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The role of oxidative stress in premalignant lesions

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Summary

Purpose of Investigation: The authors aimed to evaluate serum total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) in women with abnormal cervical cytology, to determine the association between serum oxidant and antioxidant status of these women, and the progression of abnormal cervical cytology. *Materials and Methods:* A total of 75 women enrolled in the study: 20 women with a determination of atypical squamous cells of undetermined significance (ASCUS), 20 women with low squamous intraepithelial lesions (LSIL), 15 women with high squamous intraepithelial lesions (HSIL) and 20 healthy controls. Serum TOS and TAS were determined and OSI was calculated as the indicator of degree of oxidative stress. *Results:* Serum TOS levels and OSI were highest in the HSIL group and there was a trend toward increasing serum TOS levels and OSI from ASCUS to HSIL group. *Conclusion:* The authors demonstrated that increased oxidative stress with altered antioxidant level is associated with abnormal cervical cytology. Serum oxidant and antioxidant status may provide guidance as a simple and cost-effective method for follow-up, treatment, and recommendation in all stages of lesions.

Key words: Total oxidant stress; Premalignant lesions; Abnormal cervical cytology.

Introduction

Reactive oxygen species (ROS) are normally formed as a product of oxygen metabolism in normal cells from endogenous sources such as mitochondria, peroxisomes, and activated inflammatory cell or from exogenous sources such as environmental agents. However, ROS produced by cells is counterbalanced by antioxidant defences of the cells to protect cells from the detrimental effects of oxidative damage of ROS. There is a balance between the level of oxidants and normal biological antioxidants in cells. Under pathological conditions, increasing ROS production in cells or decreasing antioxidant defences of the cell, oxidative stress, occurs in the cells because of this imbalance. Thus, oxidative stress caused by increased accumulation of ROS may damage critical bio-molecules such as proteins, lipids, sugars, and DNA [1]. Finally, degenerative alterations in cells such as tissue degradation, carcinogenesis, and aging resulting from ROS is well-documented [2].

Transformation of a normal cell to a neoplastic cell is a multi-stage process [3]. DNA damage is the initial stage of neoplastic transformation [4-7]. The increased accumulation of ROS in the cell could cause DNA damage by mutagenic effects due to unrepaired damaged DNA, increased sensitivity to mutagenic agents, modulation of gene expression-oncogene activation and tumor suppressor gene inactivation, induction of several signaling cascades, and chemo-attractant production. The relationship between increased oxidative stress and the development of cervical cancer are well-documented and it has been reported that increase in oxidative stress positively correlates with the progression of cervical lesion from normal epithelium to CIN to invasive carcinomas [8-10].

In the present study, the authors aim to evaluate serum total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) in women with atypical squamous cells of undetermined significance (ASCUS), low squamous intraepithelial lesions (LSIL), and high squamous intraepithelial lesions (HSIL), to determine the association between serum oxidant and antioxidant status of these women, and the progression of the pre-malignant lesions.

Materials and Methods

Participants

A total of 75 women were selected for a new routine PAP smear screening and were included in this study. It was conducted at Department of Obstetrics and Gynecology, Bezmialem University Hospital, Istanbul, Turkey between December 2013 and October 2014. The case-control study protocol was approved by the Ethical Committee of the Medical Faculty of Bezmialem University. Written informed consent was obtained from all the participants. Inclusion criteria were healthy, sexually active women with no cervical lesion, not pregnant, and not lactating. Exclusion criteria were women who were consuming antioxidant vitamin or mineral supplements, that underwent immunosuppressant treatment or had comorbid medical conditions such as diabetes mellitus, liver disease, rheumatoid arthritis, and smoking.

Patients were divided into three groups according to PAP smear results classified by the Bethesda system. Group 1, ASCUS group, included 20 women with a determination of ASCUS; group 2,

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Table 1. — *Demographic characteristics of women.*

	ASCUS (n=20)	LSIL (n=20)	HSIL (n=15)	CONTROL (n=20)	*p* value
Age (years)	37.85 ± 10.2	39.75 ± 8.3	43 ± 11.5	36.58 ± 6.3	0.184
Gravidity	2.63 ± 2.5	3.15 ± 2.15	3.13 ± 1.95	2.78 ± 2.6	0.534
Parity	1.48 ± 2	2.25 ± 1.25	2.33 ± 1.34	1.67 ± 2.5	0.274

All values are expressed as mean ± SD.

Table 2. — *Serum TAS. TOS levels, and OSI of women.*

	ASCUS (n=20)	LSIL (n=20)	HSIL (n=15)	CONTROL (n=20)	*p* value
TAS	2.34 (1.93−3.39)	2.26 (1.15−3.92)	2.23 (1.53−2.8)	2.84 (2.65−5.66)	< 0.001*
TOS	5.85 (3.84−34.42)	6.57(3.27−31.7)	12.63 (9.8−25.3)	4.73 (3.04−8.75)	< 0.001*
OSI	0.25 (0.16−1.5)	0.29 (0.11−1.3)	0.43 (0.34−0.52)	0.14 (0.09−1.2)	< 0.008*

All values are expressed as median (min-max). * $p \leq 0.05$, significant difference.

LSIL group, included 20 women with LGSIL; group 3, HSIL group, included 15 women with HSIL. Twenty age-matched women with negative PAP smear results were selected as the control group. Collected data were age, gravida, parity, serum TOS, TAS, and OSI.

Blood sample collection

Blood samples were collected into EDTA tubes from all women following overnight fasting. The blood samples were centrifuged at 3,000 rpm for ten minutes for plasma separation. Serum produced after centrifuge was taken to eppendorf tubes, and then quickly stored at -80°C until analysis.

Measurement of total oxidant and antioxidant status

TOS of serum was measured by using the method developed by Erel [11]. In this method, the presence of oxidants causing the oxidation of ferrous ion to ferric ion creates a colored complex with chromogens in an acidic medium. The color intensity association with the total amount of oxidant molecules in the sample can spectrophotometrically be measured. The results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L). The assay has excellent precision values lower than 2%.

TAS was measured by using the colorimetric measurement method for TAS developed by Erel [12]. In this method, which determines the antioxidative effect of the sample, the color change depends on the amount of antioxidants in the sample. The change of absorbance at 660 nm is related to the total antioxidant level of the sample. The results are expressed as millimolar Trolox equivalent per liter (mmol Trolox Equiv./L). This assay has excellent precision, lower than 3%.

OSI was defined as the percentage ratio of the TOS level to TAS level [13-15]. OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 eq/L) / TAS (mmol Trolox eq/L).

Statistical analysis

The results were analyzed using the Statistical Package for the Social Sciences, version 20. Data were reported as mean ± SD or number, or median (min-max). A $p \leq 0.05$ was considered significant. ANOVA (the one-way analysis of variance) test was used for comparison of more than two independent groups with normal distribution. For those without normal distribution, Kruskal-Wallis Test was preferred. DUNN test was chosen for comparing binary significant groups.

Results

The demographic characteristics including mean age (years), gravidity, and parity are presented in Table 1. All groups were similar to each other with regard to these characteristics. Serum TAS, TOS levels, and OSI were shown in Table 2. Serum TAS levels were significantly lower in ASCUS, LSIL, and HSIL groups when compared with control group (2.34 (1.93−3.39), 2.26 (1.15−3.92), 2.23 (1.53−2.8) *vs* 2.84 (2.65−5.66), respectively; $p = < 0.001$) and there was a trend toward decreasing serum TAS levels from ASCUS to HSIL group. Serum TOS levels and OSI were significantly higher in ASCUS, LSIL, and HSIL groups when compared with control group 5.85 (3.84−34.42), 6.57 (3.27−31.7), 12.63 (9.8−25.3) *vs* 4.73 (3.04−38.75), respectively; $p < 0.001$ and 252 (169−1530), 294 (119−1332), 430 (349−520) *vs* 194 (90−1292), respectively; $p < 0.008$). Serum TOS levels and OSI were highest in HSIL group and there was a trend towards increasing serum TOS levels and OSI from ASCUS to HSIL group.

Discussion

The present authors postulated that serum oxidant and antioxidant status of women with abnormal cervical cytology may be an important predictor for progression and clinical outcome of lesions. Therefore, they evaluated serum oxidant and antioxidant status of women with ASCUS, LSIL, and HSIL. They used serum TAS level, serum TOS level, and OSI for determination of oxidative stress. According to the present results, oxidative stress significantly increases in women with abnormal cervical cytology compared to the control group. In accordance with previous studies [16-18], the authors found that there was a trend towards increasing serum TOS level and OSI and decreasing TAS in the progression of lesion from ASCUS to HSIL group. In HSIL group, OSI was much higher than the other groups.

There are some advantages of pre-malignant cervical lesions such as slow-progression, spontaneous regression, and the availability of a simple screening method, especially in less developed countries, as cervical carcinoma is one of the most common cancers affecting women [19, 20]. Several factors such as smoking, human papillomavirus (HPV) infection, oxidative stress, and cervical trauma can be associated with the pathogenesis of progression to precancer or cancer and clinical outcome of lesions. HPV infection is one of the most important causative factors of cervical carcinoma and causes damage to the DNA and other constituents of the cell [21-23]. In all organisms oxidative reactions producing ROS during metabolic and physiological events and enzymatic or non-enzymatic antioxidant systems protecting organisms from detrimental effects of ROS are present [24). The presence of imbalance between physiological ROS production and the total antioxidant capacity resulting in oxidative stress in cells may have a role in the pathology of some diseases and cancers. It is well-known that several diseases such as neurodegenerative diseases, vascular diseases, cancer, diabetes, and osteoporosis are associated with detrimental effects of oxidative stress [2]. The relationship between changes in indicators of oxidative stress and the pathogenesis of cervical cancer is well-documented in studies in serum of women with three pre-malignant and malignant states. Interestingly, it is clear that antioxidants are able to handle the detrimental effects of ROS on cells and to protect the cells [25, 26]. The natural antioxidants can also prevent cancer development by modulation of some cellular signaling pathways [27]. These effects of antioxidants can occur by mechanisms including induction of apoptosis, growth arrest, inhibition of DNA synthesis, and modulation of signaling pathways in response to oxidative stress [28]. In addition, recent studies show the potential protective effect of antioxidants against HPV persistence and cervical dysplasia [29, 30].

In study by Looi *et al.*, similar to the present study, patients with CIN and squamous cell carcinoma (SCC) were evaluated and they found that malondialdehyde (MDA) and urinary 8-OHdG showing oxidative damage were high and levels of antioxidant enzymes such as superoxide dismutase (SOD) and catalase were decreased [17]. Another study evaluating the role of oxidative stress in women with LSIL, HSIL or cervical cancer found an increase in erythrocyte thiobarbituric acid reactive substances (TBARS), reactivation index of δ-aminolevulinate dehydratase (δ-ALA-D), and a decrease in vitamin C content as indicators of oxidative stress [18]. Also, Beevi *et al.* evaluated serum oxidant and antioxidant status of women with cervical carcinoma and demonstrated that increased oxidative stress with altered antioxidant defence system is associated with the pathogenesis of cervical cancer [9]. As mentioned above, the present authors have also confirmed the relationship between increased oxidative stress with altered antioxidant defence system and abnormal cervical cytology.

Although the relatively small sample size of the present study needs to be pointed out, the authors included women with ASCUS in the current study. To their knowledge, the current study is the first study to evaluate serum oxidant and antioxidant status of these three groups: ASCUS, LSIL, and HSIL, concomitantly, and to use TAS, TOS, and OSI for evaluation of oxidative stress for these three groups. In this study TAS, TOS, and OSI measurements were done by using Erel method in a fully automated, inexpensive, and easy fashion. In patients with abnormal cervical cytology, this method can be used as an easily accessible, fully automated, and considerably inexpensive method compared other methods which cannot be used routinely and are costly [31, 32].

Conclusion

In conclusion, the main purpose of the current study was to determinate the potential importance of serum oxidant and antioxidant status for prediction of progression and clinical outcome of lesions. According to the present results, increased oxidative stress with altered antioxidant level was associated with abnormal cervical cytology, ASCUS, LSIL, and HSIL and there was a trend towards increasing progression of lesion by increasing oxidative stress. Serum oxidant and antioxidant status may provide guidance as a simple and cost-effective method for follow-up, treatment, and recommendation in all stages of lesions.

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