

Oxidative Stress and Male Infertility in the Region of Peja in Republic of Kosovo

Afrim Zeqiraj¹, Sheqibe Beadini², Nexhbedin Beadini², Zafer Gashi¹, Sadi Bexheti³, Shkelzen Elezaj⁴, Sanije Berisha⁵ and Agim Shabani⁶

¹Department of Biochemistry, FAMA Colleague, Republic of Kosovo, Kosovo

²Department of Biology, University of Tetovo, Republic of Macedonia, Macedonia

³Faculty of Medicine, University of Tetovo, Republic of Macedonia, Macedonia

⁴Clinic of Urology, Regional Hospital of Peja, Republic of Kosovo, Kosovo

⁵Department of Biochemistry, University clinical center, Tetovo, Republic of Macedonia, Macedonia

⁶Department of Chemistry, University of Tetovo, Republic of Macedonia, Macedonia

ABSTRACT

Introduction: Oxidative stress is a pathology that is found in 40-50% of all infertile males. Oxidative stress occurs when the production of peroxides exceeds the antioxidant protection of the organism resulting in damage to the sperm. Oxidative stress is known as one of the most important causes of male infertility. Purpose of the work, it is to determine the degree of male infertility in infertile males caused by oxidative stress. The study was conducted in Regjion of Peja, in the Republic of Kosovo.

Materials and methods: Sampling was conducted for a period of 2017/2018, a total of 77 samples, 47 samples for analysis and 30 samples for control were collected. Oxidative stress analysis in the ejaculate was performed at the Biolab-Zafi Laboratory in Peja, Republic of Kosovo. Oxidative stress analysis is performed with the aid of the Oxisperm/Halotech, S.L. Madrid, Spain. The importance of the presentation is at ($p < 0.05$).

Results: The results show significant levels ($p < 0.004$) of oxidative stress between the working group and the control group.

Conclusion: In our paper we have found high significance of oxidative stress in infertile male ejaculation compared to the control group of patients taken in the study. For determining the oxidative stress regensi Oxisperm/Halotech is selected because of ease of use, both in terms of practice as well as cost. An assessment of the levels of oxidative stress in men's ejaculation should become part of routine work in clinical andrology laboratories to assist clinicians in determining infertility status and in establishing optimal medical treatment. We acknowledge that this study is a new technique in our country and requires more general studies and should be repeated by other authors and compared with other methods for evaluating sperm DNA damage. It is also necessary to make a link between oxidative stress and fragmented DNA in the sperm.

Keywords: Oxidative stress (OS), DNA-fragmentation, Oxisperm/halotech

INTRODUCTION

Oxidative stress is a pathology that is found in 40-50% of all infertile males. Oxidative stress occurs when the production of peroxides exceeds the antioxidant protection of the organism resulting in damage to the sperm [1,2]. Oxidative stress is known as one of the most important causes of male infertility [3,4]. The sperm can create ROS in two ways:

1. Nicotinamide adenine dinucleotide phosphate (NADPH) at the level of the spermatozoid plasma membrane,

2. Nicotinamide adenine dinucleotide (NADH) dependent on oxidation-reduction at the level of mitochondria [5].

The sperm is rich in mitochondria because they need constant energy supply for their movement. Increasing the production of ROS in non-dysfunctional mitochondria affects the motion and function of the sperm. Such a relationship may be due to two mutually related phenomena - ROS, causing damage to the mitochondrial membrane, where these mitochondrial membrane damage causes increased ROS production [5]. The cells are susceptible to reactive oxygen species (ROS) attacks. When manipulated *in vitro* during assisted reproductive techniques, these cells are at risk of generating and exposing to the physiological effect of ROS [6]. Deficiencies in sperm cells of ejaculate are the most common causes of male infertility [7]. Many environmental, physiological and genetic factors have been implicated in the function of spermatozoa and infertility [8,9]. Thus, it is very important to identify factors that affect normal sperm functions. Among the various causes, oxidative stress (OS) is attributed to affect the fertility status and sperm physiology [10]. The term oxidative stress is generally applied when oxidants exceed antioxidants [7]. The imbalance between the production of reactive oxygen species (ROS) and the ability of biological and physiological systems to repair the damage caused is known as oxidative stress [10]. Agents The main aspects of oxidative stress are the production of ROS, which includes free radicals and peroxides [11]. The free radicals are short reactive chemical intermediates, which contain one or more unsupported electrons [12,13]. They promote cellular damage when passing this unwavering electron to nearby cellular structures, resulting in lipid cell membrane oxidation, protein amino acids, or within nucleic acids [14]. Free radicals are also recognized as bad for intra-cellular signaling involved in the normal proliferation, differentiation and cell migration process [15,16]. In the reproductive tract, free radicals also play a dual role and can regulate different reproductive functions [6]. The growth of free radicals in most cases involves spermatogenesis error resulting in the release of spermatozoa from the germinal epithelium with high levels of cytoplasmic retention [17]. ROS are formed as the necessary by-products during the normal enzymatic inter-and cellular signaling reactions. Because of their very reactive nature, ROS can easily combine with other molecules, causing direct oxidation that can lead to structural and functional changes and result in cellular genetic damage [17,18]. Types of reactive nitrogen (nitrogen oxides, peroxyxynitrite, nitroxyl, etc.) are free radicals of nitrogen and are considered as a subclass of ROS [19]. Nitric Oxide (NO) has been shown to have adverse effects on normal sperm functions by preventing sperm mobility and ability to bind to the pellucida area [10]. Sperm dysfunction is related to increased number of leukocytes, especially neutrophils and macrophages, causing excess production of ROS [19,20]. Two of the main factors contributing to ROS accumulation in *in vitro* conditions are the lack of endogenous protective mechanism and the excretion of gametes and embryos in various manipulation techniques that can lead to oxidative stress generation [21]. ROS levels may rarely increase within the ejaculate of a fertile male, but this increase does not affect sperm concentration and mobility. This may be due to the presence of adequate antioxidant protection mechanisms for healthy individuals. Movements at ROS levels may be due to temporary subclinical infection and temporary temporal abnormalities of spermatogenesis such as cytoplasmic retention or periodic admission of abnormal sperm into sperm [22]. The middle part of the sperm is covered by mitochondria that generates energy from the ATP cellular nuclear depot [23]. The excess ROS damages the mobility of the sperm and the fertilization capacity. In addition, ROS plays an important role in the formation of membranes by determining the dynamic status that affects the joining of the plasma membrane of male and female gamete [23]. Nowadays the assumption that ROS can affect male fertility has received considerable scientific support from the world [19]. The sperm membranes are rich in polyunsaturated fatty acids (PUFAs) and are susceptible to ROS attack which results in the slowing of the sperm movement, apparently by a rapid loss of intracellular ATP leading to a reduction in the sustainability of the spermatozoa sperm and growth of morphological defects in spermatozoa, adverse effects on sperm ability and acrosome reaction [6]. Thus ROS are independent markers of male infertility [24].

PURPOSE OF THE WORK

It is to determine the degree of male infertility caused by oxidative stress in the ejaculate. The study was conducted in the Region of Peja in Republic of Kosovo.

MATERIALS AND METHODS

Sampling was conducted for a period of 2017/2018, a total of 77 samples. 47 samples for analysis and 30 samples

for control were collected. The age of the patients taken for analysis is 20-45 years, whereas the age of the patients received for the control group was 22-35 years. Oxidative stress analysis in the ejaculate was performed at the Biolab-Zafi Laboratory in Peja, Republic of Kosovo. Patients received: Name, surname, year of birth, a period of infertility (years, primary or secondary infertility) and collection of ejaculate for analysis. The oxidative stress analysis of DNA was performed according to the procedure mentioned in the instruction of the Oxisperm/Halotech DNA reagent, S.L. Madrid, Spain. First, agarose ependorf tubes are placed in the water for 5 min at 90-100°C until the agarose is fluidized, then the agarose ependorf tube is placed in the water bath at 37°C and left for 5 min, the mixture is taken up into 35 µL of ejaculate and placed on the tubes of the ependorf in the refrigerator for 5 min to solidify the agarose. Then the ependorf epithelial is obtained and settled at 37°C for 45 min and finally the color obtained in the ependorf is compared to the colors in the Oxisperm/Halotech DNA reagent guide. In the reagent guide are divided four levels of oxidative stress (L1-L4). The importance of the presentation is at $p < 0.05$ (Figure 1).

RESULTS

From the results obtained and presented in Table 1, we found significant levels ($p < 0.004$) of oxidative stress between the working group and the control group. From the results obtained in our study, we have found high levels of oxidative stress in all patients with leukocyte values up > 1 million in semen. In our study, we have not found high levels of oxidative stress in patients with under < 1 million leukocytes in semen (Table 1 and Figure 2).

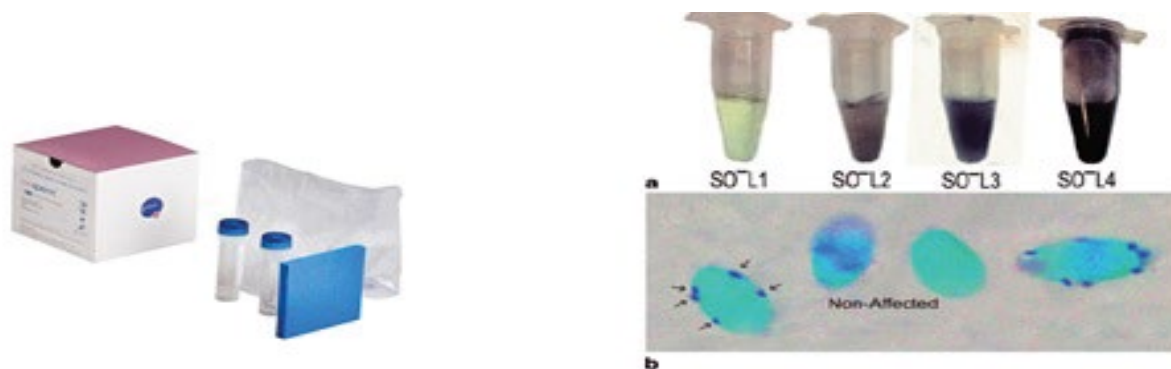


Figure 1: Oxidative stress analysis

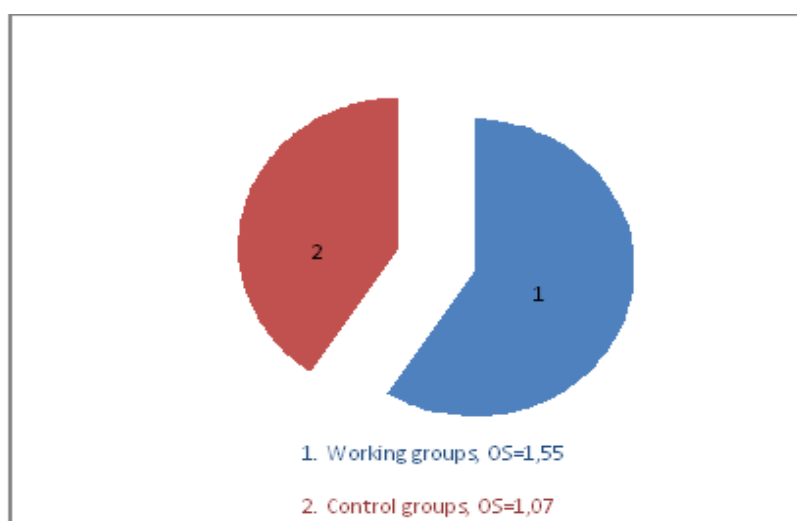


Figure 2: Presentation of acquired values of oxidative stress in infertile male ejaculation in Region of Peja, in the Republic of Kosovo

Table 1: Acquired values of oxidative stress in infertile male ejaculation in Region of Peja in the Republic of Kosovo

	Working groups [47 patients]	Control groups [30 patients]	t-test	Significant $p < 0.05$	S-significant N-no significant
Oxidative stress	Average/Std 1,55 ± 0,82	Average/Std 1,07 ± 0,26	2,814	< .004	S

DISCUSSION

From our study results we have gained high frequency of oxidative stress (L2-L4) at significant levels ($p < 0.004$) in infertile males compared with male males, results that matched the results obtained from Lu et al. [25]. Our results obtained at significant levels of oxidative stress are consistent with the results obtained by Badade et al. [26], which have found high frequency of oxidative stress in ejaculate of patients with oligoasthenospermia compared to the control group. The results of our study correlate with the results obtained from Athayde et al. [27], which found high frequency of significant oxidative stress ($p < 0.001$) in ejaculate samples of patients with leukocytospermia. Also Sharma [28], have found high frequency of significant oxidative stress ($p = 0.001$) in ejaculate samples of patients with leukocytosis, which are consistent with our study results. Despite the antioxidant activity of seminal plasma, epididymis, and spermatozoa, oxidative stress undermines the function and integrity of the DNA molecule [29,30]. The findings of these authors are consistent with our results we found in our paper that patients who have been diagnosed with varicocele have dominated cases with oxidative stress in values (L2, L3).

CONCLUSION

In our paper we have found high significance of oxidative stress in infertile male ejaculation compared to the control group of patients taken in the study. For determining the oxidative stress is selected regensi Oxisperm/Halotech because of ease of use, both in terms of practice as well as cost. An assessment of the levels of oxidative stress in men's ejaculation should become part of routine work in clinical andrology laboratories to assist clinicians in determining infertility status and in establishing optimal medical treatment. We acknowledge that this study is a new technique in our country and requires more general studies and should be repeated by other authors and compared with other methods for evaluating sperm DNA damage. It is also necessary to make a link between oxidative stress and fragmented DNA in the sperm.

REFERENCES

- [1] Bozhedomov VA, Gromenko DS. Oxidative stress of spermatozoa in pathogenesis of male infertility. *Urologia*, **2009**, 51-56.
- [2] Tremellen K. Oxidative stress and male infertility. *Hum Reprod*, **2008**, 14: 243-258.
- [3] Desai N, Sabanegh Jr, Kim T, Agarwal A. Free radical theory of aging. *Urology*, **2009**, 14-19.
- [4] Tempest HG, Homa ST, Routledge EJ. Plants used in Chinese medicine for the treatment of male infertility possess antioxidant and anti-oestrogenic activity. *Biol Reprod*, **2008**, 54: 185-195.
- [5] Gavella M Lipovac V. NADH-dependent oxidoreductase [diaphorase] activity and isozyme pattern of sperm in infertile men. *Arch Androl*, **1992**, 28: 135-141.
- [6] Evenson DP, Darzynkiewicz Z, Melamed MR. Simultaneous measurement by flow cytometry of sperm cell viability and mitochondrial membrane potential related to cell motility. *J Histochem Cytochem*, **1982**, 30: 279-280.
- [7] du Plessis SS. Impact of oxidative stress on IVF. *Expert Review of Obstetrics*, **2008**, 3: 539-554.
- [8] Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. *Front Biosci*, **1996**, 1: 78-86.
- [9] Vine MF, Tse CK, Hu P, Truong KY. Cigarette smoking and semen quality. *Fertil Steril*, **1996**, 65: 835-842.
- [10] Kenkel S. Occupational risks for male fertility: An analysis of patients attending a tertiary referral centre. *Int J Androl*, **2001**, 24: 318-326.
- [11] Agarwal A. Role of reactive oxygen species in the pathophysiology of human reproduction. *Ferti Steril*, **2003**, 79: 829-843.
- [12] Valko M. Metals, toxicity and oxidative stress. *Curr Med Chem*, **2005**, 12: 1161-1208.
- [13] Kefer JC. Role of antioxidants in the treatment of male infertility. *Int J Urol*, **2009**, 16: 449-457.
- [14] Sanocka D. Reactive oxygen species and sperm cells. *Reprod Biol Endocrinol*, **2004**, 2: 12-26.

-
- [15] Ochsendorf FR. Infections in the male genital tract and reactive oxygen species. *Hum Reproduction*, **1999**, 5: 399-420.
- [16] Agarwal A. Role of antioxidants in treatment of male infertility. *Reprod BioMed*, **2004**, 16: 616-627.
- [17] Ford WC. Reactive oxygen species and sperm. *Hum Fertil*, **2001**, 77-78.
- [18] Guerin P. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Hum Reprod*, **2001**, 7: 175-189.
- [19] de Lamirande E, Gagnon C. Impact of reactive oxygen species on spermatozoa. *Hum Reprod*, **1995**, 10: 15-21.
- [20] Sikka SC. Relative impact of oxidative stress on male reproductive function. *Curr Med Chem*, **2001**, 8: 851-862.
- [21] Saleh RA, Agarwal A, Kandirali E. Leukocytospermia is associated with increased reactive oxygen species production by human spermatozoa. *Fertil Steril*, **2002**, 78: 1215-1224.
- [22] Desai NR, Mahfouz R, Sharma S, Gupta, Agarwal A. Reactive oxygen species levels are independent of sperm concentration, motility and abstinence in a normal, healthy proven fertile man: A longitudinal study. *Fertil Steril*, **2010**, 94: 1541-1543.
- [23] Bucak MN, Ates, s, ahin A, Yuce A. Effect of anti-oxidants and oxidative stress parameters on ram semen after the freeze-thawing process. *Small Rumin Res*, **2008**, 61: 128-134.
- [24] Bucak MN. The influence of trehalose, taurine, cysteamine and hyaluronan on ram semen. *Theriogenology*, **2007**, 67: 1060-1067.
- [25] Lu JC, Zhang. A survey on the status of semen analysis in 118 laboratories in China. *Asian J Androl*, **2010**, 12: 104-110.
- [26] Badade ZG, Kavita More. Oxidative stress adversely affects spermatogenesis in male infertility. *Biomed Res*, **2011**, 22: 323-328.
- [27] Athayde KS Cocuzza M, Agarwal A. Development of normal reference values for seminal reactive oxygen species and their correlation with leukocytes and semen parameters in a fertile population. *J Androl*, **2007**, 28: 613-620.
- [28] Sharma RK. Sperm DNA damage and its clinical relevance in assessing reproductive outcome. *Asian J Androl*, **2004**, 6: 139-148.
- [29] Lanzafame FM, Vignera SL. Oxidative stress and medical antioxidant treatment in male infertility. *Reprod Biomed*, **2009**, 19: 638-659.
- [30] Desai NE. Sabanegh Jr, Kim T, Agarwal A. Free radical theory of aging. *Urology*, **2009**, 14-19.