# Evaluation of Dynamic Thiol-Disulfide Homeostasis on Hpv Positive-Women in Progression to Cervical Intraepithelial Lesion

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**Research Article**

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# Abstract

**PURPOSE:** Dynamic thiol disulfide homeostasis (TDH) is critical in cervical carcinogenesis at HPV infection as a sign of antioxidant consumption native and total thiol levels decrease in progress to cervical intraepithelial lesions. TDH is the main actor in signaling pathways, apoptosis, antioxidant and detoxification reactions. In this study, we aimed to evaluate the effect of TDH intraepithelial progression of cervical precancerous lesions on HPV positive women.

**METHODS:** This was a prospective cross-sectional study. Subjects were selected from newly diagnosed high risk HPV DNA-positive patients. TDH results were calculated as the levels of disulfide, native and total thiol, the ratios of disulfide/total thiol (SS/SH+SS), disulfide/native thiol (SS/SH) and native thiol/total thiol (SH/SH+SS).

**RESULTS:** A total of 146 women were included in the study. Study groups were as group one; control included 66 participants, group two; HPV DNA-positive women without preinvasive cervical lesion included 30 participants and group three; HPV DNA-positive women with preinvasive cervical lesion included 50 participants. Native and total thiol levels were elevated on HPV-positive women without preinvasive cervical lesions. There were no significant differences between groups related to the ratios of SS/SH, SS/ Total SH, SH/ Total SH levels.

**CONCLUSIONS**: HPV infection related to oxidative stress has effects on oxidant/antioxidant balance and could be demonstrated in systemic circulation by TDH parameters. Consumption of thiol substances play role in the cervical neoplastic process, replacement with antioxidants would be a treatment option for HPV infections.


# Introduction

Human Papilloma Virus (HPV) is a double-stranded DNA virus that involves the squamous epithelium. HPV is a sexual transmitted infection and the main cause of the cervical cancer. There are about 40 HPV types indicating anogenital involvement. High oncogenic types of HPV persistence cause cervical intraepithelial lesions and cancer formation. More than 90% of HPV infection is cleared from the body in about two years (1–3). On the other hand, up to 10% of the infection persists and causes a cervical intraepithelial lesion.

HPV targets basal layer and metaplastic cells to create infection and reaches there through the microabrasions formed in the stratified squamous epithelium. After transmission of the virus to the cervical epithelium integrates to host genome and oncogenic differentiation occurs by expressing E6 and E7 oncogenes in the cell. Dysfunction at native immune response of the host, chronic inflammation and oxidative stress also have an effect on HPV persistence and carcinogenesis. Impairment at oxidant-antioxidant balance and elevated oxidative status were found to be correlated with CIN and cervical carcinoma (4, 5).

The primary target of oxygen radicals are proteins such as cysteine, methionine, glutathione called sulfides containing sulfide groups. These proteins oxidize to form reversible disulfide bonds. Structural and functional changes occur in these proteins during losing thiol groups. (6, 7). Plasma and tissue levels of thiol groups decrease in the course of prevention from the destructive effects of free oxygen radicals (8). In many cellular events as signaling pathways, apoptosis, antioxidant and detoxification reactions dynamic TDH is the main actor. (9, 10)

In recent years, TDH has maintained its popularity and has been the subject of many studies. In the literature, there are a growing body of studies showing the effect of TDH in many acute and chronic disorders. In this study, we evaluated the effect of TDH intraepithelial progression of cervical precancerous lesions at HPV-positive women. This is the first study that investigates the potential impact of TDH on cervical carcinogenesis.

# Material And Method

This prospective cross-sectional study was performed in the Gynecology and Obstetrics Department of Trabzon Kanuni Training and Research Hospital. The study was organized in accordance with the Helsinki Declaration guide and received approval from the local ethics committee. The informed consent form was subscribed by all participants after giving information about the study.

A total of 146 women were included in the study. The participants consist of three groups. Group one was control group included 66 participants, group two was HPV DNA positive women without preinvasive cervical lesion included 30 participants and group three was HPV DNA positive-women with preinvasive cervical lesion included 50 participants. Age, gravidity, body mass index (BMI) were evaluated as demographic features.

Subjects were selected from newly diagnosed high-risk HPV DNA-positive patients without concomitant active sexually transmitted infections, previous history of cervical preinvasive lesions or cervical cancer. Pregnancy, lactation, smoking, any disease associated with immune deficiency, corticosteroid usage, having any genitourinary system infection were the other exclusion criteria. Age matched women who were admitted for routine gynecological check-ups were enrolled as a control group. All participants were interrogated about sexual, reproductive, medical and surgical history.

Gynecological evaluation was performed including bimanual palpation and transvaginal ultrasonographic assessment. During speculum examination cervicovaginal swaps obtained for Liquid-based (ThinPrep Pap Test, Hologic) cervicovaginal smear test and the Hybrid Capture 2 DNA test (Qiagen, Hilden, Germany) for High-Risk HPV detection. Bethesda 2001 classification system was used in the evaluation of smear tests. HPV carriers underwent to colposcopic evaluation (Leica MSV 197, Germany), 3–5% acetic acid administered for visualization of cervical intraepithelial lesions and punch biopsies were taken from suspected areas.

About 4 mL of blood picked up by venopuncture from the antecubital region. The blood samples were centrifuged at 4000 rpm for 10 minutes, and the serum was decomposed and stored at -80°C until an assessment of TDH. Serum TDH was evaluated with an automated spectrophotometric measuring technique defined by Erel (11). TDH results were figured out as μmol/L.

Firstly, disulfide links were reduced by using sodium borohydride to form free functional thiol groups. For prevention of the reduction of 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) reductant sodium borohydride was consumed and removed with formaldehyde. Reduced and native thiol groups were identified after the reaction with DTNB. The dynamic disulfide amount provided from the difference between the total and native thiols and calculated by the relationship between disulfide and thiol groups. If the ratio increases in favor of thiols, it indicates oxidative stress, while an increase in favor of disulfides indicates elevation of antioxidant capacity. The parameters for calculation of dynamic TDH were disulfide/total thiol percent ratios (SS/SH + SS), disulfide/native thiol percent ratios (SS/SH), and native thiol/total thiol percent ratios (SH/SH + SS), disulfide, native and total thiol quantity.

# Statistical analysis:

Version 18 of SPSS was used for statistical analysis. Kolmogorov-Smirnov method was used to determine the normal distribution of data. Mean ± standard deviation was used for normally distributed data, and median ± interquartile range values were used for non-parametric data. Comparison of TDH between study groups was done with One Way ANOVA test with post hoc LSD analysis.

## Results

Mean age of the participants was 42.22 ± 7.16, gravidity 3.6 ± 0.61 and BMI 25.56 ± 3.27. No significant differences were obtained amongst the groups ($p > 0.05$).

TDH was measured by native thiol and disulfide values and SS/ total SH, SH/ total SH, SS/ SH ratios. Mean native thiol levels were 246.84 ± 81.80, disulfide 22.07 ± 7.26, SS/ total SH ratio was 8.21 ± 3.81, SH/ total SH ratio was 83.52 ± 7.63 and SS/ SH ratio was 10.49 ± 7.67. Comparison of TDH parameters between study groups were given in Table 1. There were no significant differences between group according to native thiol, disulfide, SS/SH, SS/ Total SH, SH/ Total SH levels. Native and total thiol levels were elevated in HPV positive women without preinvasive cervical lesions. Contrarily in cervical intraepithelial lesion group native and total thiol levels were decreased, however the results could not reach to statistical significance (Fig. 1).

Table 1
Thiol disulfide homestasis parameters in study groups

| | Group 1 (n = 66) | Group 2 (n = 30) | Group 3 (n = 50) | P value |
|---|---|---|---|---|
| **Native Thiol** | 249,96 ± 74,06 | 273,26 ± 68,45 | 233,21 ± 82,72 | 0,07 |
| **Total Thiol** | 293,96 ± 80,14 | 317,86 ± 68,17 | 271,08 ± 93,52 | 0,05 |
| **Disulfide** | 22,84 ± 7,68 | 22,29 ± 5,76 | 21,31 ± 6,85 | 0,51 |
| **SS/SH** | 10,05 ± 5 | 8,90 ± 3,69 | 10,75 ± 5,76 | 0,29 |
| **SS/Total SH** | 8,14 ± 3,20 | 7,40 ± 2,58 | 8,70 ± 3,98 | 0,25 |
| **SH/ Total SH** | 83,81 ± 6,52 | 85,18 ± 5,17 | 82,35 ± 8,67 | 0,21 |

# Discussion

In this study, we compared the differences between TDH parameters, which are the main components of antioxidant protection, in HPV infected women without cervical lesions and those who developed cervical dysplasia.

Our results demonstrated that TDH parameters affected in HPV positive- women with cervical intraepithelial lesions. Evaluations from peripheral blood samples of HPV positive-women represented that total and native thiol levels decreased at cervical intraepithelial lesions. Our findings supported that HPV infection related cellular changes activates detoxification systems and causes consumption of cellular antioxidant. However, our results were not statistically significant, differences between study groups were thought that cervical neoplastic progression effects oxidant/antioxidant status and that could be shown in systemic circulation. These findings demonstrated that HPV infection caused cervical neoplastic process is closely related with oxidant and antioxidant regulation. According to this data we concluded that replacement of antioxidants in HPV infection could be a strategy for treatment of infection and prevention from cellular changes.

Experimental and observational studies show that a large portion of HPV infection are spontaneously regressed however a small part of them progress and generate cervical cancer. Being HPV infected is not just enough for cancer development, there is still lack of information about other individual and environmental factors and their mechanisms on pathogenesis. Free oxygen radicals are important factors those effective in carcinogenesis by signaling pathway up regulation, cell differentiation, proliferation and change of cellular survival. HPV reproduces in infected and transformed cells and disrupts the redox balance (12–15). Past studies showed that immune response of the host to HPV infection influence oxidative stress and could be demonstrated with alteration of stress markers. Siegel et al. (16) evaluated the relation between oxidant load and HPV clearance, and they determined a high oxidant status. On the contrary, an increase in oxidant levels, antioxidant enzymes have been shown to be lowered in patients with CIN and cervical carcinoma due to excessive consumption (17–19). According to

an in-vivo study evaluating the effect of the Redox system on carcinogenesis, an increased oxidant environment has been shown to be effective in HPV 16 neoplastic progression, and oxidative modification of DNA and proteins in dysplastic tissues have influenced cellular differentiation, leading to neoplastic progression. In cancerous tissue, controlling oxidative damage could be provided by selective reduction of key detoxification proteins. (13).

Histopathological evaluations show an increased inflammatory infiltration in severe HPV-induced lesions. In the early stages, the virus causes infection at basal cells not associated with circulating immune cells therefore inflammation does not play a central role in the pathogenesis of HPV infection. Persistent infection causes chronic inflammation and triggers an imbalance between pro-oxidant and antioxidants (14).

During intracellular reactive oxygen species increase, local antioxidant capacity and numerous intracellular adaptive mechanisms upregulate to prevent the development of apoptosis and to protect the tissue. Throughout free radicals rise above physiological levels, the regulation of redox homeostasis, which was the cellular protection system of the organism, is disrupted and initiates the process of uncontrolled cell growth and carcinogenesis. (20, 21). Redox homeostasis is controlled by oxidizing and reducing of free radicals and thiol-containing proteins in the cell.

Thiols are the parts of the natural antioxidant enzyme system in the organism that contain Sulfhydryl and form disulfide in antioxidant activation (22). Attachment of sulfur and hydrogen atoms to a carbon atom forms sulfhydryl and oxidation reactions form disulfide bonds between two sulfhydryl groups (23). This binding is reversible and disulfides can reduce to thiol groups to sustain homeostasis (24). This homeostasis plays a crucial role in antioxidant protection, detoxification, signal transmission, programmed cell death, organizing enzymatic reaction, transcription factors and intra and inter-cellular signaling mechanisms (25).

Many compounds like cysteine, methionine, glutathione, homocysteine, cysteinyl-glycine and glutamylcysteine are containing thiol groups and have structural alterations under oxidative stress. These proteins oxidize to form reversible disulfide bonds. Structural and functional changes occur in these proteins during losing thiol groups. (6, 7). Plasma and tissue levels of thiol groups decrease in the course of prevention from the destructive effects of free oxygen radicals (8). The transformation of thiols into disulfides is an early indicator of protein oxidation from reactive oxygen radicals. Measurement of total thiol level and determination of TDH is a mirror of excessive free oxygen radical formation in many illnesses (26).

Disruption of this ratio acts a part in the pathogenesis of many inflammatory diseases such as diabetes mellitus, inflammatory arthritis, renal failure, cancer, Parkinson, Alzheimer, multiple sclerosis. Shifting thiol/disulfide balance to disulfide direction was seen in degenerative diseases such as diabetes, obesity, and pneumonia, to thiol direction poses risk factor neoplastic processes such as multiple myeloma, bladder, colon and kidney cancer (27).

The colorimetric method improved by Erel and Neşelioğlu (11) renders possible to provide information about oxidative stress by identifying the total plasma thiol/disulfide ratio. The easy, inexpensive and practical method is carried out with a fully automatic analyzer that does not require separation. It can be used to evaluate free radicals synthesized by many different metabolic pathways, including aerobic respiration in mitochondria. (12). Before this measurement technique was developed, only low molecular weight thiol components which were cysteine, glutathione, and homocysteine could be measured. This method allows measuring the majority part of thiol and disulfides in albumin and proteins. According to the old method, thiol / disulfide measurement did not reflect true homeostasis.

In enzymes containing thiol, free radicals formed after normal metabolites or pathological processes cause structural and functional disturbance and alterations in thiol/disulfide balance. A decrease in plasma thiol concentration indicates an increase in free radical formation. A small proportion of HPV-infected cells progress to cancer, the expression of E6 and E7 oncogenes play role in this process. Camporeale et al. (28) studied the molecular mechanism of potential damages of the oxidative environment in HPV infected cells. They reported that carboxy-terminal of E7 oncoprotein is rich in the domain of cysteine and sensitive to ROS. Exposure to free radicals regulates the transition from the cytoplasm to the nucleus by creating disulfide bonds.

HPV infection has a local effect at cervical epithelium and evaluation of TDH parameters at cervical secretions could be most informative for detection of oxidant/ antioxidant status. This was one of the limitations of our study. The other limitation was cross-sectional design of the study, the levels of TDH parameters previous the infection and cervical lesion were not included in the study.

In conclusion, HPV infection related oxidative stress has systemic effects and could be demonstrated in the systemic circulation by TDH parameters. Consumption of thiol substances play role in cervical neoplastic process, replacement with antioxidants would be a treatment option for HPV infections.

## Declarations

# Funding

Not applicable

# Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

# Availability of data and material (data transparency)

All authors are confident that all data and materials and the software application support their published claims and comply with field standards.

# Code availability (software application or custom code)

Not a applicable

# Authors' contribution

ERİN R: Project development, Data Collection, Manuscript writing

TEKİN YB: Project development, Data Collection, Manuscript writing

KÜÇÜK H: Data collection, Data analysis

EREL Ö: Data analysis

# Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Local Ethics Committee of Trabzon Kanuni Research and Education Hospital (No. 23618724-799)

# Consent to participate

Informed consent was obtained from all individual participants included in the study.

# Consent for publication

Patients signed informed consent regarding publishing their data and photographs.

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# Figures

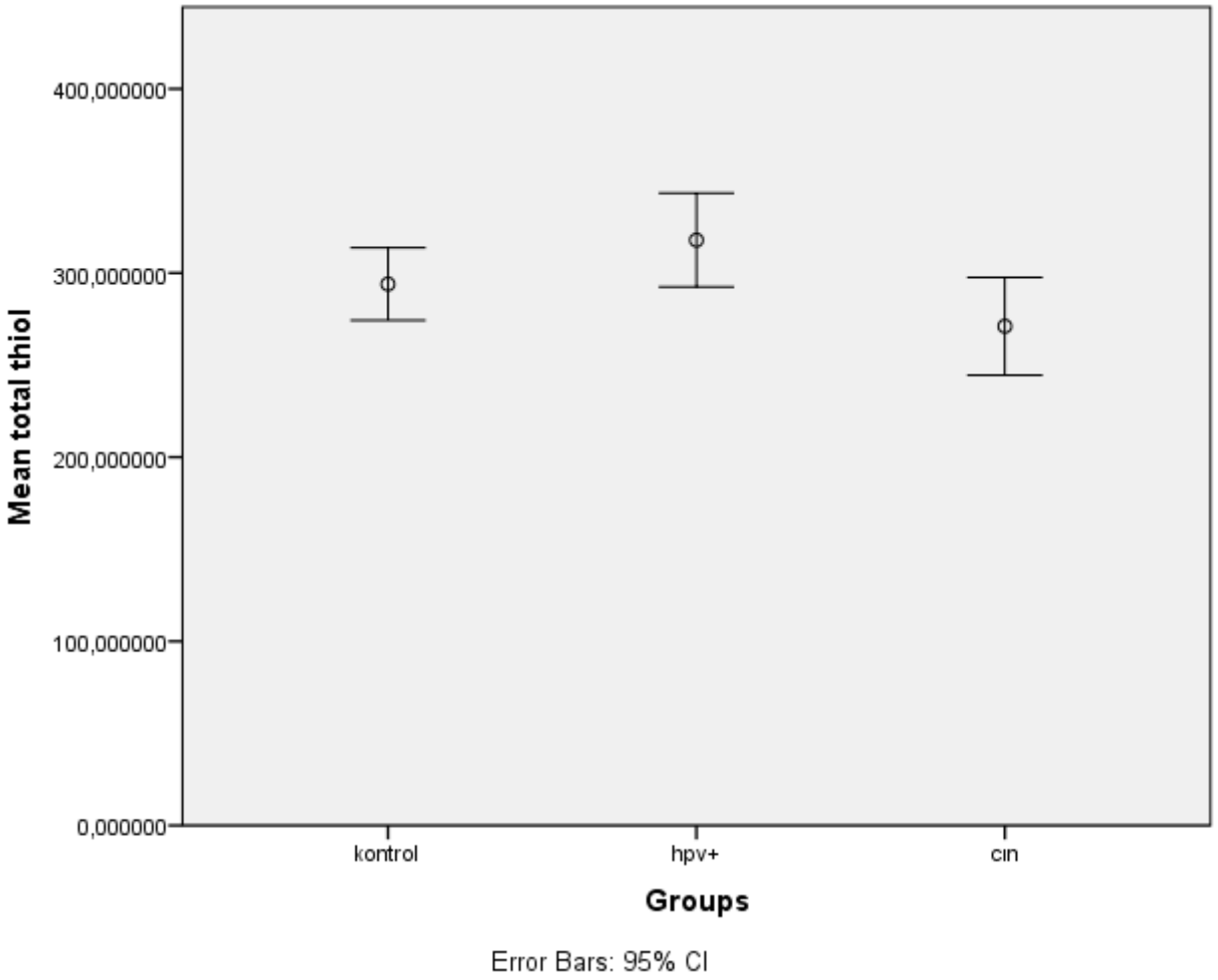


**Figure 1**

The graph of total thiol levels distrubition in study groups