ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# Evaluation of the role of oxidative DNA damage marker 8-hydroxy-2-deoxyguanosine in patients with breast cancer

**Sanaa A. El-Benhawy [1], Heba G. El-Sheredy [2]**

**1.** Lecturer of radiobiology, Radiation Sciences Department, Medical Research Institute, Alexandria University, Egypt.
**2.** Lecturer of medical oncology, Cancer Management and Research Department, Medical Research Institute, Alexandria University, Egypt.

## *Manuscript Info*

***Manuscript History:***

Received: 12 September 2014
Final Accepted: 29 October 2014
Published Online: November 2014

***Key words:***

8-hydroxy-2-deoxyguanosine, Breast cancer, Oxidative DNA damage.

***Corresponding Author***

**Heba G. El-Sheredy**

## *Abstract*

**Background:** DNA damage by oxygen free radicals, frequently leads to initiation and progression of human cancer. Among all the purine and pyrimidine bases, guanine base tends to be the more oxidized one. In previous studies, 8-hydroxy-2-deoxyguanosine (8-OHdG) is the most commonly measured marker to indicate the degree of endogenous oxidative DNA damage. 8-OHdG can be measured at high sensitivity, and its levels are correlated with oxidative stress and cancer incidence in the target tissues.
**Objective:** Our purpose was to assess the possible role of serum 8-hydroxy-2-deoxyguanosine in patients with breast cancer.
**Subjects and Methods:** Blood samples were collected from 40 females newly diagnosed with breast cancer and 30 healthy females as control group. The level of serum 8-OHdG was measured by enzyme linked immunossorbent assay (ELISA).
**Results:** There was highly significant difference ($P<0.001$) in the mean serum levels of 8-OHdG in breast cancer patients in comparison to controls. Serum 8-OHdG had high diagnostic performance in breast cancer using ROC curve analysis and associated with breast cancer risk using logistic regression analysis. Serum 8-OHdG had no impact on survival outcome in breast cancer patients.

**Conclusions:** Serum 8-OHdG was highly elevated in breast cancer patients in comparison to controls and it may be a carcinogenic factor in breast cancer. Reduction of oxidative stress is thought to be a very important measure for primary prevention of breast cancer.



## Introduction

The development of cancer in humans is a multistep process. The complex series of cellular and molecular changes participating in cancer development are mediated by a diversity of endogenous and exogenous stimuli. One type of endogenous damage is that arising from intermediates of oxygen-free radicals [Valko ML, 2004]. There is extensive experimental evidence that oxidative damage permanently occurs to lipids of cellular membranes, proteins, and DNA. In nuclear and mitochondrial DNA, 8-hydroxy-2-deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions. It has therefore been widely used as a biomarker for oxidative stress and DNA damage [Valavanidis A, 2009]. The compound 8-OHdG causes G→T and A→C transversions [Unfried K, 2002]. These substitutions have been reported as the sites of spontaneous oncogene

expression and may be largely responsible for the onset of carcinogenesis and cell proliferation, ultimately leading to cancer development. [Jaurand MC, 1997]

8-OHdG is a pivotal marker for measuring the effect of exogenous and endogenous oxidative damage to DNA. It has been also studied as a factor of initiation and promotion of carcinogenesis [Halliwell B,2007]. The appearance of 8-OHdG in the urine is the consequence of DNA-repair mechanism in the cell. It is excreted in the urine without metabolizing any further. Thus, the urinary excretion of 8-OHdG reflects oxidative DNA damage and the body repair of DNA [Cooke MS, 2005]. Since urine concentration of 8-OHdG is very low ,and urine as such is a complex matrix, measurement of 8-OHdG in urine is an analytical challenge. In recent years, 8-OHdG has been used widely in many studies not only as a biomarker for the measurement of endogenous oxidative DNA damage but also as a risk factor for many diseases including cancer. The assessment of 8-OHdG involves a simple method for the evaluation of oxidative DNA damage. Many studies showed that urinary 8-OHdG levels were significantly higher among breast cancer patients than among the control subjects [Kuo HW,2007].

The objective of the present work was to assess the possible role of serum 8-hydroxy-2-deoxyguanosine in patients with breast cancer.

## Patients and Methods

This study was conducted on 40 newly diagnosed breast cancer patients with stage I-III admitted to the Department of Cancer Management and Research, Medical Research Institute, University of Alexandria. Thirty healthy females of matched age with breast cancer patients were selected on a voluntary basis as control group. Adjustments for confounders such as smoking, coffee consumption and use of oral contraceptives were made between the two groups.

A written informed consent for participating in the study was taken according to the declaration of Helsinki and approved by the ethical committee of the Medical Research Institute. 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 from this study.

Blood samples were collected before the surgical operation. Circulating serum levels of 8-hydroxy-2-deoxyguanosine were measured by enzyme linked immunosorbent assay using anti 8-OHdG monoclonal antibodies according to the manufacturer's instructions (Enzo Life Sciences, USA).

Patients were subjected to preoperative evaluation including history taking, clinical examination to detect the site of the tumor and the presence of enlarged lymph nodes. Radiological investigations including mammogram, abdominal ultrasound and chest x-ray. The diagnosis of invasive breast cancer was made by fine needle aspiration cytology (FNAC) or core-needle biopsy of the breast tumor. Patients were subjected to surgery (modified radical mastectomy or conservative surgery). Staging was defined by the seventh edition of the Cancer Staging Manual of the American Joint Committee on Cancer [Edge SB, 2011]. Postoperative pathological evaluation of the tumor including type of the tumor, grading, size of the tumor, axillary lymph node status (with or without capsular invasion), presence or absence of vascular invasion. Assessment of estrogen, progesterone receptors (ER, PR) and human epidermal growth factor receptor-2 (Her2/neu) status was also confirmed. Follow-up of the patients was carried out for 30 months to assess the prognostic role of 8-hydroxy-2-deoxyguanosine.

**Statistical analyses:** Statistical analyses were conducted using the statistical software package of SPSS version 15 (SPSS Inc, Chicago, USA). Differences between groups were assessed by the Mann Whitney U test for nonparametric variables. Logistic regression analysis was used to assess the association between 8-OHdG and breast cancer risk. The adjusted odds ratio (OR) and exact computation of 95% confidence intervals (95% CI) were calculated. Serum 8-OHdG diagnostic performance was evaluated using ROC curve analysis. The Kaplan-Meier method was used to calculate disease-free survival (DFS) and overall survival (OS). The significant difference between curves was evaluated by the log-rank test. Statistical significance was set at $p \leq 0.05$.

## Results

### Patients´ clinico-pathological characteristics

Clinico-pathological characteristics of the studied patients are summarized in table (1).

**Table (1):** Patients´ clinico-pathological characteristics.

| | **Breast cancer patients (n=40)** |
|---|---|
| **Age** | |
| Mean ± S.D | 49.76 ± 11.52 |
| Range | 33– 75 |
| **Menopausal status** | |
| Pre | 17 (42.5%) |
| Post | 23 (57.5%) |
| **Nuclear grade** | |
| I-II | 25 (62.5%) |
| III | 15 (37.5%) |
| **Stage** | |
| I-II | 30 (75%) |
| III | 10 (25%) |
| **ER status** | |
| Positive | 27 (67.5%) |
| Negative | 13 (32.5%) |
| **PR status** | |
| Positive | 25 (62.5%) |
| Negative | 15 (37.5%) |
| **Her-2/neu status** | |
| Positive | 18 (45%) |
| Negative | 22 (55%) |
| **Vascular invasion** | |
| Yes | 26 (65%) |
| No | 14 (35%) |
| **Tumor size** | |
| ≤ 3 | 23 (57.5%) |
| >3 | 17 (42.5%) |
| **LN metastasis (%)** | |
| Yes | 33 (82.5%) |
| No | 7 (17.5%) |
| **Histology** | |
| Ductal | 34 (85%) |
| Other | 6 (15%) |

Abbreviations: ER: estrogen receptor, PR: progesterone receptor, LN: lymph node.

**Serum 8-OHdG levels in breast cancer patients**

Table (2) represents serum 8-OHdG in breast cancer patients and control group. In this table, the mean values of serum 8-OHdG in breast cancer patients was significantly higher than that in control group (14.13±5.10 vs 1.50±0.7, P<0.000).

**Table (2):** Serum 8-OHdG in breast cancer patients and control group.

| | | Control Group (n=30) | Breast cancer Patients (n=40) |
|---|---|---|---|
| **8-OHdG (ng/mL)** | **Range** | 0.4 – 2.5 | 3 – 25 |
| | **Mean±S.D** | 1.50±0.7 | 14.13±5.10 |
| | **Median** | 1.6 | 14 |
| | **P** | **<0.000*** | |

*: Significantly different from control group.
Significance was considered at level of P≤ 0.05.

**Serum 8-OHdG and the risk of breast cancer**

Table (3) showed the estimated odd ratio of breast cancer risk according to tertiles of serum 8-OHdG levels using logistic regression analysis. The risk of breast cancer was significantly increased by 7.129 times in the highest tertile group for serum 8-OHdG compared to lowest tertile group (odd ratio: 7.129 [95% CI: 1.085-46.851]).

**Table (3):** Analysis of multivariate regression with risk of breast cancer.

| | OR | 95%CI | | P |
|---|---|---|---|---|
| | | Lower | Upper | |
| **8-OHdG** | **7.129** | **1.085** | **46.851** | **<0.001*** |

OR: odd ratio. CI: confidence interval.
*: Statistically significant at p ≤ 0.05.

**Diagnostic performance of serum 8-OHdG.**

As can be seen in (Table 4) serum 8-OHdG showed significant area under the ROC curve (AUC) (0.990) (P<0.000), sensitivity (97.1 %) and specificity (100%), at a cutoff value of (5.5) ng/ml.

**Table (4):** The area under the ROC curve, sensitivity and specificity for serum 8-OHdG.

| | AUC | Asymptomatic significance | Cutoff value | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|
| **8-OHdG** | 0.990* | <0.000 | 5.5 | 97.1 | 100 |

*: Statistically significant at p ≤ 0.05.

**Correlation between serum 8-OHdG and clinico-pathological characteristics of the disease.**

A significant positive correlation was found between serum 8-OHdG and lymph node metastasis (P=0.047) and vascular invasion (P=0.014), while there was no significant correlation was observed with tumor grade (P=0.300), tumor size (P=0.211), disease stage (P=0.487), ER (P=0.113), PR (P=0.291) or Her-2/neu status (P=0.133) (Table 5).

**Table (5):** Correlation between serum 8-OHdG and clinico-pathological characteristics of the disease.

| | 8-OHdG | |
|---|---|---|
| | $r_s$ | P |
| **Lymph node metastasis** | 0.291 | 0.047* |
| **Vascular invasion** | 0.377 | 0.014* |
| **Grade** | 0.093 | 0.300 |
| **Tumor size** | 0.142 | 0.211 |
| **Stage** | 0.006 | 0.487 |
| **ER** | 0.213 | 0.113 |
| **PR** | 0.098 | 0.291 |
| **Her-2/neu** | 0.283 | 0.133 |

*: Statistically significant at $p \leq 0.05$.
rs: Spearman coefficient.

**Disease- free survival according to serum 8-OHdG**

Figure (1) represents the Kaplan Meier curve, which showed that the mean disease free survival time for breast cancer patients with high serum 8-OHdG (24.18 months, CI:20.75-27.60) was not significantly different from the mean disease free survival time for the patients with low serum 8-OHdG (27.50 months, CI:23.03-31.97). The difference was not statistically significant (p=0.497) using the log-rank test.

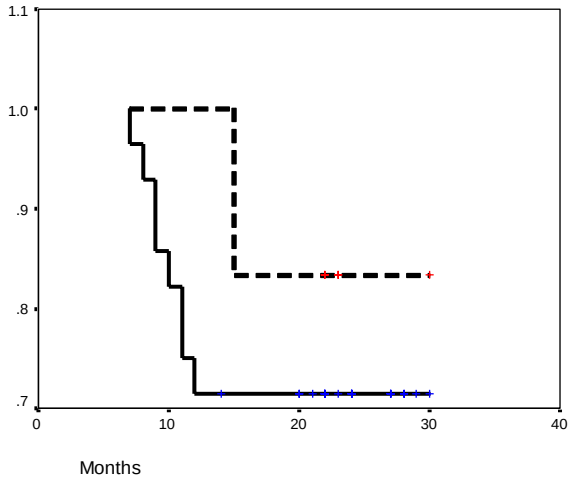


**Figure (1):** Disease free survival according to the serum 8-OHdG in 40 patients with breast cancer.
Dotted line: Low serum 8-OHdG (n=12), [27.50 months, CI: 23.03-31.97].
Thick line: High serum 8-OHdG (n=28), [24.18 months, CI: 20.75-27.60].
The log-rank value was (0.46) and test significance was (p=0.497).

**Overall survival according to serum 8-OHdG**

Figure (2) represents the Kaplan Meier curve for overall survival which showed insignificant difference between breast cancer patients with high serum 8-OHdG (27.49 months, CI: 24.84-30.13) and patients with low serum 8-OHdG (28.33 months, CI: 25.35-31.32) in mean overall survival time. The difference not statistically significant using the log-rank test (p=0.978).

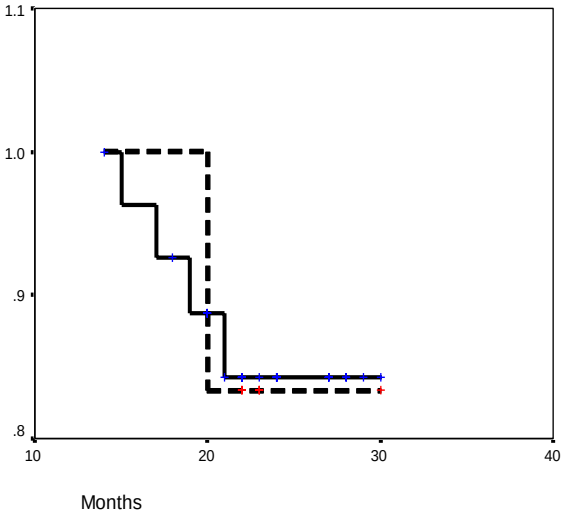


**Figure (2)**: Overall survival according to the serum 8-OHdG in 40 patients with breast cancer.
Dotted line: Low serum 8-OHdG (n=12), [28.33 months, CI: 25.35-31.32].
Thick line: High serum 8-OHdG (n=28), [27.49 months, CI: 24.84-30.13].
The log-rank value was (0.0) and test significance was (p=0.978).

## Discussion

8-hydroxy-2-deoxyguanosine (8-OHdG) is the most abundant DNA lesion caused by reactive oxygen species (ROS). It is highly mutagenic resulting in GC to TA transversions. After cleavage from DNA and as a result of DNA repair, 8-oxodG is excreted in urine. Another significant source of extracellular 8-oxodG may be oxidation of the nucleotide pool [Haghdoost S, 2005]. Urinary 8- OHdG levels are therefore considered as a general biomarker of oxidative stress. Because increased levels were observed among patients with small-cell lung cancer [Shen J, 2007], bladder cancer [Kaczmarek PL, 2005], and prostate cancer [Miyake H, 2004], 8- OHdG may be useful as an indicator of cancer risk. 8-OHdG can be measured by immunohistochemistry, enzyme-linked immunosorbant assay (ELISA) and high pressure liquid chromatography [Wu LL, 2004].

Our study showed that serum 8-OHdG in breast cancer patients was significantly higher than that in the control group. Elevated 8-OHdG levels in this malignant disease compared with healthy individuals may be a sign of increased oxidative stress, impaired antioxidant defense or inadequate repair of oxidative DNA damage. In line with our results Kuo et al. [Kuo HW, 2007] found that urinary 8-OHdG levels were significantly higher among breast cancer patients than among control subjects, after making adjustments for confounders such as coffee consumption and use of oral contraceptives. Furthermore, Musarrat et al. [Musarrat J, 1996] reported that the malignant breast cancer tissue had a statistically significant 9.76-fold higher level of 8-OHdG than normal tissue. A statistically significant 12.9-fold higher endogenous formation of 8-OHdG was also observed in cultured breast cancer cells compared with normal breast epithelial cells. Contradictory to our results, Lee et al. [Lee KH, 2010] reported that urinary levels of 8-OHdG in breast cancer patients were not significantly different as compared to controls. Moreover, Karihtala et al. [Karihtala PI, 2011] reported that 8-OHdG expression diminished significantly in invasive

breast carcinomas compared to non-invasive lesions possibly resulting from the induction of DNA repair in these invasive lesions.

The 8-hydroxy-2′-deoxyguanosine is one of the best characterized oxidized bases. 8-OHdG in DNA could lead to mis-incorporation of adenines opposite the 8-OHdG lesion thus inducing G: C to T: A mutations in genomic DNA. 8-oxoguanine DNA glycosylase-1 (OGG1), a bifunctional glycosylase, is mainly involved in repairing 8-OHdG from oxidative damage. Polymorphisms and loss of heterozygozity of OGG1 gene were detected as susceptibility factors for sporadic epithelial ovarian cancer. The defects in this gene had been also revealed in several case-control studies [Tuhkanen H (2004), Arcand SL (2005), Sokhansanj BA,(2006)]. Increasing serum 8-OHdG in breast cancer patients may be due to defects in OGG1 repair gene leading to accumulation of mutations, cancer manifestation and progression.

Regarding serum 8-OHdG and breast cancer risk, our study revealed that the risk of breast cancer was significantly increased by 7.129 times in the highest tertile group for serum 8-OHdG compared to lowest tertile group (odd ratio: 7.129 [95% CI: 1.085-46.851]). Kuo et al. [Kuo HW, 2007] found that urinary 8-OHdG levels significantly correlated with the risk of development of breast cancer which agrees with our result. In contrast to our study, Lee et al. did not find a significant association between urinary 8-OHdG levels and risk of breast cancer [Lee KH, 2010].

The intracellular redox environment plays an important role in the maintenance of proper cellular homeostasis and functions. Disturbances in redox equilibrium of cells result in pro-inflammatory conditions, and these inflammatory conditions can induce carcinogenesis or increase the malignant potential of the tumor [Karki K, 2014]. Reactive oxygen species (ROS) generated indirectly from inflammation induce activation of transcriptional factors (AP1, NF-κB). These factors contribute to the regulation of inflammatory cytokines, which in turn interact with their receptors by stimulating the production of growth factors, such as vascular endothelial growth factor (VEGF). Free radicals generated during inflammation cause DNA damage, including point mutations in cancer-related genes, modifications in cellular proteins that are involved in DNA repair, and apoptosis. Mutagenic DNA lesions that are not repaired accumulate in the genomic DNA of cells thus leading to their transformation [Tomasetti M, 2010].

In the present study, the diagnostic performance of serum 8-OHdG was high with significant AUC (0.990) ($P<0.000$), sensitivity (97.1 %) and specificity (100%), at a cutoff value of 5.5 ng/ml. So serum 8-OHdG level is sensitive and specific marker in diagnosis of breast cancer patients, suggesting that serum 8-OHdG levels may be used as non-invasive method for breast cancer screening. Karki et al. [Karki K, 2014] reported that high accuracy, specificity, and sensitivity of oxidative stress markers including serum 8-OHdG can be used as discriminatory markers for efficient diagnosis of breast cancer which agrees with our finding.

Our study showed a significant positive correlation between high serum 8-OHdG levels and conventional prognostic factors for aggressive breast cancer as lymph node metastasis and vascular invasion. This correlation reflects the role of 8-OHdG in progression of the tumor and enhancing their metastatic potential. Pylvas e al. [Pylväs M, 2011] found that high 8-OHdG levels both in the serum and in the tumor tissue was associated with traditional factors of poor prognosis in ovarian cancer patients which in line with our findings. In contrast to our results, Sova et al. [Sova H, 2010] found that the low immunohistochemical expression and low preoperative serum 8-OHdG levels were significantly associated with lympho-vascular invasion and positive lymph node status. This association was more obvious among ductal carcinomas.

Regarding the impact of serum 8-OHdG on survival outcome, our study revealed that there was insignificant difference between patients with high serum 8-OHdG level and patients with low serum 8-OHdG level in mean disease- free or overall survival time. In agreement with our finding, Beketic OL et al. [Beketic OL, 2011] reported that immunohistochemical expression of 8-OHdG did not provide sufficient evidence as a prognostic indicator in breast cancer patients

On the other hand, Sova et al. [Sova H, 2010] found that negative 8-OHdG immunostaining from the tumor tissue could be used as an indepependent prognostic factor for poor breast cancer-specific survival. Furthermore, Shen et al. [Shen J,2007] and Matsumoto et al. [Matsumoto K,2003] found that high 8-OHdG levels from tumor tissue DNA have been reported as an independent prognostic factor of poor survival in lung cancer and hepatocellular carcinoma which disagree with the present study.

## Conclusions:

- Serum 8-OHdG was highly elevated in breast cancer patients in comparison to controls and increased 8-OHdG levels may have significant influence in the development of breast cancer.
- 8-OHdG could be a potential biomarker for evaluating risk of breast cancer.
- Measuring of serum 8-OHdG may be non-invasive, highly specific and sensitive screening method for breast cancer diagnosis.
- Reduction of oxidative stress is thought to be a very important measure for primary prevention of breast cancer.
- Serum 8-OHdG did not provide sufficient evidence as a prognostic marker in breast cancer patients.
- Larger studies are needed to validate our findings, and mechanistic studies are needed to elucidate the underlying molecular mechanisms of oxidative stress, DNA damage and breast cancer.

## Acknowledgement:

All authors have contributed significantly to this work.

## References

Arcand SL, Provencher D, Mes-Masson AM, Tonin PN. (2005): OGG1 Cys326 variant, allelic imbalance of chromosome band 3p253 and TP53 mutations in ovarian cancer. Int J Oncol ; 6:1315–20.

Beketic OL, Ozretic P, Rabbani ZN, Jackson IL, Sarcevic B, Levanat S, et al. (2011): Prognostic significance of carbonic anhydrase IX (CA-IX), endoglin (CD105) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in breast cancer patients. Pathol Oncol Res ; 17:593-603.

Cooke MS, EvansMD, Dove RmRozalski R, Gackowski D, Siomek A, et al. (2005): DNA repair is responsible for the presence of oxidativeely damage DNA lesions in urine. Mutat Res.;574:58-66.

Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FI, Trotti A. (2011): Breast. In AJCC Cancer Staging Manual, seventh edition. New York, Dordrecht, Heidelberg, London, Springer, pp. 347 - 76.

Haghdoost S, Czene S, Naslund I, Skog S, Harms- Ringdahl M. (2005): Extracellular 8-OHdG as a sensitive parameter for oxidative stress in vivo and in vitro. Free Radic Res ; 39(2):153– 62.

Halliwell B. (2007): Oxidative stress and cancer: have we moved forward? Biochem J ; 401:1–11.

Jaurand MC. (1997): Mechanisms of fiber-induced genotoxicity. Environ Health Perspect ; 5:1073–84.

Kaczmarek PL, Błaszczyk J, Fijałkowski P, Sierakowska-Fijałek A, Niemirowicz J, Kasprzak A, Baj Z. (2005): Assessment of 8-hydroxy-2-deoxyguanosine concentration in bladder cancer patients treated with intravesical BCG instillation. Pol Merkur Lekarski. ; 19 (112):526-8.

Karihtala PI, Kauppila S, Puistola U, Jukkola-Vuorinen A. (2011): Divergent behaviour of oxidative stress markers 8-hydroxydeoxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal (HNE) in breast carcinogenesis. Histopathology 2011 ; 58:854-62.

Karki K, Pande D, Negi R, Khanna S, Khanna RS, Khanna HD. (2014): Expression of Serum Toll-Like Receptor 9 and Oxidative Damage Markers in Benign and Malignant Breast Diseases. DNA Cell Biol 2014 ahead of print doi:10. 1089/dna. 2405.

Kuo HW, Chou SY, Hu TW, Wu FY, Chen DJ. (2007): Urinary 8-hydroxy-2-deoxy guanosine (8-OHdG) and genetic polymorphisms in breast cancer patients. Mutat Res ; 631:62–8.

Lee KH, Shu XO, Gao YT, Ji BT, Yang G, Blair A, et al. (2010): Breast cancer and urinary biomarkers of polycyclic aromatic hydrocarbon and oxidative stress in the Shanghai Women's Health Study. Cancer Epidemiol Biomarkers Prev ; 19:877-83.

Matsumoto K, Satoh Y, Sugo H, Takamori S, Kojima K, Fukasawa M, et al. (2003): Immunohistochemical study of the relationship between 8-hydroxy-2-deoxyguanosine levels in noncancerous region and postoperative recurrence of hepatocellular carcinoma in remnant liver. Hepatol Res; 25: 435– 41.

Miyake H, Hara I, Kamidono S, Eto H. (2004): Oxidative DNA damage in patients with prostate cancer and its response to treatment. J Urol ; 171:1533-6.

Musarrat J, Arezina-wilson J, Wani A. (1996): Prognostic and aetiological relevance of 8-hydroxyguanosine in human breast carcinogenesis. Eur J Cancer; 32:1209-14.

Pylväs M, Puistola U, Laatio L, Kauppila S, Karihtala P. (2011): Elevated serum 8-OHdG is associated with poor prognosis in epithelial ovarian cancer. Anticancer Res ; 31:1411-5.

Shen J, Deininger P, Hunt JD, Zhao H. (2007): 8-Hydroxy-2-deoxyguanosine (8-OHdG) as a potential survival biomarker in patients with non-small cell lung cancer. Cancer; 109:574–80.

Sokhansanj BA, Wilson DM. (2006): Estimating the effect of human base excision repair protein variants on the repair of oxidative DNA base damage. Cancer Epidemiol Biomarkers Prev ; 6:1000–8.

Sova H, Jukkola VA, Puistola U, Kauppila S, Karihtala P. (2010): 8-Hydroxy- deoxyguanosine: a new potential independent prognostic factor in breast cancer. Br J Cancer; 102:1018-23.

Tomasetti M, Santarelli L. (2010): Biomarkers for Early Detection of Malignant Mesothelioma: Diagnostic and Therapeutic Application. Cancer ; 2:523-48.

Tuhkanen H, Anttila M, Kosma VM, Ylä-Herttuala S, Heinonen S, Kuronen A, et al. (2004): Genetic alterations in the peritumoral stromal cells of malignant and borderline epithelial ovarian tumors as indicated by allelic imbalance on chromosome 3p. Int J Cancer ; 6:247–52.

Unfried K, Schurkes C, Abel J. (2002): Distinct spectrum of mutations induced by crocidolite asbestos: clue for 8-hydroxydeoxyguanosine-dependent mutagenesis in vivo. Cancer Res ; 62:99–104.

Valko ML, Izakovic M, Mazur M, Rhodes CJ, Telser J. (2004): Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem ; 266:37-56.

Valavanidis A, Vlachogianni T, Fiotakis C. (2009): 8-hydroxy-2-deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev; 27:120-39.

Wu LL, Chiou CC, Chang PY. (2004): Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta ; 339:1- 9.