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Influence of Semen Parameters and Malondialdehyde on Infertile Males in Iraq

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Abstract

Seventy samples were collected from male individuals suffering from infertility either primary or secondary, as well as 35 samples were collected from fertile persons served as a control group.

Criteria referred to by WHO for semen parameter were applied through-out the course of the study, these criteria includes: Age, volume of the specimen, total sperm count/mL, morphology of sperm, pH of the specimen, liquefaction time, and activity of sperm. The effect of oxygen reactive species was estimated by measuring of the concentration of malondialdihyde.

Computerized system (General Sperm Analyzer, motic type) was used to determine semen parameter mentioned above. Seminal plasma of liquefied samples were collected from all samples by centrifugation at 3000 rpm for 7 min, plasma were stored frozen (-80°C) utile use. Enzyme Linked Immune-Sorbent Assay technique (Elisa) was used to detect MDA level in seminal plasma.

Keywords: Malondialdihyde; Seminal parameters; Infertile males

Introduction

Infertility is a state when two couples failed to have child for 12 months of marriage with unprotected sex [1]. A great percentage of infertility is caused by male partner. Several studies have emphasized in general, on the role of Reactive Oxygen Species (ROS) influence on seminal parameters such as motility, count, volume, activity, etc. [2]. ROS is a free radical of different types produced naturally by many cells and tissues of human body in controlled levels called (physiological levels), that are essential for many natural processes such as apoptosis and sperm maturation and so forth, these free radicals have got another function that is, destroying microbial agents happened to infect many sites of human body [3]. Access of ROS production, on other hand, has a devastative effect on several types of cells in human body, spermatozoa are at the top of these cell. Effect of ROS on seminal parameters is evident among infertile males more than that of fertile [4]. ROS is a highly unstable material, and it needs an immediate measuring, it is well known that free radicals are causing peroxidation of unsaturated lipids, spermatozoa containing a significant amount of unsaturated lipid in their heads which makes them venerable to the action of ROS; the result of this action is the production of Malondialdehyde (MDA) [5].

Aim of Study

Efforts had been directed in this study to high-light the influence of malondialdehyde semen parameters on infertile males.

Materials and Methods

Materials

General sperm analyzer: Motic-China.

Malondialdehyde: Eliza assay kit-Human Germany was used to detect the concentration of Malondialdehyde (MDA) throughout the course of research.

Methods

Sample collection

Seventy semen samples were collected form male individuals suffering from infertility either primary or secondary, and fertile individuals who had conceive pregnancy within last 12 months or those who fathered a baby within this period were served as control group. All individuals were advised to collect semen samples in wide mouth cup after a period of absence of three days [6]. Sample collection lasted for 3 months started from April 2017-June 2017. All samples were examined for semen quality and sperm parameters. Using General sperm analyzer (motic/ china). Centrifugation of semen samples was done at 3000rpm for 7 min, seminal plasma then collected in a sterile tube and frozen at -80°C until used [7].

This test was done using sandwich ELIZA technique and the steps were as indicated by the manufactures instructions. The standard curve for the Malodialdehyde concentration was drawn using the standard concentration provided with the kit.

Statistical analysis

Data tabulation, input and coding was done by the use of IBM© SPSS© (Statistical Package for the Social Sciences) Statistics Version 22.

Result

Samples were divided equally to include 50% (35 sample) for each group of research (primary and secondary infertile groups) with (35 samples from fertile males served as control group).

The result presented in Tables 1 and 2 showed the mean values and the statistical correlation for all parameters included in this study.

	Controls	Primary Infertility	Secondary Infertility
Variables	Mean ± SD	Mean ± SD	Mean ± SD
Age	29.17 ± 5.5	28.49 ± 4.9	33.09 ± 5.88
Volume	3.23 ± 1.28	3.02 ± 1.21	2.95 ± 1.30
Total sperm	149.73 ± 87.09	63.34 ± 61.8	86.87 ± 88.161
Motility	50.30 ± 8.12	39.17 ± 15.31	29.80 ± 18.02
Abnormal Morphology	53.11 ± 11.69	33.69 ± 14.65	31.78 ± 13.19
PH	7.52 ± 0.22	7.65 ± 0.267	7.6 ± 0.264
Liquefaction Time	26.86 ± 11.76	23.43 ± 12.41	25.79 ± 11.54
Activity	70.22 ± 10.40	50.15 ± 20.26	44.06 ± 17.79
MDA	8.36 ± 2.97	8.13 ± 4.33	9.72 ± 3.66

Table 1: Semen parameters of study groups.

Variables	Controls *vs.* PI	Controls *vs.* SI	PI *vs.* SI
Age	0.859	**0.01**	**0.002**
Volume	0.766	0.616	0.968
Total sperms count	**0**	**0.004**	0.438
Motility	**0.005**	**0**	**0.021**
Abnormal Morphology	**0**	**0**	0.818
PH	0.09	0.413	0.673
Liquefaction time	0.454	0.925	0.687
Activity	**0**	**0**	0.284
Key: PI: Primary Infertility; SI: Secondary Infertility.			

Table 2: Statistical significance of variables.

Age

It is clear that the mean of age in all groups was in the range proposed for this study, though the P-value indicated the presence of significant difference in this respect.

Volume of seminal fluid

The result presented in Table 2 indicated that there was no significant difference between volumes of seminal fluid in all individuals participated in this study.

Total sperm count

The total count of spermatozoa in all three groups of research laid in the range of normal value, though there was quite difference between individuals in control group comparing to primary or secondary groups (149.73 ± 87.09, 63.34 ± 61.8, 86.87 ± 88.161) sperm/mL respectively.

Motility of spermatozoa

Motility referred to in Table 1 was the mean total percentage of motility, regardless the type of motility (forwards, zigzag, or no motile). It was obvious that motility represents the health status of spermatozoa in all three groups, since a clear statistically difference observed between control group and either infertile groups, on one hand and between the two infertile groups (primary and secondary infertile), on the other.

Abnormal morphology

The result of this study concerning teratozoospermia ratio showed that there was a positive relationship between the ratio of teratozoospermia and male infertility since there was a significant difference between control group and other groups of infertility.

PH of seminal fluid

The result of this study showed that there was no significant difference between the study groups. That is because the selection of PH of the samples was somewhat bias (samples with pH <7.2 or >8.6) were excluded from the study, so in a-way the effect of PH concerns male infertility was neutralized.

Liquefaction time of seminal fluid

The result of this study showed that the mean value of liquefaction time was (26.86 ± 11.76 for control groups, and 23.43 ± 12.41 for primary infertile group, and 25.79 ± 11.54 for secondary infertile group), the criteria proposed by WHO for normal semen values indicated that liquefaction time is considered abnormal if it exceeded 60 min.

Activity of spermatozoa

Computerized system used in this study classified the activity of sperm in to four classes A-D when A means sperms move ahead quickly and D means the sperms are immotile (software weili dynamic/USA). Sperms considered active when the sum of classes A and B (The active motile plus sluggish motile) greater or equal to 50%.

The result presented in this study concerning the activity of sperms showed that the mean activity of sperms in control group was equal to 70%, while it was 50% among primary infertile patients and it decreased to 44% among secondary infertile patients. the result of this study announce loudly that infertile individuals seem to suffer from decreasing activity of their sperms and that could be a reason for their

situation, but to this point it was rather difficult to put this reason at the top of factors leading to infertility.

Lipid peroxidation of sperms lipid contents

Malondialdehyde levels was measured for all groups, the mean value of this substance was (8.36 ± 2.97, 8.13 ± 4.33, 9.72 ± 3.66) mmol/L for control group, primary and secondary infertile group respectively. Malondialdehyde concentrations measured for all individuals participated in this study, showed no significant difference between all groups of study.

Correlation of MDA with semen parameters

In this study, correlation of MDA in seminal fluid with age and other parameters, Table 3 revealed a statistically significant positive correlation of MDA with abnormal morphology of sperms in all groups, but it was more evident in primary infertile group (r=-0.483) compared to control and secondary infertile groups, which was almost similar (r =-0.336 and -0.335, respectively, yet, a negative correlation was obtained between MDA level and active sperms in primary group, but it was statistically significant in control group (r=-0.385) and in secondary infertile group (r=-0.372). It seemed that the effect of MDA on semen parameters is some-how controversial.

Variables	Controls		Primary Infertility		Secondary Infertility	
	R	P-value	R	P-value	R	P-value
Age	-0.225	0.194	-0.146	0.403	0.267	0.121
Volume	0.077	0.66	-0.144	0.409	-0.106	0.546
Total Sperm	-0.108	0.538	-0.016	0.928	-0.078	0.655
Motility	-0.25	0.147	-0.146	0.403	-0.333	0.05
Normal Morphology	-0.336	0.049	-0.483	0.003	-0.335	0.049
PH	0.062	0.721	0.021	0.905	-0.154	0.376
Liquefaction Time	0.325	0.057	-0.039	0.826	-0.046	0.794
Activity	-0.385	0.022	-0.267	0.121	-0.372	0.028

Table 3: Correlation between mean MDA seminal level with different variables in patients with Primary and Secondary Infertility.

Discussion

The results presented in this study concerning age of individuals were in agreement with the American fertility society that recommends the age limit for good quality of sperm is 50 years old or less [8]. To the best of knowledge of this study there was no recommended age for fertility of male in Iraq. So it was believed that there was no observable effect of age on male fertility, least at this point of study.

No effect was observed of volume of semen sample no matter what the group is; the result presented in this study had fallen in the normal range of seminal fluid referred by WHO [9].

This result concerning the total count of sperms showed a clear difference between fertile and infertile men; yet, this difference had no obvious effect on male fertility, because even infertile men could have normal sperm count according to WHO report [9].

It is rather unfair to take the result of motility mentioned above as a cause of infertility, because this parameter is subjected to so many factors that may interfere or hinder the motility of sperm, among these factors (Calcium ion, Magnesium ions, Iron, and Vitamin D&C) deficiency [4]. It is well known that temperature, may affect severely the motility of sperms as indicated by [10].

From the point of view of this study, the abnormality of sperms in all research groups did not exceed the acceptable ratio referred to by WHO report which indicated that person is considered infertile, when the percentage of normal sperms is less than 4% [1].

It seemed that liquefaction time of seminal fluid could have no role in evaluation of male infertility in the light of the present result and what had been mentioned by [11].

It seemed from the results of this study concerning MDA concentration, that it had almost the same activity in all study groups, that in a-way may reflect the idea that all groups of the study are under similar conditions, on other hand, the correlation of MDA with the semen parameters gave a conflict result since it showed a positive effect with semen abnormality in all groups with a marked effect among primary infertile person, while a negative correlation was recorded between MDA concentration and sperm activity among primary infertile group. But it correlated positively with control group and secondary infertile group, which makes the influence of malondialdehyde controversial [12].

Conclusion

Infertility is a multi-factorial process. Since no single parameter is safe enough to be considered as a cause of male infertility either primary or secondary. MDA on the other hand, has no significant effect as a factor for male infertility.

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