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Serum Levels of Melatonin and Oxidative Stress Markers and Correlation between Them in Infertile Men

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ABSTRACT

Introduction: Infertility is the problem of 15% of young couples in different societies. One of the factors that could affect fertility is oxidative stress. Therefore, the aim of the present study is to investigate the level of Melatonin, a free radical scavenger, and its correlation with oxidative biomarkers in infertile men.

Methods: For this purpose, fertile and infertile men in 2 groups, 30 people in each group, were studied. The fertile men were selected from husbands of patients admitted to Alzahra obstetric and gynecology hospital, according to WHO standards. The infertile men were selected from patients referred to infertility ward. Blood sampling from the participants carried out at a specific time, sera collected and the levels of malondialdehyde, total antioxidant capacity and Melatonin were detected in the sera. The data were analyzed using t-test and Sperman's correlation method.

Results: Melatonin level in the sera from fertile men were 522 (39.32) ng/L and in infertile men were 511.78 (34.6) ng/L. MDA level in fertile and infertile men were 2.26 (0.34) vs 2.99 (0.44) nmol/ml which was significantly different. The level of TAC in the sera from fertile men were significantly higher than in infertile men. The result obtained for correlation coefficient Spearman's test revealed a significant, strong and direct correlation between Melatonin and TAC and a significant and reverse correlation between melatonin and MDA.

Conclusion: It is concluded that melatonin could be involved in infertility. In other word, melatonin treatment and antioxidant-rich nutrition could help fertility by combating oxidative stress.

Introduction

Infertility is the problem of 15% of young couples in different societies.[1] Therefore, any assistance in this matter would promote the health status of families and society. Since spermatogenesis and oogenesis are the basis of fertility, increasing our knowledge about factors that are involved in them, would be helpful in solving the fertility problem.[2]

Oxidative stress (OS) is one of the known factors which is involved in reproduction. The imbalance in the production of oxidants or free radicals and antioxidant molecules in the body would result in OS state.[3,4] Increasing of free radicals such as reactive oxygen species (ROS) by peroxidation of membrane lipids and DNA damage could affect sperm motility, sperm ability to bind oocyte and sperm capacitition.[5-7] The association between decreased sperm quality and increased ROS level in infertile men has previously been shown.[6] On the other hand, it is reported that reduction of antioxidant level results in infertility and antioxidants

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could be used for treatment of infertility in men.[8,9]

Melatonin, a hormone secreted by pineal gland, is one of the strongest endogenous antioxidants in the body.[10] In addition to its antioxidant activity, the hormone is involved in regulation and secretion of sex hormones via its receptors on reproductive organs.[6, 11-13] While most recent studies on melatonin are focused on its antioxidant activity, there are studies reported that melatonin is necessary for a normal pregnancy and is also involved in increasing of pregnancy.[14,15] However, the level of melatonin in infertile men and its relation to OS biomarkers have precisely not been established and further studies are needed to clarify the matter.

Materials and methods

This case-control study was carried out in Alzahra obstetric and gynecology hospital, Tabriz- Iran, from Jan 2012 – Jan 2013. The fertile men are considered as control group and the infertile men are considered as case group. Sample size was chosen based on infertility prevalence and according to discrete equation (α=0.05, d=1.95) and include 30 men in each group. The proposal was approved in ethic committee of the Tabriz University of Medical Sciences and registered as 5.4.8622-90.11.6 . Accordingly, the consent was obtained from volunteers and the aim of the study was explained for every participants.

The husbands of the patients, who were referred to Alzahra hospital for any gynecological reason other than infertility, were chosen as fertile men. The men considered as fertile if had a child during the past year or had normal spermogram. According to WHO standard, a normal spermogram should contain at least 15 million sperm per ml of semen including motile sperms with normal morphology.

The infertile men were chosen from the patients who were referred to Alzahra hospital for infertility treatment. These men were oligospermic and their wife had not an apparent infertility problem. Furthermore, they had no child during one year after marriage without using any contraception method. The men with azospermia and those with oligospermia and varicocele and smoker men (in both groups) were excluded from the study.

Inclusion criteria, in addition to being fertile or infertile include: fertile age (15-45) and having of normal, up to 25, BMI.

For hematological determinations, 5^{cc} blood was obtained from each person, transferred to sterile tube, centrifuged and their serum was separated for measurement of Malondialdehyde (MDA) and total antioxidant capacity (TAC), as OS biomarkers, and melatonin. The sera were kept in – 80 degree centigrade refrigerator until the time of measurement.

The MDA measurement was carried out according to Bilic and Yagi.[16,17] The measurement was based on thiobarbituric acid (TBA) reaction and extraction with normal bothanol, spectrophotometric detection of absorbance at 532 wave length and comparing with standard curve. TBA was purchased from Merck.

TAC measurement in serum was carried out according to miller et al.[18] using Randox kit. Melatonin measurement was carried out using human melatonin (MT) Elisa Kit, purchased from Glory Company.

The data obtained for case and control groups were presented as Mean±SD and analyzed using t-test. For evaluation of association between MDA and TAC with melatonin, non parametric correlation sperman test, SPSS ver.13 was used. $P<0.05$ is considered as significant.

Results

The results of hematologic parameters in case and control groups are as follow:

Serum level of melatonin

The melatonin levels in sera from fertile and

infertile men are shown in figure 1. As the figure shows, mean concentration of melatonin in the serum from fertile men is 522± 39.32 mg/l and in infertile men is 511.78 ± 34.6 mg/l. While the melatonin level in infertile men is lower than that in fertile men however, the difference is not significant (p=0.08).

Serum level of TAC

The results of TAC measurement in the sera from both groups are shown in figure 2. As it is shown in the figure, the mean concentration of TAC in the serum from fertile men is 1.88 (0.21) nmol/L and in infertile men is 1.51 (0.19) nmol/L. Statistical analysis revealed a significant difference between two groups (P=0.001).

Serum level of MDA

Figure 3 presents the results of MDA measurement in the sera from both groups. As it is seen in the figure the mean concentration of MDA in the serum from fertile men is 2.26 (0.34) nmol/ml and in infertile men is 2.99 (0.44) nmol/ml. Statistical analysis revealed that the MDA level in fertile men is significantly (P=0.001) lower than those in infertile men.

Correlation of melatonin, TAC and MDA

Correlation of melatonin with TAC and MDA are shown in figures 4-7. Figure 4 shows the Sperman's correlation coefficient ratio between TAC and melatonin in fertile men. As the figure shows there is a strong (r=0.93) and significant (p=0.001) direct correlation between TAC and melatonin.

The examination of the same parameters in infertile men is shown in figure 5. As the figure shows there is a moderate (r=0.6) and significant (P=0.008) direct correlation between TAC and melatonin in infertile men.

Figure 6 shows the Sperman's correlation coefficient ratio between MDA and melatonin in fertile men. As the figure show there is a strong (r=-0.96) and significant (P=0.001) reverse correlation between MDA and melatonin in this group. The examination of the same parameters in infertile men is shown in figure 7. As the figure shows there is a strong (r=-0.94) and significant (P=0.001) reverse correlation between MDA and melatonin in infertile men.

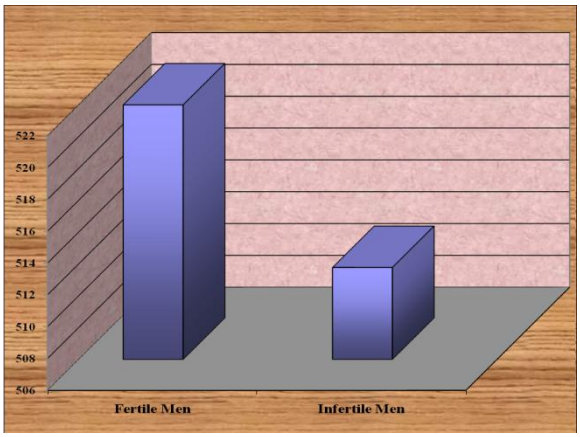

Figure 1: Serum level of melatonin in fertile and infertile men presented as ng/L.

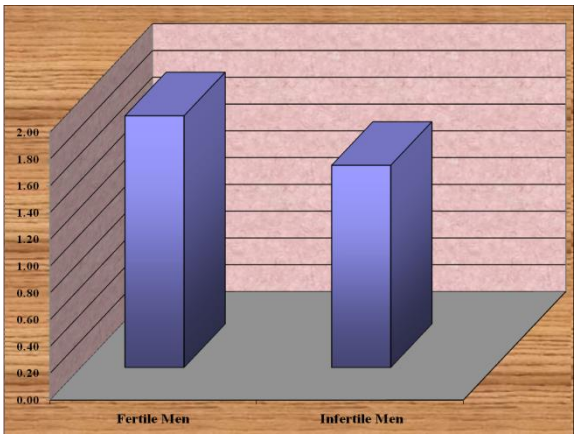

Figure 2 : Serum level of TAC in fertile and infertile men presented as nmol/L.

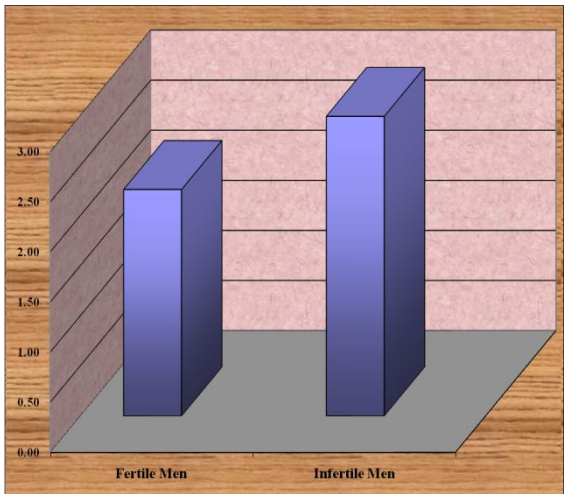

Figure 3 : Serum level of MDA in fertile and infertile men presented as nmol/L.

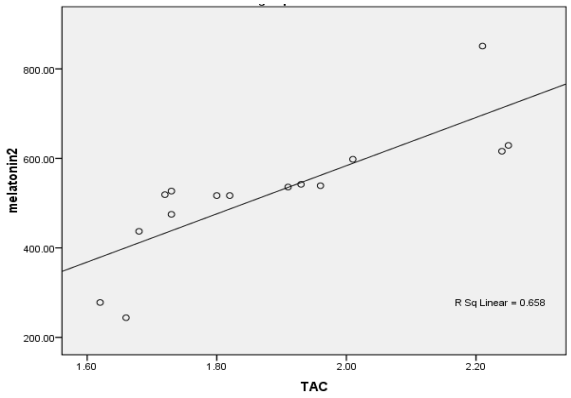

Figure 4: Scatter plot showing correlation of melatonin and TAC in fertile men.

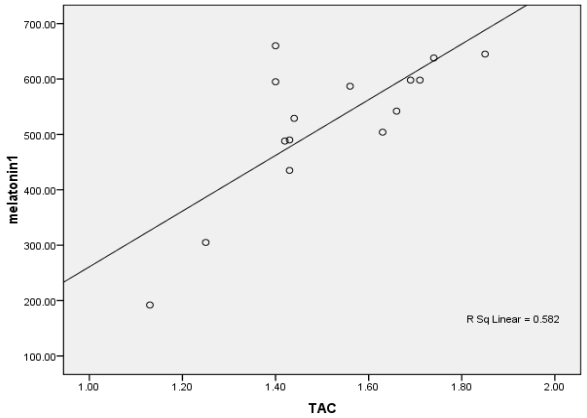

Figure 5: Scatter plot showing correlation of melatonin and TAC in infertile men.

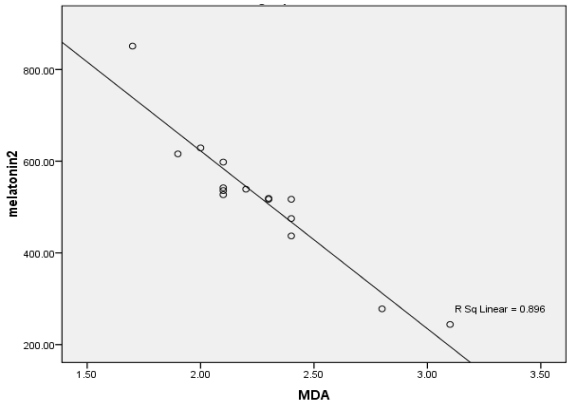

Figure 6: Scatter plot showing correlation of melatonin and MDA in fertile men.

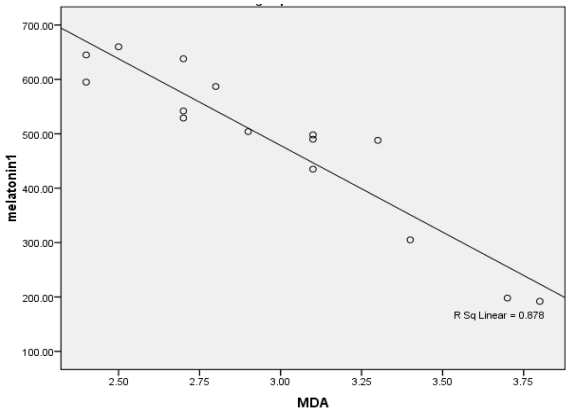

Figure 7: Scatter plot showing correlation of melatonin and MDA in infertile men.

Discussion

Knowing of the factors that are involved in infertility would greatly be helpful in the treatment of infertility problem. The present study was carried out to investigate the possible role of melatonin in infertility.

The hypothesis was based on the fact that oxidative stress has an adverse effect on fertility and melatonin is a strong scavenger of oxidative factors and therefore could improve fertility.

The result of the present study revealed that while the melatonin level in the serum from infertile men is notably lower than the fertile men (Figure 1), but the difference is not significant. In correlation with our result, it has been reported that melatonin level in the semen and serum from infertile men is lower than that in fertile men.[19] The low level of melatonin in infertile men, in the present study, could still be defended in that; as the mean values of melatonin level show melatonin level ranges widely in individuals. On the other hand, the level of melatonin in human blood is affected greatly by varying numerous factors. Such factors include the amount of light, stress, body position,

physical activity, time of the day and so on.[20,21]

Although it is tried that demographic characteristic of participants such as BMI and age to be in a limited range and the time of blood collection was restricted to a limited time, but the control of other factors was almost impossible. As so, melatonin level in the participants of the present study ranged from 192-645 ng/L, a 3.5 fold difference between individuals in a group. It is obvious that with such a big variation the difference between the groups could hardly be significant unless to have a very large groups. The other finding of the present study is that the concentration of TAC in infertile men was significantly ($P<0.001$) lower in comparison to fertile men and concentration of MDA in infertile men was significantly ($P<0.001$) higher in comparison to fertile men. The significant correlation between melatonin and OS factors is indicating that low melatonin level in infertile men possibly is involved in production of OS. In accordance with our findings it is reported that increased ROS, a main molecule involved in OS production, has a detrimental effect in human reproduction.[22] Other effects of OS on reproduction is disturbing physiologic process of sperm maturation and sperm capacitation.[23-25] It is also reported that high concentration of ROS in the media increases DNA damage in infertile men during ART process.[4,23,25] Glutathione peroxidase (GSH) is one of the main intracellular antioxidants and using of glutathione in infertile men would result in improvement of fertility.[4,26]

In vitro studies confirmed the above findings by showing that ROS incensement in sperm preparation media would result in increased DNA damage, changes in acrosome reaction and sperm attachment to zona pellucid.[4,27] It is believed that most of the alterations are produced by the lipid peroxidation in the sperm membrane which is the resultant of free radicals.[27,28] In agreement with above findings, it is shown that reduction of antioxidants result in increased apoptosis in spermatogenic cells and atrophy of seminiferous tubules.[9] Decreasing of germinal cells due to OS-induced apoptosis is reported by several researchers.[29-31]

The above findings are in favor of our results in that in oligospermic infertile men the level of melatonin and TAC was lower but MDA level was higher in comparison to fertile men. Involvement of age related stress oxidative to glutathione metabolism and decreased germinal epithelium in aged mice have been reported.[32,33]

Chaudhari et al., in 2008 have shown that the level of glutathione and related enzymes in sperm and seminal plasma of men who are oligospermic is lower than that in healthy men.[34] This report is agreed with our finding that TAC and melatonin levels in infertile men are lower in comparison to fertile men. There are also reports showing that in infertile men there is a significant correlation between antioxidant concentration and sperm number and motility and an increased lipid peroxidation evidenced by higher MDA level in seminal plasma.[34,35] These findings are well correlate with our finding in infertile men. Agarwal et al., in 2007 have stated that ROS should be considered as an independent marker for male infertility.[6]

Conclusion

According to the results of the present study, it is concluded that due to low level of melatonin and antioxidants in infertile men, melatonin usage in the treatment of infertility could be taken into consideration.

Furthermore, for decreasing of infertility rate in the society care should be taken to modulate stress producing factors and promote using of antioxidant–rich nutrients.

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Ethical issues

None to be declared.

Conflict of interest

The authors declare no conflict of interest in this study.

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