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EARLY DETECTION OF SKIN SQUAMOUSCELL CARCINOMA USING H_2O_2 BIOMARKER

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ABSTRACT

Skin squamous cell carcinoma (SCC) is growing and a method for an early detection of this disease is vital. One of the harming causes which lead to SCC is exposure in UV radiation. Peroxidation of lipids and protein crosslinking with UV, results in lesions which some of them are SCC. The damaging effects of UV radiation are DNA destruction. Visible light and UVA radiation are liable to cause DNA destruction indirectly by producing reactive oxygen species such as superoxide anion, singlet oxygen, and hydrogen peroxide via biochemical reactions. As a result the presence of hydrogen peroxide in patient's urine can be an indication of SCC. A new biomarker based on reaction between potassium iodide and hydrogen peroxide was prepared and the presence of hydrogen peroxide in urine of patients was tested using this biomarker. Results from this study indicated that H_2O_2 in urine of SCC patients can be detected by this biomarker.

Keywords: Skin squamous cell carcinoma; urine; biomarker; H_2O_2

INTRODUCTION

Skin cancer is prevailingly the most common type of human cancer and its extent is expanding every year [1]. Squamous cell carcinomas (SCC) are originated from stratified epithelia of the skin and oral cavity. Some cases of aggressive SCC become metastatic and give rise to death [2]. The rate of metastasis in skin SCC ranges from 0.1% to 10% [3]; cutaneous SCC is the second most common non-melanoma skin cancer and is growing up every year due to environmental conditions. The rising incidence of SCC is a reason for increased urgent requirement for any medical care

related to skin cancer [4]. Raising public awareness involves creating the concernof skin cancer as a potentially fatal disease should be fostered, and the growing economic burden of rising skin cancer incidence to the societies should be taken into consideration in medical care's [5-6]. In routine practice, diagnosis is based on clinical evaluation plus histologic examination of a biopsy specimen. In the past decade, several noninvasive diagnostic tools for the diagnosis of SCC have been developed [7]. One group of noninvasive detection includes fluorescence detection with instruments. The use of PpIX fluorescence for the diagnosis of SCC is known as photo dynamic diagnosis (PDD) [8].To develop a rapid non invasive method for early detection are valuable to dermatologists. The most rapid noninvasive methods are the usage of biomarkers. Investigating about novel biomarkers to improve the accuracy of diagnosis for progression of SCC is very important. It can be deduced that a single biomarker probably will not be sufficient, but a panel of biomarkers is needed for making clinical decisions, since SCC is not a uniform disease but rather a heterogeneous group of tumors [9].H_2O_2 is one of most abundant ROS in human cells [10]. This compound would serve as an essential signaling molecule which can regulates cellular biological processes. This aspect of H_2O_2 allows it to be an important intra- and intercellular second messenger in signaling events. It was shown that endogenously produced H_2O_2 directly contributes to a signaling response. The idea that this molecule is an important component of biology was highlighted in 1990s with the development of molecular biology techniques.Thesenew tools revealed that increased expression of manganese superoxidedismutase suppresses the malignant and dangerous phenotype of human melanoma cells [11]. Indeed, cancer initiation and progression have been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation [12]. The use of urine samples provides many advantages in research studies as their collection does not require any invasive technique [13,14]. In this project, we introduce the reaction between H_2O_2 and KI in the presence of peroxidase to detect H_2O_2 in human urine. It seems that H_2O_2 detected in SCC patients' urine can be a new biomarker for early detection of skin SCC disease.

MATERIALS AND METHODS:

Ten cases of skin SCC and ten cases of healthy people were nominated for detecting the presence of H_2O_2 in freshly voided urine.

Chemicals

Pure cellulose filter paper (0.22 μm) , potassium iodide and peroxidase from horseradish were obtained from GE Health care life sciences, MP Biomedicals Netherlands and Fluka, respectively.

Hydrogen peroxide used in this study was obtained from MP Biomedicals Netherlands.

Making H2O2 detection indicator

Potassium iodide solution (2%): 2 g of KI was dissolved in 98 g of deionized water.

Peroxidase solution: 10 mg of horseradish peroxidase was dissolved in deionized water.

The solutions were stores in brown bottles to protect from the light.

Pure cellulose filter paper was cut in to strips. 1 µL of KI solution was place on the filter paper. After drying, 1 µL of peroxidase solution was applied to the coated filter paper and allowed it to be dried completely.

Study design

For treatment group patients referred to Emam Khomeini Hospital, Tehran, Iran, during the year 2014 for skin SCC were considered for eligibility in the study. The inclusion criterion was the existence of skin SCC.Patients with other types of skin disorders or those who were receiving other chemical drugs at the time of admission were excluded.

For control group 10 healthy people were included in the study.

Patients admitted to Emam Khomini Hospital were asked to be included in the study. Provided the patients met the eligibility criteria, they signed the informed consent before to be included in the study.

Eligible participants were then given the sample bottle and were instructed, by a trained pre-instructed nurse, to collect their urine. In addition, the pre-instructed nurse was constantly in contact with the patients monitoring patient condition and recording all the findings.

The same pre-instructed nurse also treated participants, who were in control group. For either case, a data collection form was filled out, recording the patient's disorder, age, gender, color change of strips.

Ethical considerations

Before being included in the study, the eligible participants accepted to sign the informed consent. Each participant was allowed to withdraw from the study whenever they desired. The used strips and sample bottles were provided for the participants free of charge. Finally, all of the patients' information was classified and the findings were reported anonymously.

Study procedure

1 μL of urine samples from both treatment and control groups were placed on the prepared indicator.

Deionized water and pure hydrogen peroxide were applied to the indicator strip as negative and positive controls, respectively.

All above procedures were carried out on polyethylene substrate, under sterile condition.

RESULTS

A total of 10 patients complied with the eligibility criteria and were included in the study. The result for each patient was compared with healthy samples.

H_2O_2 presents in patient's urine react with KI and also peroxidase enzyme. Cellulose filter paper turned dark brown in the edges (figure 1).

Pure hydrogen peroxide creates a more intense brown color than urine samples (figure 2).

Impregnated cellulose filter paper used for control group urine did not go in to any reaction and just wash the indicator like water as negative control (figure 3).

DISCUSSION

This study was conducted aiming to explore the significance of H_2O_2 as biomarker for skin SCC in patients with these abnormal cells. Results from this study indicated that H_2O_2 in urine of SCC patients can be detected using the biomarker prepared.

It is well known that oxidative stress is an important cause of cell damage associated with the initiation and progression of many diseases including cancer. Hydrogen peroxide acts as a signaling molecule in the regulation of different biological processes.

In this study we investigate H_2O_2 as a probable biomarker for early detection of skin SCC.

H_2O_2 in the patients' urine would react with KI in the presents of peroxidase enzyme. This reaction release iodine (I_2):

$H_2O_2 + 2\ KI \rightarrow I_2 + H2O$

I_2 in the present of water and KI are brown and its intensity is depending on initiate H_2O_2 concentration. Since pure peroxide hydrogen has more concentration that urine samples, the more intensive brown color would cause.

Although the effectiveness of H_2O_2 as biomarker has been evaluated in previous studies [15, 16], none of them had sought to investigate its effectiveness in SCC.

This work was the first comparative study exploringthe effectiveness of investigating H_2O_2 in urine of patient and health people.

CONCLUSION

It appears hydrogen peroxide can be a biomarker in SCC patients. We investigate that it can be detected in patients' urine.

Obtained results from this test can be used for further medical studies.

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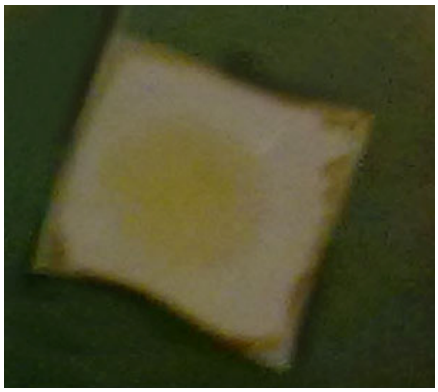

Figure 1: patient's Urine: Cellulose filter paper turned dark brown in the edges

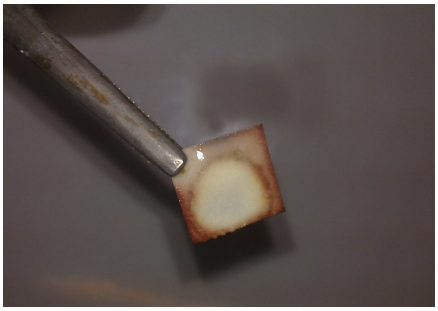

Figure 2: pure hydrogen peroxide as positive control: Cellulose filter paper turned dark brown entirely

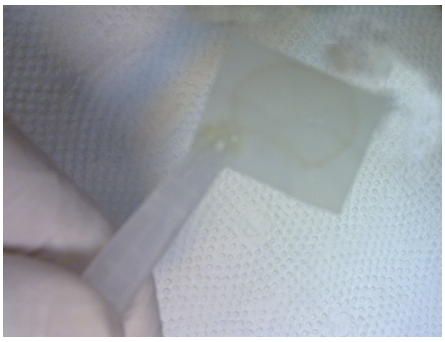

Figure 3: control group urine: Cellulose filter paper did not go in to any reaction