ORIGINAL ARTICLE

Annals of Agricultural and Environmental Medicine 2013, Vol 20, No 4, 865–868
www.aaem.pl

Biological and environmental conditionings for sperm DNA fragmentation

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Bojar I, Witczak M, Wdowiak A. Biological and environmental conditionings for sperm DNA fragmentation. Ann Agric Environ Med. 2013; 20(4): 865–868.

Abstract

The objective of the presented study was determination of the effect of selected agents on sperm DNA fragmentation – superoxide dismutase in seminal plasma, the patients' age, and burdening with the tobacco smoking habit. An attempt was also undertaken to evaluate the effect of DNA fragmentation on the effectiveness of infertility treatment.

The study covered 186 men who received treatment due to infertility. The database and statistical analyses were performed using computer software STATISTICA 7.1.

A relationship was observed between sperm DNA fragmentation and superoxide dismutase activity, the higher the SOD activity, the lower the percentage of sperm fragmentation (rs=−0.324; P=0.000; r = −0.2110). A statistical relationship was found between sperm DNA fragmentation and the percentage of pregnancies obtained during the first year of treatment – patients with the lower DFI more frequently became fathers during the first year of trying, compared to the remainder (t=2.51; P=0.013). A statistically significant relationship was confirmed (rs=−0.370; P=0.000) consisting in an increase in the DFI with respondents' age. No significant differences were noted between the DFI and the tobacco smoking habit (Chi2=0.29; P=0.926).

The percentage of sperm DNA fragmentation was inversely proportional to superoxide dismutase activity in seminal plasma. DNA fragmentation becomes intensified with patients' age. Cigarette smoking has no effect on sperm DNA fragmentation. DNA fragmentation exerts an effect on the effectiveness of infertility treatment.

Key words

DNA damage, infertility, semen, superoxide dismutase

INTRODUCTION

The possession of offspring is the most important human biological goal which conditions the survival of the human species. The problem of the lack of offspring is a phenomenon which concerns approximately 15% of married couples in Poland. In a half of the cases, the causative agent is the male factor. There is evidence that certain states of male fertility problems are associated with genetic disorders, as well as with an excessive production of reactive oxygen species. An appropriate concentration of reactive oxygen intermediates is regulated by means of antioxidant enzymes, to which belong, among others, superoxide dismutase (SOD). Disorders in the balance in the oxidoreduction system in the human body may cause an excessive peroxidation of lipids, destruction of cellular membranes and organelles, as well as damage to enzymes and nucleic acids [1]. Tobacco smoking is also an agent weakening the system of the body's defence against oxidants. As a result of nicotinism there occur modifications of nitrogenous bases and formation of DNA adducts. The level of natural antioxidants in blood plasma decreases (vit. C and vit. E), and there is an increase in lipid peroxidation (F2-isoprostanes) in the cardiovascular system. The process of ageing is inseparably associated with the weakening of human antioxidant defence. With age, an increase occurs in the number of DNA lesions, cholesterol deposits, lipid peroxidation, and atherosclerosis intensifies. An elevated cell membrane lipid peroxidation with age deteriorates the fluidity and functions of the cell and mitochondrial membranes. This leads to electron leakage and disorders in energy production [2].

It is considered that in approximately 20% of patients with idiopathic infertility and elevated level of sperm DNA fragmentation may be the cause of failure in reproduction [1, 2]. The quality of the genetic material carried in male sperm becomes a prognostic factor in the area of the effectiveness of treatment of an infertile couple, bearing a healthy child, as well as the risk of contracting cancer in the future generations [3].

The objective of the presented study was determination of the effect of selected agents on sperm DNA fragmentation – superoxide dismutase in seminal plasma, the patients' age, and burdening with the tobacco smoking habit. An attempt was also undertaken to evaluate the effect of DNA fragmentation on the effectiveness of infertility treatment.

MATERIAL AND METHOD

The presented study was conducted in the Non-Public Health Care Unit 'Ovum Reproduction and Andrology' in Lublin, and covered 186 men treated due to infertility. The patients had no history of other diseases which could disturb fertility and semen parameters. Patients qualified for *in vitro* fertilization were also excluded from the study. The sperm was obtained by way of masturbation, and examined directly

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Received: 20 February 2012; accepted: 2 July 2013

after liquidation according to the WHO standards. Seminal plasma was obtained by centrifugation of the cellular elements. SOD activity was determined spectrophotometrically as the degree of inhibition of the competitive reaction using RANSOD kit (Randox Laboratories Ltd.). The structure of sperm chromatin was evaluated using the technique of flow cytometry – Sperm Chromatin Structure Assay (SCSA). The result of the examination was the sperm DNA Fragmentation Index (DFI), i.e., the percentage of sperm with DNA lesions (DNA fragmentation). The percentage of pregnancies obtained in the study group was also analyzed.

The studies were approved by the Ethics Committee. All patients gave their informed consent to participate in the study.

The sperm DNA Fragmentation Index was from 7%-32%. The patients were divided into 4 groups: Group A – DFI below 15% (normal percentage of sperm without chromatin damage), Group B – DFI from 15%-19% (moderate chromatin damage), Group C – DFI from 20%-25% (considerable chromatin damage), and Group D – DFI over 25% (severe chromatin damage). The SOD activity in the study group was from 0.28–6.7 U/mg. The patients were divided into 3 groups according to the SOD activity in sperm plasma: Group 1 – values below 0.34 U/mg (considerably decreased SOD activity), Group 2 – from 0.35–0.49 U/mg (normal SOD activity), and Group 3 – over 0.5 U/mg (high SOD values). After one year of infertility treatment, in the group examined, 151 pregnancies were obtained, while in 35 cases the therapy was unsuccessful. In the group of males in the study, 35 were aged under 26, 109 – 26–35 years old, and 41 were older. 72.58% of the patients examined were non-smokers, whereas the remaining 27.42 were smokers.

The results of the studies obtained were subjected to statistical analysis. The values of the parameters analyzed were determined by means of frequency and percentage. For uncorrelated nominal variables, in order to investigate differences between the classes compared, the c2 goodness of fit test was applied. The relationships between the values examined were analyzed by means of the c2 test for independence. The P values P<0.05 were considered statistically significant. The database and statistical analysis were performed using computer software STATISTICA 7.1 (StatSoft).

RESULTS

Table 1 and Figure 1 present the level of sperm DNA fragmentation according to superoxide dismutase activity. A relationship was observed between sperm DNA fragmentation and superoxide dismutase activity – the higher the SOD activity, the lower the percentage of sperm fragmentation (rs=–0.324; P=0.000; r =–0.2110).

Table 1. Relationship between DFI and SOD activity

DFI	SOD activity below 0.34 U/mg		SOD activity 0.35–0.49 U/mg		SOD activity over 0.5 U/mg	
	n	%	n	%	n	%
Below 15%	16	26.67	48	48.98	19	70.37
15%–19%	14	23.33	20	20.41	4	14.81
20%–25%	18	30.00	17	17.35	3	11.11
Over 25%	12	20.00	13	13.27	1	3.70
Total	60	100.00	98	100.00	27	100.00
M+SD	10.57±4.42		8.20±4.81		6.19+4.18	

rs=–0.324; P=0.000
DFI, DNA Fragmentation Index; SOD, superoxide dismutase.

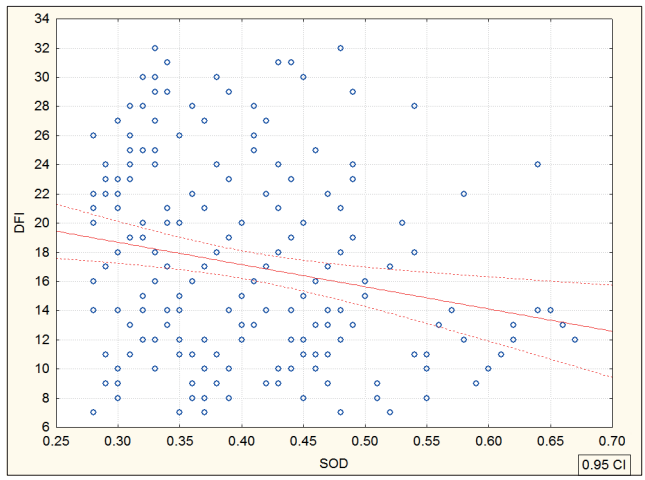

Figure 1. The level of sperm DNA fragmentation according to superoxide dismutase activity

Table 2. Relationship between DFI and percentage of pregnancies obtained after one year of infertility treatment.

DFI	Lack of pregnancy after one year of treatment		No. of pregnancies during 1 year of treatment	
	n	%	n	%
Below 15%	8	22.86	75	49.67
15%–19%	10	28.57	29	19.21
20%–25%	10	28.57	28	18.54
Over 25%	7	20.00	19	12.58
Total	35	100.00	151	100.00
M+SD	10.49±4.64		8.26±4.74	

t=2.51; P=0.013
DFI – DNA Fragmentation Index.

Table 2 presents the effectiveness of infertility treatment according to sperm DNA fragmentation. A statistically significant relationship was confirmed between sperm DNA fragmentation, and the percentage of pregnancies obtained during the first year of treatment; patients with a lower DFI more frequently became fathers during the first year of trying, compared to the remainder (t=2.51; P=0.013).

Table 3 shows the relationship between the percentage of sperm fragmentation and the patients' age. A statistically significant relationship was observed (rs=–0.370; P=0.000), in that DFI increased with age.

Table 3. Relationship between DFI and patients' age.

DFI	Age over 35		Age 26–35		Age under 26	
	n	%	n	%	n	%
Under 15%	9	21.95	49	44.95	25	71.43
15%–19%	7	17.07	29	26.61	3	8.57
20%–25%	14	34.15	19	17.43	4	11.43
Over 25%	11	26.83	12	11.01	3	8.57
Total	41	100.00	109	100.00	35	100.00
M±SD	11.49±4.58		8.39±4.44		6.20±4.59	

rs=–0.370; P=0.000
DFI, DNA Fragmentation Index.

Table 4. Relationship between DFI and tobacco smoking habit.

DFI	Non-smoker		Smoker	
	n	%	n	%
Below 15%	61	45.19	22	43.14
15%–19%	29	21.48	10	19.61
20%–25%	27	20.00	11	21.57
Over 25%	18	13.33	8	15.69
Total	135	100.00	51	100.00
M+SD	8.63±4.68		8.80±5.13	

Chi^2=0.29; P=0.926
DFI, DNA Fragmentation Index.

Table 4 presents sperm DNA fragmentation in the groups of smoking and non-smoking males; no significant differences were found between the DFI and burdening with the tobacco smoking habit (Chi2=0.29; P=0.926).

DISCUSSION

Many scientific studies suggest that a decrease in male fertility is frequently associated with genetic abnormalities, which may cause disorders of balance in the oxidoreductive system [3, 4]. The harmful effect of the reactive oxygen species may occur at various stages of the process leading to an effective reproduction. Among the causes of sperm DNA fragmentation is lipid peroxidation of cell membranes by the reactive oxygen species [1].

Antioxidant enzymes are an important form of cellular defence against damage caused by reactive oxygen species, to which belongs, among others, superoxide dismutase.

The results obtained in the presented study are consistent with the results obtained by Atig *et al.* [5] who observed an elevated DFI and decreased activity of enzymes of the oxidoreductive system in a group of patients treated for infertility, compared to the control group, which confirms the effect of disorders in the oxidoreductive system on the intensification of sperm DNA fragmentation. Similar conclusions were drawn by Tartibian *et al.*, [6] who compared semen parameters in healthy males practicing sports recreationally, and those engaged in professional sports.

The researchers found that DNA fragmentation decreased with an increase in SOD in seminal plasma. Similar results were obtained by Omran *et al.*, [7] who confirmed smaller chromatin damage in the spermatozoa of men whose semen had a higher density, motility, and activity of the oxidoreductive enzymes. In the presented study, a greater effectiveness of infertility treatment was observed among patients with lower DNA fragmentation, which may be related with the SOD activity and which, according to Shiva *et al.*, [8] and also Shamsi *et al.*, [9], is responsible for sperm motility and density of semen, thus explaining the results obtained in the presented study. In studies performed by Bungum *et al.* [2], sperm DNA fragmentation as measured by SCSA proved to be an independent predictor of successful pregnancy in first pregnancy planners, as well as in couples undergoing intrauterine insemination, and can be used as a tool in investigation, counselling and treatment of involuntary childlessness, which is consistent with the presented results. Similar values of the level of superoxide dismutase activity were obtained by Murawski *et al.*, [10] and Zelen *et al.* [11] who confirmed its decrease in infertile males. Ménézo *et al.*, [12], while investigating the oral supplementation of antioxidants, noted a decreased DNA fragmentation in males taking these dietary supplements, compared to the control group, which may indirectly explain the results of the presented study. Acosta *et al.* [13] carried out an experiment by the addition of substances with activity similar to SOD, and obtained better results in freezing and refreezing of animal sperm from alpaca, and improved the motility parameters. Studies concerning other animals – bulls, were conducted by Nagy *et al.* [14] The researchers found a relationship between DNA fragmentation and semen parameters – decreased semen parameters accompanied an increased DNA fragmentation. In a study conducted by Erenpreiss *et al.* [15], 97 men (28% of the whole study group) had a DFI>20%, and 43 men (12%) had a DFI>30%. The presented study shows similar results: 20.43% of the whole study group had a DFI>20%, and 13.98% had a DFI>25%. Borini *et al.* [16] obtained results concerning the effectiveness of infertility treatment according to DFI similar to those in the presented study; however, his studies were conducted among patients undergoing IVF therapy. The presented study did not show any relationship between tobacco smoking and sperm DNA fragmentation; nevertheless, it should be presumed that such relationships may exist – Kłuciński *et al.* [17] noted decreased SOD in smokers, which could indirectly affect DFI, also Li *et al.* [18] confirmed an unfavourable effect of smoking on the sperm's cell membrane. Elshal *et al.* [19] proved that the classical semen parameters were negatively correlated with lipid peroxidation in spermatozoa; motility and morphology were negatively correlated with DFI% (P<0.05), DFI% was significantly higher in the infertile smokers group than in infertile non-smokers (P=0.032). Wu *et al.* [20] investigated a decrease in semen parameters in smokers. Zhu *et al.*, [21] in the studies conducted on mice exposed to tobacco smoke, observed an intensification of oxidative stress and deterioration of semen parameters. Patients who were smokers constituted 27.41% of the patients examined, which on the background of the studies carried out in the same region by Panasiuk *et al.*, [19] who, in an analogous age group, found 35.6% of smokers, which may evidence a purposeful discontinuation of smoking by some males planning the conception of a child in the group examined in this study. Encisio *et al.*, [22] in their studies observed an elevated percentage of abnormalities in genetic tests in males with DNA fragmentation, increased beyond the standard, which was confirmed by the studies by Khadem *et al.* [23] carried out among males whose female partners chronically miscarried. Dobrzyńska *et al.* [4] observed a deterioration of sperm motility parameters with an increase in DNA damage, whereas Pan *et al.* [24], in their studies, did not unequivocally confirm any relationship between DFI and sperm motility, which may suggest that the scope of studies of the causes of sperm DNA fragmentation will require further research.

CONCLUSIONS

1. The percentage of sperm DNA fragmentation was inversely proportional to superoxide dismutase activity in seminal plasma.
2. DNA fragmentation becomes intensified with patient's age.

3. Cigarette smoking has no effect on sperm DNA fragmentation.
4. DNA fragmentation exerts an effect on the effectiveness of infertility treatment.

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