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Original Article

Elevated levels of urinary 8-hydroxy-2′-deoxyguanosine and 8-isoprostane in esophageal squamous cell carcinoma

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Abstract

Aims: To measure oxidative DNA and lipid damages, urinary levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), and 8-isoprostane in esophageal squamous cell carcinoma (SCC) patients and compare the values with that in controls. **Materials and Methods:** The urinary concentrations of 8-OHdG and 8-isoprostane were measured in 32 SCC patients (13 female/19 male; mean age: 61.4 ± 10.5 years) and 45 controls (22 female/23 male; mean age: 58.1 ± 8.3 years). **Results:** Squamous cell carcinoma patients showed significantly higher levels of urinary 8-OHdG (15.6 ± 5.1 ng/mg creatinine) than controls (5.8 ± 2.1 ng/mg creatinine) (P<.001). Increased urinary concentrations of 8-isoprostane were also detected in SCC patients (35.4 ± 6.5 ng/mmol creatinine) as compared to the controls (16.9 ± 4.0 ng/mmol creatinine) (P<.001). **Conclusions:** Our results show the presence of oxidative DNA and lipid damage in the SCC patients. This may have a connection to carcinogenesis in the esophagus.

Keywords: Esophageal squamous cell carcinoma, 8-hydroxy-2′-deoxyguanosine, 8-isoprostane

BACKGROUND

Esophageal cancer in humans occurs worldwide with a variable geographic distribution and ranks eighth among various cancers in the order of occurrence. This malignancy exists in two main forms, with distinct etiological and pathological characteristics: squamous cell carcinoma (SCC) and adenocarcinoma. More than 90% of cancers of the esophagus worldwide are SCCs.[1] SCC remains prevalent worldwide, particularly in the endemic areas of China, Russia, Turkey, and northern Iran.[2] Despite widespread efforts at developing methods for the early diagnosis of SCC over the past two decades there has been limited success.[3]

All cells in the body are exposed constantly to oxidants from both endogenous and exogenous sources and to free radicals that are continuously produced *in vivo*. Reactive oxygen and nitrogen species can attack various substrates in the body, such as lipids and nucleic acids. Oxidation of any of these substrates can theoretically contribute to chronic diseases like cancer.[4]

The most representative product that may reflect oxidative damage induced by reactive oxygen species (ROS) is 8-hydroxy-2′-deoxyguanosine (8-OHdG), a product of oxidatively modified DNA base guanine.[5] Urinary 8-OHdG has been reported to be strongly association with diabetes mellitus,[6] chronic renal failure,[7] and cancer.[8]

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F2-Isoprostanes, a group of bioactive prostaglandin F2-like compounds generated by the oxidatively catalyzed reaction of arachidonic acid, are considered as reliable markers of lipid peroxidation *in vivo*. The 8-isoprostane (8-isoprostaglandin F2α, the major F2-isoprostane), the well-known compound belonging to the F2-isoprostane class is, in practice, usually quantified in urine instead of plasma because of the short half-life of plasma F2-isoprostane. Elevated levels of urinary 8-isoprostane has been reported in several conditions, such as diabetes, alcoholic liver disease, cardiovascular disease, and cancer.[9]

In the present study, we measured oxidative DNA and lipid damage by determining the levels of 8-OHdG and 8-isoprostane in SCC as compared to controls.

SUBJECTS AND METHODS

Thirty-two patients with SCC proven by biopsy and pathological examination (19 male/13 female; mean age: 61.4 ± 10.5), referred to our hospital for treatment, were selected for the study. Forty-five volunteers (23 male/22 female; mean age: 58.1 ± 8.3), hospitalized for non-kidney diseases, were used as controls. Written informed consent was obtained from patients and control participants. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, Iran. Hospital records were used to verify patients' data. Subjects who had any history of stroke, diabetes, ischemic heart disease, asthma, or kidney disease and those who were taking any kind of medicines or supplements such as vitamins were excluded. Twenty-four hours urine samples from patients with SCC were collected before surgery. Similarly, 24-hours urine samples collections were obtained from controls. All the samples were centrifuged at 10000 ***g*** for 10 min to remove any precipitate. The supernatants were stored at −40°C until analyses. A competitive ELISA kit (Cayman Chemicals, Ann Arbor, Michigan, USA) was used for determination of 8-OHdG in the urine samples. Determination of 8-OHdG level was conducted according to the kit protocol, using the mouse anti-mouse IgG-coated plate provided in the kit and a standard curve of 8-OHdG. The urinary concentration of 8-OHdG was expressed by creatinine to avoid the effect of urine volume fluctuation [8-OHdG (ng/ml): creatinine (mg/ml)]= 8-OHdG (ng/ mg creatinine). Similarly, a competitive ELISA kit (Cayman Chemicals, Ann Arbor, Michigan, USA) was used to detect the levels of 8-isoprostane produced within the esophageal cancer patients and the controls. Determination of 8-isoprostane levels in each sample was conducted according to the kit protocol, using the mouse anti-rabbit IgG coated plate provided in the kit and a standard curve of 8-isoprostane. The urinary concentration of 8-isoprostane was expressed by creatinine to avoid the effect of urine volume fluctuation [8-isoprostane (ng/ml): creatinine (mmol/ml)]= 8-isoprostane (ng/mmol creatinine). Statistical analysis was performed using SPSS software (version 16). Statistical comparison of the results was performed using an independent samples *t*-test. Data were expressed as mean ± SD for each group. Statistical significance was determined at $P<.05$.

RESULTS

Demographic and clinical characteristics of controls and SCC patients are shown in Table 1.

SCC patients had significantly elevated urine levels of 8-isoprostane compared with controls (35.4 ± 6.5 vs 16.9 ± 4.0 ng/mmol creatinine, respectively; $P=.000$) [Figure 1].

As shown in Figure 2, increases in 8-OHdG were found in patients with SCC (15.6 ± 5.1 ng/mg creatinine). The mean values obtained in SCC patients were significantly different when compared with that of controls (5.8 ± 2.1 ng/mg creatinine) ($P=.000$) [Figure 2].

DISCUSSION

The incidence of esophageal cancer varies widely among different geographic regions. The high risk areas include the so-called Asian esophageal cancer belt from eastern Turkey, through the southern regions of the former Soviet union (Kazakhstan, Turkmenistan, Uzbekistan, and Tajikistan), Iraq, Iran into western and northern China, Hong Kong, Japan, southeastern Africa, France, and parts of South America (Brazil and Bermuda). It has been reported that in certain regions of northern China or the Caspian Sea littoral in Iran, SCC is 200 times more likely to develop than in other low-risk areas of the world.[3] The etiology of SCC, particularly in people living in high-risk regions such as the Caspian Sea littoral region of Iran, is unclear.[10]

Numerous epidemiological studies and intervention studies with antioxidants indicate that oxidative modification

Table 1: Demographic and clinical characteristics of controls and SCC patients

	Controls	SCC
Age (years)	58.1 ± 8.3	61.4 ± 10.5
Gender (male/female)	23/22	19/13
Location of tumor (n)		
Proximal esophagus	0	3
Middle esophagus	0	12
Distal esophagus	0	17

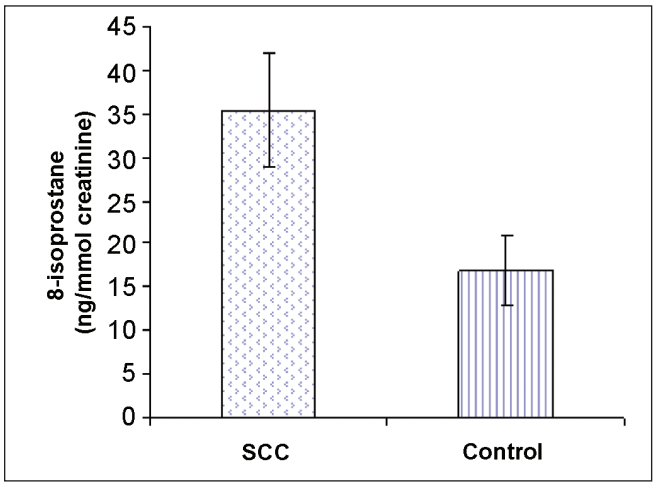

Figure 1: Elevated levels of urinary 8-isoprostane (ng/mmol creatinine) in SCC patients as compared with controls (*P*<.001). Values are expressed as mean ± SD.

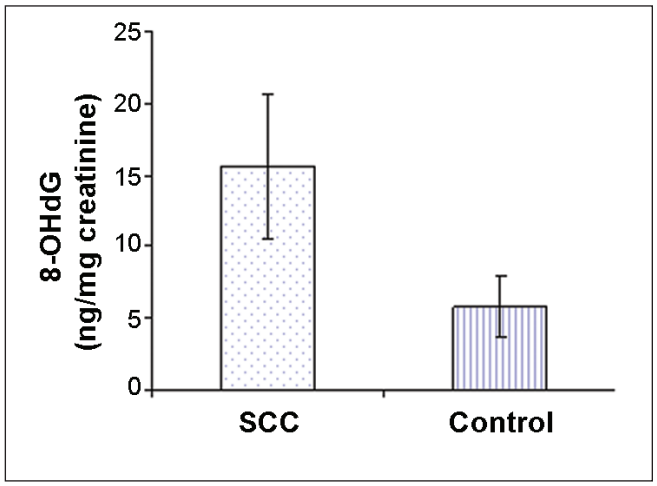

Figure 2: Levels of urinary 8-OHdG (ng/mg creatinine) in SCC patients and controls (*P*<.001). Values are expressed as mean ± SD.

is an important factor in cancer development at certain sites.[11] Oxidative stress is well documented as a biological phenomenon. It has been demonstrated that oxidative stress leads to oxidation of important macromolecules, and this has been hypothesized to be an important pathogenic factor in the development of cancer.[12] The present study investigated the association of oxidative stress, as assessed by 8-OHdG and 8-isoprostane, with carcinogenesis in the esophagus. The substances 8-OHdG and 8-isoprostane are known to indicate oxidative damage of DNA and membrane lipid, respectively. There have been no studies that have evaluated the association of this oxidative stress–related 8-OHdG and 8-isoprostane in the urine of SCC patients.

Estimation of 8-OHdG is an important factor in the evaluation of oxidative DNA damage. It has been shown that hydroxyl radicals, singlet oxygen, and direct photodynamic action produce 8-hydroxylation of the guanine base. Damaged DNA is repaired *in vivo* by endonucleases and free water-soluble 8-OHdG is excreted into the urine without further metabolism.[13] We observed that the urinary 8-OHdG level was significantly higher in SCC patients compared with controls.

In 1995 Toyokuni *et al.* reported that human carcinoma cells (breast, lung, liver, kidney, brain, stomach, and ovary) have a higher content of 8-OHdG than adjacent non-tumorous tissue.[14] Moreover, investigators have reported a high concentration of 8-OHdG in renal cell carcinoma cells,[15] as well as in urine samples from patients with carcinoma of female genitalia,[16] in malignant breast tissues with invasive ductal carcinoma,[17] and in colorectal tumor tissues,[18] gastric cancer tissues,[19] and lung cancer tissues.[10] They hypothesized that the tumor cells themselves produce ROS spontaneously, which results in an increase of 8-OHdG in DNA. Tagesson *et al*. in 1995 reported significant elevation of urinary 8-OHdG excretion following chemotherapy in various cancer patients (i.e., patients with laryngeal cancer, osteosarcoma, and gastric cancer).[20] Erhola *et al*. in 1997 reported the elevation of urinary 8-OHdG in lung cancer patients, which decreased to pre-therapy levels after treatment. In contrast, no significant increase in urinary 8-OHdG was observed in patients whose disease were in progression.[21] It appears that urinary 8-OHdG is useful for indicating cancer risk.[11] Although the cause of increased urinary 8-OHdG levels in cancer patients is still unclear, this marker may be a good prognostic indicator.[13]

It has since become clear that measurement of F2-isoprostanes provides a valuable and reliable approach for assessing oxidative stress status *in vivo*. Measurement of F2-isoprostanes has firmly established the occurrence of oxidative stress in a wide variety of disease states, often for the first time.[22] Evidence is increasing that isoprostanes, a novel class of prostaglandin-like compounds produced upon peroxidation of lipoproteins, play a causative role in carcinogenesis. In human controls, levels of the 8-isoprostane range from 5–50 pg/ml plasma and 500–3000 pg/g urinary creatinine, respectively. The *in vivo* concentration of F2-isoprostanes increases dramatically in animal models of lipid peroxidation. Urine is generally considered a better matrix than serum to quantify isoprostane status.[23]

An increase in 8- isoprostane was not detected in the urine of patients with prostate cancer compared with normal controls.[24] In our study, there was a significant increase of urinary 8-isoprostane levels in SCC patients as compared with controls.

CONCLUSIONS

The results indicate that elevated levels of 8-OHdG and 8-isoprostane are seen in SCC patients, supporting the hypothesis that the evaluation of oxidative stress may represent an additional information. This may indicate an association between oxidative DNA and lipid damage and carcinogenesis of esophagus, and more studies are needed to clarify the picture.

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