SEMINAL PLASMA LEVELS OF FREE 8-ISOPROSTANE AND ITS RELATIONSHIP WITH SPERM QUALITY PARAMETERS

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ABSTRACT

There is growing evidence that damage to spermatozoa by reactive oxygen species play a key role in male infertility. The aim of this study was to assess seminal plasma free 8-Isoprostane levels in men with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia compared to normozoospermic males and its correlation with seminal parameters. The case group consisted of men with asthenozoospermia (n=15), asthenoteratozoospermia (n=16) and oligoasthenoteratozoospermia (n=15). The control group consisted of 16 males with normozoospermia. After Purification of Free 8-Isoprostane by affinity column, its concentration was measured by enzyme immunoassay method. Free 8-Isoprostane evaluation showed significantly greater values in the total case group (n=46) versus control group (18.23 ± 3.56 vs 2.6 ± 0.38 ng/ml). In each case group free 8-Isoprostane also showed a significant increasing compared to normozoospermic males. Free 8-Isoprostane showed an inversely significant correlation with sperm motility and sperm morphology. Lipid peroxidation could have significant role in etiology of sperm abnormalities. Measurement of 8-Isoprostane can be used as a specific biomarker for assessing lipid peoxidation in sperm.

KEY WORDS

Isoprostane, Reactive Oxygen Species, Lipid peroxidation, Semen, Sperm.

INTRODUCTION

In the etiology of male infertility, there is growing evidence that damage to spermatozoa by Reactive oxygen species (ROS) play a key role (1, 2). Spermatozoa contain large quantities of polyunsaturated fatty acids (PUFA) therefore, they are susceptible to ROS-induced damage. It has been suggested that ROS induce membrane lipid peroxidation in sperm. The toxicity of generated fatty acid peroxides are important causes of sperm malfunction (3-9). Available data on the impact of oxidative stress on sperm are based on the measurement of seminal plasma and sperm malondialdehyde (MDA) by the thiobarbituric acid-reacting substance (TBARS) assay (10-17).

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Recently, it has been shown that 8-Isoprostane is a specific, chemically stable, and quantitative marker of oxidative stress in vivo. 8-Isoprostane is formed in situ in cell membranes following free radical attack on the arachidonic acid. So it can be considered as a specific biomarker of lipid peroxidation in cell membrane (18-20). In semen, cell membranes of spermatozoa and genital tract lining cells could be sources of 8-Isoprostane.

The aim of this study was to assess seminal plasma free 8-Isoprostane levels in men with asthenozoospermia (impaired sperm motility), asthenoteratozoospermia (impaired sperm motility and morphology) and oligoasthenoteratozoospermia (low sperm concentration and impaired sperm motility and morphology) compared to normozoospermic males and its correlation with seminal parameters.

MATERIALS AND METHODS

Semen samples : A case-control study with a simple random sampling was designed. Following Institutional Review board

approval the semen samples were collected from the case and control groups. All specimens were collected into sterile plastic containers by masturbation after an abstinence period of 3-5 days and were analyzed within 1h of collection. After allowing at least 30 min for liquefaction to occur, semen analysis was performed to measure sperm concentration, normal sperm morphology and percentage sperm motility using Sperm Quality Analyzer IIC (SQA IIC, United Medical Systems Inc, Santa Ana, CA, USA) (21, 22). Samples with a leukocyte concentration $>10^6$ /ml of ejaculate were excluded from this study (23). The differentiation of leukocytes and spermatogenic cells was performed by the myeloperoxidase staining (LeucoScreen; FertiPro). Patients with recent fever episodes, genital tract inflammation, varicocele, recurrent infections were excluded from the present study (24). The criteria for sperm normality were as follows: sperm concentration= 20×10^6 /ml of ejaculate, progressively motile sperms=50% and normal sperm morphology=30%. The case group consisted of men with asthenozoospermia (n=15) (age 31.33 ± 4.84 yr), asthenoteratozoospermia (n=16) (age 34.31 ± 5.20 yr) and oligoasthenoteratozoospermia (n=15) (age 35.75 ± 5.33 yr). The control group consisted of 16 healthy normozoospermic males (age 32.06 ± 3.91 yr). Samples were considered asthenozoospermic when motility was $<50\%$ and sperm concentration and morphology were normal. Samples with sperm concentration 20×10^6 /ml of ejaculate, motility $<50\%$ and morphology 30% were considered asthenozoospermic. Samples with sperm concentration 20×10^6 /ml of ejaculate, motility $<50\%$, morphology $<30\%$ were considered asthenoteratozoospermic. Samples with sperm concentration $<20 \times 10^6$ /ml of ejaculate, motility $<50\%$ and morphology $<30\%$ were considered oligoasthenoteratozoospermic. Liquefied semen samples were centrifuged at 3000g for 10 minutes. The supernatant seminal plasma was then frozen at -80°C until examination.

Free 8-Isoprostane purification : Purification of Free 8-Isoprostane was carried out using commercially available affinity column (Cayman Chemical, Ann Arbor, MI, USA). All samples were centrifuged at 15000g for isolating of particulates and precipitates. Then the supernatant was diluted 1:5 with column buffer and applied to the column. Other procedures were according to the instructions provided by the manufacturer. The ethanol washed 8-Isoprostane was stored at -80°C until measuring it.

Free 8-Isoprostane measurement : At first, the elution solution was evaporated to dryness using a vacuum centrifugation. Then, the concentration of 8-Isoprostane was measured using commercially available enzyme immunoassay

(EIA) method (Cayman Chemical, Ann Arbor, MI, USA). The procedure for the EIA was according to the instructions provided by the manufacturer. The sample volume that used was 50 ml. Absorbance was measured at a wavelength of 405 nm using enzyme-linked immunosorbent assay (ELISA) reader (STAT FAX 2100, USA). The levels of 8-Isoprostane are presented as ng/ml. The intra-assay coefficient of variation was $<10\%$. The sensitivity and specificity of the 15-F_{2t}-isoprostane assay were 5 pg/ml and 100%, respectively (Cayman Chemical, Ann Arbor, MI, USA).

Data analysis : Differences between groups were assessed using Mann-Whitney U test and Kruskal-Wallis test. Coefficients of correlation were calculated using Spearman's correlation analysis. All hypothesis tests were two-tailed with statistical significance assessed at the p value <0.05 level with 95% confidence intervals. The data are expressed as the Mean $\pm$ SEM. Statistical computations were calculated using SPSS 11.5 for windows software (SPSS Inc, Chicago, IL, USA).

RESULTS

Seminal parameters of the subjects are reported in Table 1. Seminal plasma levels of free 8-Isoprostane of the specimens are shown in Table 2. Asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic males had significant higher values of free 8-Isoprostane when compared to normozoospermic males. Seminal plasma free 8-Isoprostane levels were also significantly higher in total case group (n=46) than the control group (18.23 ± 3.56 vs 2.6 ± 0.38 ng/ml). No significant changes were seen in seminal plasma levels of free 8-Isoprostane when asthenozoospermic, asthenoteratozoospermic and oligoasthenoteratozoospermic males were compared among themselves. We further examined the correlation between seminal parameters and seminal plasma free 8-Isoprostane levels. Seminal plasma levels of free 8-Isoprostane showed an inverse correlation with

Table 1: Seminal parameters in normozoospermic (N), asthenozoospermic (A), asthenoteratozoospermic (AT) and oligoasthenoteratozoospermic (OAT) males.

Subjects	Concentration (10^6 /ml)	Motility (%)	Morphology (%)
N (n=16)	97.06 ± 5.38	57.81 ± 1.39	39.62 ± 1.04
A (n=15)	62.40 ± 1.90	46.07 ± 0.30	31.07 ± 0.25
AT (n=16)	39.00 ± 2.75	36.75 ± 1.38	23.56 ± 0.89
OAT (n=15)	13.73 ± 1.19	18.53 ± 1.28	16.80 ± 0.33

Data are reported as Mean $\pm$ SEM; n= no. of cases.

Table 2: Seminal plasma levels of free 8-Isoprostane in normozoospermic (N), asthenozoospermic (A), asthenoteratozoospermic (AT) and oligoasthenoteratozoospermic (OAT) males

variable	N (n=16)	A (n=15)	AT (n=16)	OAT (n=15)
Free 8-Isoprostane (ng/ml)	6.95±2.10	14.66±4.10*	16.71±5.58**	3.42±8.36***

Data are reported as mean±SEM; n= no. of cases.

*p= 0.01, **p=0.008, and ***p=0.04 in comparison with normozoospermic men.

percentage progressively motile sperms ($r=-0.26$) (Fig. 1) and percentage normal sperm morphology ($r=-0.27$) (Fig. 2).

DISCUSSION

The most relevant finding from this study was a significant increase in seminal plasma levels of free 8-Isoprostane in males with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia compared to normozoospermic men. Our results are agreed with some previous studies that show increasing of lipid peroxidation by measuring MDA in sperm and seminal plasma in males with asthenozoospermia, asthenoteratozoospermia and oligoasthenoteratozoospermia (13, 15). Similar to MDA (11, 16), 8-Isoprostane also showed an indirect correlation with sperm motility.

MDA is widely used index of lipid peroxidation due to its simplicity. The TBARS test application to body fluids and tissue samples is unreliable. Application of a gas chromatography/mass spectrometry (GC/MS) assay for MDA has indicated that the commonly used TBARS assay overestimates the actual MDA levels by more than 10-fold, possibly resulting from cross

reactivity with other aldehydes and the harsh conditions used in sample preparation (18).

Recent studies have focused on 8-Isoprostane as an index of lipid peroxidation. Isoprostanes are formed in situ in cell membranes following free radical attack on the arachidonic acid. Unlike prostaglandins, which are formed from arachidonic acid following its release from the *sn*-2 position of phospholipids by phospholipase A2, isoprostanes are formed initially in situ, where they may contribute to the effects of oxidative stress on membrane biophysics. Measurement of 8-Isoprostane may provide a reliable marker of lipid peroxidation in vivo, because, it is a stable compound. In addition, 8-Isoprostane is specific product of free radical-induced lipid peroxidation. 8-Isoprostane has also been found to be present in detectable quantities in all normal biological tissues and in free form in all normal biological fluids. This is important because it allows the definition of a normal range such that small increase in its formation can be detected in situations of mild oxidant stress. Finally, the levels of 8-Isoprostane is unaffected by lipid content of the diet (18, 20).

Evidence is beginning to emerge suggesting that isoprostanes are not only markers of oxidative injury, but active participants in the pathophysiology of some disorders. The capacity of isoprostanes to readily esterify cell lipid membranes and the resulting marked distortion of membrane structure and function, undoubtedly contribute to their pathophysiologic potential. As well, the existence of specific receptor for isoprostanes has been proven (18, 25). As isoprostanes are biologically active, they may have significant role in the etiology of some sperm abnormality. A significant limitation of the present study was that we measured 8-Isoprostane only in

Fig 1 : Correlation between sperm motility and seminal plasma levels of free 8-Isoprostane ($r=-0.26$).

Fig 2 : Correlation between normal sperm morphology and seminal plasma levels of free 8-Isoprostane ($r=-0.27$).

the seminal plasma. Another limitation of our study was a small sample size. However, the results of our study could provide a database for further research in this area. Therefore, we propose further study with higher sample size and measuring 8-Isoprostane in both spermatozoa and seminal plasma to investigate whether it could be considered as a specific biomarker of lipid peroxidation in spermatozoa.

ACKNOWLEDGEMENT

This research was supported by a grant from the Drug Applied Research Center of Tabriz University of Medical Sciences.

REFERENCES

1. Agarwal A, Saleh RA. Role of oxidants in male infertility: rationale, significance, and treatment. *Urol Clin North Am* 2002;29:817-27.
2. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 2003;79: 829-43.
3. Gil-Guzman E, Ollero M, Lopez MC, Sharma RK, Alvarez JG, Thomas AJ Jr, *et al.* Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. *Hum Reprod* 2001;16: 1922-30.
4. Sanocka D, Kurpysz M. Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol* 2004;2:12.
5. Zarghami N, Khosrowbeygi A. Seminal plasma levels of 15-F2a-isoprostane, malondialdehyde and total homocysteine in normozoospermic and asthenozoospermic males. *Ind J Clin Biochem* 2005;20:86-91.
6. Agarwal A, Prabakaran SA. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Ind J Exp Biol* 2005;43:963-74.
7. Baker MA, Aitken RJ. Reactive oxygen species in spermatozoa: methods for monitoring and significance for the origins of genetic disease and infertility. *Reprod Biol Endocrinol* 2005;3:67.
8. Agarwal A, Prabakaran S, Allamaneni SS. Relationship between oxidative stress, varicocele and infertility: a meta-analysis. *Reprod Biomed Online* 2006;12:630-33.
9. Agarwal A, Gupta S, Sikka S. The role of free radicals and antioxidants in reproduction. *Curr Opin Obstet Gynecol* 2006;18:325-32.
10. Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. *J Androl* 1996;17:530-37.
11. Gomez E, Irvine DS, Aitken RJ. Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. *Int J Androl* 1998;21:81-94.
12. Rhemrev JP, Vermeiden JP, Haenen GR, De Bruijne JJ, Rekers-Mombarg LT, Bast A. Progressively motile human spermatozoa are well protected against in vitro lipid peroxidation imposed by induced oxidative stress. *Andrologia* 2001;33:151-8.
13. Fraczek M, Szkutnik D, Sanocka D, Kurpysz M. Peroxidation components of sperm lipid membranes in male infertility. *Ginekol Pol* 2001;72:73-9.
14. Dandekar SP, Nadkarni GD, Kulkarni VS, Puneekar S. Lipid peroxidation and antioxidant enzymes in male infertility. *J Postgrad Med* 2002;48:186-9.
15. Nakamura H, Kimura T, Nakajima A, Shimoya K, Takemura M, Hashimoto K, *et al.* Detection of oxidative stress in seminal plasma and fractionated sperm from subfertile male patients. *Eur J Obstet Gynecol Reprod Biol* 2002;105:155-60.
16. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghazzi H, Hammami S, *et al.* Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2003;49:83-94.
17. Tavilani H, Doosti M, Saeidi H. Molondialdehyde levels in sperm and seminal plasma of asthenozoospermic and its relationship with semen parameters. *Clin Chim Acta* 2005;356:199-203.
18. Meagher EA, FitzGerald GA. Indices of lipid peroxidation in vivo: strengths and limitations. *Free Radic Biol Med* 2000;28:1745-50.
19. Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med* 2000;28:505-13.
20. Pratico D, Lawson JA, Rokach J, FitzGerald GA. The isoprostanes in biology and medicine. *Trends Endocrinol Metab* 2001;12: 243-7.
21. Shibahara H, Suzuki T, Obara H, Hirano Y, Onagawa T, Taneichi A, *et al.* Accuracy of the normal sperm morphology value by Sperm Quality Analyzer IIC: comparison with the strict criteria. *Int J Androl* 2002;25: 45-8.
22. Suzuki T, Shibahara H, Tsunoda H, Hirano Y, Taneichi A, Obara H, *et al.* Comparison of the Sperm Quality Analyzer IIC variables with the computer-aided sperm analysis estimates. *Int J Androl* 2002;25:49-54.
23. Zalata AA, Christophe AB, Depuydt CE, Schoonjans F, Comhaire FH. The fatty acid composition of phospholipids of spermatozoa from infertile patients. *Mol Hum Reprod* 1998;4:111-8.
24. Cavallini G. Male idiopathic oligoasthenoteratozoospermia. *Asian J Androl* 2006;8:143-57.
25. Habib A, Badr KF. Molecular pharmacology of isoprostanes in vascular smooth muscle. *Chem Phys Lipids* 2004;128: 69-73.