

**URINARY 8-OHDG (8-HYDROXY-2' -DEOXYGUANOSINE) AS BIOMARKER OF  
CANCER INDUCED OXIDATIVE DNA DAMAGE****<sup>1</sup>Deependra Singh Raghuwanshi and <sup>2\*</sup>Reddy P. B.**<sup>1</sup>Department of Chemistry, Government Madhav Science College, Ujjain, M.P. India.<sup>2\*</sup>Department of Zoology, Government PG Arts and Science College, Ratlam, M.P. India.**\*Corresponding Author: Dr. Reddy P. B.**

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**ABSTRACT**

Cancer induced oxidative DNA damage is subject of interest today. The oxidized bases like 8-hydroxy-2'-deoxyguanosine are formed due to DNA damage which is continuously excreted through urine. 8-OHdG (8-hydroxy-2' -deoxyguanosine) is one of the major forms of free radical-induced oxidative cuts, and for that reason it has been extensively used as a biomarker for oxidative stress and carcinogenesis. The present study was intended to assess the oxidative DNA damage in lung cancer patients by using a urinary 8-OHdG as biomarker. A case study was carried out on 18 lung cancer patients (12 men and 6 women) who were undergoing chemotherapy. Patients were identified on the basis of various hospital records. Urinary 8-OHdG levels of lung cancer patients were estimated by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody. Eighteen urine samples of pulmonary cancer patients were collected in and around Nagda town (Ujjain, M.P.India) and were examined for 8-oxo-2'-deoxyguanosine by ELISA technique. The urinary 8-OHdG levels in lung cancer patients were significantly higher than in control ( $119.48 \pm 13.14$  ng/ml versus  $5782.2 \pm 195.3$  ng/ml). We conclude that estimation of urinary 8-OHdG is easy and useful biomarker for the diagnosis of cancer induced DNA damage. In our opinion a follow-up of urinary 8-OHdG might be a useful future tool for the evaluation of DNA damage.

**KEYWORDS:** Reactive oxygen species (ROS), 8-Hydroxydeoxyguanosine (8-OHdG), ELISA, Lung cancer.**INTRODUCTION**

DNA is perhaps the most important target of oxidative attack. Oxidative stress (OS) has been associated to more than 100 human diseases including cancer. The endogenous cellular DNA damage occurs due to exposure of environmental mutagenic chemicals and certain types of radiation.<sup>[1]</sup> It is generally believed that continuous oxidative damage to DNA is a significant contributor to development of the major cancers.<sup>[2]</sup> Earlier research literature on carcinogenesis reveals that significant levels of DNA damage taking place from internal cells.<sup>[3] [4]</sup> Modern analytical chemistry has provided remarkable sensitive and specific methodology for identification and quantification of cellular DNA damage. Among different types of oxidative DNA damage, the formation of 8-hydroxydeoxyguanosine (8-OHdG) is an omnipresent biomarker of oxidative stress.<sup>[5]</sup> It is physiologically formed and increased by chemical carcinogens as the byproducts oxidative DNA damage. The resulting 8-OH-dG is removed through urine without further metabolism. 8-Oxo-2'-deoxyguanosine (8-oxo-dG) is an oxidized derivative of deoxyguanosine and is one of the major byproducts of DNA oxidation and occurs at an average frequency of 2,400 per cell.<sup>[6]</sup> In recent years, the urinary 8-OHdG has been extensively used as a biomarker of endogenous

oxidative DNA damage and also as a predictive risk factor for many diseases including cancer. In the present study we examined cancer induced DNA damage due oxidative stress in patients by using urinary 8-OHdG (8-hydroxy-2' -deoxyguanosine) as biomarker.

**MATERIALS AND METHODS**

Eighteen cancer patients and ten healthy human were recruited for the present investigation. Urine samples from all experimental subjects from Nagda and its surrounding villages were collected and divided into 2 groups. The cancer patients were identified on the basis of various hospital records. Prior to the study, an informed permission was taken from each participant. The mean age of patients is  $58.72 \pm 7.62$  years. Most of the patients were males (66.67%), had a previous family history of cancer (61%), non smokers (85%), married (98%), and had a primary level of education (81%). In the cross-sectional study, the lung cancer patients were slightly older than the healthy controls (58 versus 55 years).

**Collection of urine samples**

Urine samples were collected before the chemotherapy from 6 a.m. to 9 a.m. in disposable Eppendorf tubes and

centrifuged for 15min at 1000xg at 2-8°C to remove any particulate matter. The supernatant kept and stored at (-80°C) deep freeze until assaying in a new disposable Eppendorf tubes.<sup>[7]</sup>

#### Test kit for Human 8-Hydroxy- deoxyguanosine

8-OHdG was purchased from Allied Scientific Products, Kolkata, and performed according to the instruction of manufacture (Product code ab201734) produced by Abcam as the quantitative determination of endogenic human 8-OHdG concentration in the urine.

#### Procedure

All the materials and reagents were equilibrated to room temperature prior to use. The unused strips are placed back in the plate packet and store at +2-4°C. 50- $\mu$ l of urine samples and standards were added to precoated 8-oxodG protein conjugate plates followed by 50  $\mu$ l of the primary antibody, anti-8-oxodG monoclonal antibody solution. After incubation for 50-60 minutes at room temperature, the plates were washed and the enzyme labeled secondary antibody (100  $\mu$ l) was applied for 1 h at 37°C. After washing, 100  $\mu$ l of the substrate, 3,3',5,5'-tetramethylbenzidine were added to the plate and allowed to react at room temperature for 15 min. Plates were allowed for enzymatic color reaction to develop at room temperature in the dark for 30-40 minutes. The substrate reaction yielded a blue colour. After 30 minutes, we carefully removed the plate cover, and stopped the reaction by adding 100  $\mu$ l of stop solution to each well. They are gently to mixed by which the solution in the wells was changed from blue to yellow. The concentration of color produced for each sample on the ELISA plate was measured at the absorbance of 450 nm. Samples were assayed in triplicate. Separate tips were used to pipette the buffer, standard, sample, tracer, and antibody. Each plate was covered with the plate cover and incubated for 1 hour at room temperature (+20- 25°C). We subtracted the average blank absorbance value (OD) of each sample and standard, and calculated the average for each of the replicates. The average absorbances versus 8-OHdG concentration of the standards were also plotted for calculation.

#### Statistical analysis

The data was analyzed by using mean calculation (M) and standard error (SE) using Microsoft Excel windows 7 version. Data were expressed as mean  $\pm$  SE for each group. Statistical significance was determined at  $P < 0.05$ .

#### RESULTS

Patients demographic profiles are summarized in Table 1. The results of mean urinary 8-OHdG concentration of both experimental and control groups are shown in Fig 1 and Table 2 respectively. The mean values of urinary 8-OHdG concentration in normal and healthy individuals were found to be 119.48 $\pm$ 13.14 ng/ml Control, Group I). While this value was 5782.2  $\pm$  195.3 ng/ml) in lung cancer patients (Group II) which was

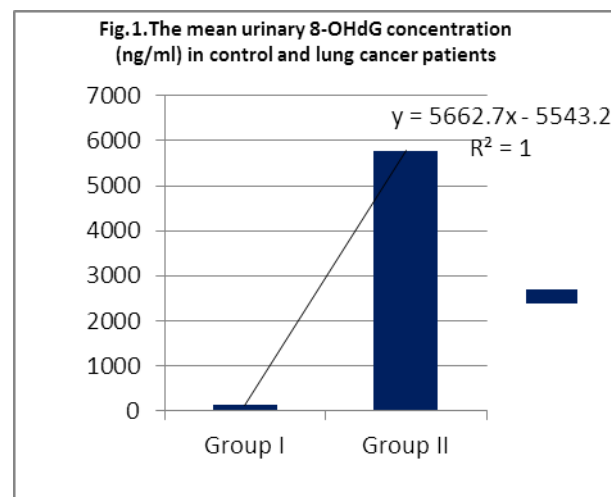
significantly much higher ( $P < 0.001$ ) as comparing with control.

**Table 1: Demographic profile of Patients Recruited in this Study.**

Parameter	Patient Group n= 100 (%)
Gender Males	12 (66.66%)
Females	6 (33.4%)
Mean Age (year) $\pm$ SD	58.72 $\pm$ 7.62
Cancer Family History Yes	61 (61%)
No	39 (39%)
Marital State Married	98 (98%)
Single	2 (4%)
Smoking ---- Yes	8 (44.44%)
No	10 (55.66%)
Education Level Yes (Primary)	13 (72%)
Yes (Secondary)	2 (11%)
No	3(16%)

**Table 2: The mean and SE of the urinary 8-OHdG concentration in the urine of two groups.**

Group	Mean $\pm$ SE
Group I	119.48 $\pm$ 13.14
Group II	5782.2 $\pm$ 195.3



**Fig 1: Concentration of 8-OHdG in lung cancer patients compared with control subjects.**

#### DISCUSSION

Oxidative stress (OS) is very well acknowledged as a biological phenomenon which leads to oxidation of important macromolecules.<sup>[8] [9] [10]</sup> The oxidation of biologically important macromolecules can lead a pathogenic condition in the development of cancer.<sup>[11]</sup>

The urinary 8-Hydroxy-deoxyguanosine (8- OHdG) is known as the biomarker of oxidative damage of DNA.<sup>[12][13][14][15]</sup> But only limited studies reveal the association of oxidative stress with 8-OHdG in the urine of lung cancer patients.<sup>[12][16][17][18][19]</sup> Hence, assessment 8-OHdG concentration is a significant factor in the

evaluation of oxidative DNA damage. Reports also reveal that that hydroxyl radicals<sup>[20]</sup>, singlet oxygen<sup>[21]</sup> and direct photodynamic action<sup>[22]</sup> can produce 8-hydroxylation of the guanine base and free water-soluble 8-OHdG is removed by excretion along with the urine.<sup>[22]</sup> Earlier published data clearly reveals reveal that urinary 8-OHdG is not only a biomarker of general cellular oxidative stress but it also indicate a risk factor for various cancers, atherosclerosis and diabetes.<sup>[12][16][19][23][24]</sup>

In the present investigation we observed higher concentration of urinary 8-OHdG in lung cancer patients significantly comparing with control individuals. Our results are in agreement with many others who reported that different human carcinomas cells (breast, liver, lung, stomach, kidney, brain and ovary) have a higher content of urinary 8-OHdG than neighboring healthy tissue.<sup>[12][15][16][19][22][23][24]</sup>

Few studies reveal the increase in 8-OHdG in DNA is due to spontaneous production of reactive oxygen species (ROS) by carcinomas cells (Halliwell and Gutteridge, 2007).<sup>[25]</sup> Published reports reveal that during chemotherapy, some of the regularly used anticancer drugs can enhance the production of ROS which resulted the higher level of 8-OHdG.<sup>[26][27]</sup> Our results are in agreement with various authors who reported higher level of urinary 8-OHdG in various types of cancer patients.<sup>[13][23][16][19][27][28]</sup>

## CONCLUSIONS

Our study revealed that the estimation of urinary 8-OHdG has clinical utility in assessing oxidative stress and predicting the cancer related disorders. Hence, the urinary 8-OHdG ELISA assay can be considered more supportive tool for the identification of cancer as tumor cells produce radical oxygen species (ROS) by its own spontaneously. The information obtained from this study may contribute to our understanding of the risk elevation from carcinogenesis and conceivably be used in a potential style to evaluate individual risk.

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