteratozoospermia (8.6±1.65).

Lastly, a significant ($P < 0.05$) difference in percentage of sperm chromatin abnormality was recorded among infertile groups. The decline value was showed with unexplained (32.1±3.09) while higher value was observed with asthenoteratozoospermia (45±6.18) .

Table(1) Semen parameters, Seminal MDA Levels and Sperm chromatin abnormality percent for infertile patients.

Patients / Variables	UXI n=30	O n=30	A n=30	T n=25	OA n=20	AT n=20
Semen volume (ml)	3.8±0.59 [a]	3.6±0.54 [b]	3.5±0.83 [c]	3.3±0.7 [d]	3.2±0.62 [e]	2.9±0.89 [f]
Liquefaction time (min)	26.4±7.05 [a]	35.4±3.03 [b]	39.1±3.99 [c]	37.8±4.41 [d]	40.5±4.65 [e]	43.9±6.61 [f]
Sperm concentration (x 10^6/ml)	69.1±6.46 [a]	16.7±1.51 [b]	56.7±10.33 [c]	51±9.15 [d]	15.3±2.55 [b]	50.6±10.63 [d]
Progressive motility percent(A+B)%	65.1±5.99 [a]	57.5±5.3 [b]	38.73±3.9 [c]	57±6.68 [b]	26.7±7.81 [d]	32.3±6.18 [e]
Normal morphology percent %	58.5±7.76 [a]	44.8±8.2 [b]	42.8±5.9 [c]	24±3.75 [d]	42.45±6.62 [c]	22.4±3.29 [e]
Leukocyte concentration (x 10^6/ml)	1.66±0.76 [a]	2.5±0.85 [b]	2.7±0.83 [b]	3.3±0.65 [c]	3.8±0.71 [d]	4.37±1.29 [e]
MDA concentration (µmol/l)	3.7±0.44 [a]	5.52±1.5 [b]	6.52±0.89 [c]	7.6±1.09 [d]	8.1±1.11 [e]	8.6±1.65 [f]
Sperm chromatin abnormality percent%	32.1±3.09 [a]	35±3.8 [b]	38±3.95 [c]	40±4.44 [d]	42.6±4.18 [e]	45±6.18 [f]

Value are expressed as mean ±S.D.

different letters means significant difference ($P<0.05$).

UXI: Unexplained infertility , O: Oligozoospermia , A: Asthenozoopermia , T: Teratozoopermia,

OA: Oligoasthenozoopermia ,AT: Asthenoteratozoospermia

Discussion

Defective sperm parameters was indicated to important role in diagnosis fertility and was closely correlated to fertilization and pregnancy rate in the natural fertilization process as well as in ART[20]. As shown in (table 1) there is a significant increase ($P < 0.05$) in semen volume, sperm concentration, progressive motility and normal morphology for unexplained compared to five abnormal semen groups including (oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia and asthenoteratozoospermia respectively) while the results in the same table was appeared a significantly decrease ($P < 0.05$) in liquefaction time, leukocytes concentration, MDA concentration and sperm chromatin abnormality for unexplained compared with abnormal semen groups. This may be due to increased concentrations of leukocytes in semen report the presence of a genital tract infection and have been associated with an increase in immature germ cell concentration.The effects of Leukocytospermia on sperm may be lead to increased oxidative stress and DNA damaged demonstrated in various studies. The production of ROS causing defect of sperm chromatin/high DNA damage can be attributed to poor sperm quality and lower pregnancy rate following ART[21]. [22] have suggested that leukocytes is the main source of oxidative stress in seminal plasma, this is because ROS at high values are toxic to sperm quality and function. Some studies have reported that ROS attack the DNA in sperm nucleus by causing base modification, DNA strand breaks and chromatin cross – linking [23]. Leukocytospermia are also associated with increased levels of sperm DNA damage, especially in abnormal ejaculation patients.

Our studies are considered oxidative stress is the main cause of sperm DNA defect with others aspects like apoptosis, sperm chromatin remodelling and defective packaging processes [24]. In the present study, significant higher values of MDA levels and sperm chromatin abnormality together with poor semen quality in the raw semen samples of leukocytospemic patients may be indicated that lipid peroxidation has making adverse alter in sperm membrane also lead to failed of sperm motility, morphology and sperm concentration, it clear that lipid peroxidation may change sperm membrane function and cause abnormal sperm quality. The finding of increased MDA levels may proposed that seminal oxidative stress could be involved in the pathogenesis of sperm DNA damge in abnormal semen patients, sperm chromatin defect may affect the quality of the ejaculated spermatozoa and could have implications on their fertility potential. These results of the present study showed agrees with many previous studies that explanation for the link between seminal oxidative stress and sperm DNA damage. May be associated to defect in spermiogenesis that making the release of spermatozoa that are immature and have abnormal chromatin structure/high DNA damage and abnormal morphology[25]. Our study showed a significant elevation in the seminal MDA levels in infertile groups. Therefore, lipid peroxidation degradation of sperm membrane integrity could held responsible for abnormal sperm motility, concentration and morphology[26]. Some studies appeared the negative significant correlation was noticed between lipid peroxidation with sperm concentration, and normal morphology between control and infertile men[27][28]. Regarding to sperm concentration, many studies have suggested clear related between abnormal chromatin and sperm count which refelective impact of protamine deficiency in cousing apoptosis or impaired spermatogenesis[29][25].

On the other hand, sperm chromatin defect included by increase levels of OS may enhance the processe of germ cell apoptosis leading to reduce in sperm concentration related with male infertility[30]. Some investigators suggest that early apoptosis is a related with immature nuclear development and then abnormal nuclear decondensed chromatin[31].

In relation to sperm motility, studies have found that excessive the level of OS causes ATP to deplete rapidly leading to decrease phosphorylation of axonemal proteins and lead to tranansient impairment of motility [32][33]. Also some researchers indicated significant negative correlation between sperm chromatin decondensation with motility[29][34]. This result is in accordance with that of [35] who found that infertile men with protamine deficiency display Lower sperm counts, motility and higher abnormal sperm morphology. In the opposite,[19]. demonstrated that there is no correlation between sperm chromatin condensation assessed by aniline blue staining and some parameters, including motility, vitality and sperm count. This come in agreement with other studies that did not find any correlation between sperm parameters[36][37].

Moreover, the data in this study indicate a significant decrease ($P < 0.05$) of semen volume with a significant increase ($P < 0.05$) of liquefaction time for five abnormal semen group compared to unexplained. Abnormal synthesis and secreation in accessory sex glands may reflect low semen volume [38] our study was indicate to increase leukocyte concentration especially monocyte in the infertile patient may be causes low semen volume[39]. This study agree with [40]. That proposed increase leukocyte concentration lead to oxidative stress which related with decrease semen volume and making decline sperm quality and male infertility.

Regarding to liquefaction time, male accessory glands are contain potential proteins important to the coagulation and liquefaction of semen. Abnormal viscosity of semen reflect hypofunction of the seminal vesicles or prostate, therefore hyperviscous seminal fluid may result because infection and high levels of seminal leukocytes [41]. Oxidative damage may play a role in semen hyperviscous during excessive ROS production by increase amount of seminal leukocyte. [42].

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