Levels of Plasma 8-Hydroxy-2'-Deoxyguanosine—A Marker of Oxidative DNA Damage—Are Not Associated with Primary Angle-closure Glaucoma

Altaf A. Kondkar

Department of Ophthalmology, Glaucoma Research Chair, King Saud University Medical City, College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/JAMMR/2021/v33i1631009

Editor(s): (1) Dr. Barbara Giambene, Al Emadi Hospital, Qatar.
Reviewers: (1) Bhandari Akshay Jawahirlal, Pravara Institute of Medical Sciences, India. (2) Laila Hassan Mohamed Elshazly, Memorial Institute for Ophthalmic Researches, Egypt. (3) Brinda Haren, Gujarat University, India. (4) Jairo Eduardo Márquez Díaz, Cundinamarca University, Colombia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71169

Received 12 May 2021
Accepted 17 July 2021
Published 20 July 2021

ABSTRACT

Aim: The study aimed to estimate plasma 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, a marker of oxidative deoxyribonucleic acid (DNA) damage, and evaluate their potential association with primary angle-closure glaucoma (PACG) and related clinical markers.

Study Design: This is retrospective case-control study.

Place and Duration: The study was performed on samples recruited from King Abdulaziz University Hospital, Riyadh, Saudi Arabia between August 2018 through September 2019 in the participants of Saudi origin.

Methodology: Plasma 8-OHdG levels were measured in duplicates using automated ELISA analyzer in 43 PACG patients and 45 non-glaucomatous controls.

Results: The mean levels of 8-OHdG were 13.59 ng/mL [±4.50] in PACG patients compared to 16.95 ng/mL [±10.66] in controls. The differences did not achieve statistical significance (P = 0.06). Similarly, no significant differences (P = 0.42) were observed in the median levels of 8-OHdG between PACG and controls. Furthermore, except for sex, where males exhibited a significant negative correlation (R = -0.35, P = 0.02), there was no significant correlation between 8-OHdG and other clinical markers.

*Corresponding author: E-mail: akondkar@gmail.com;
levels and age ($P = 0.85$), intraocular pressure ($P = 0.58$), cup/disc ratio ($P = 0.39$) and number of antiglaucoma medications ($P = 0.41$) in the PACG group. Regression analysis showed no significant influence of age, sex, and 8-OHdG levels on PACG outcome. Besides, the area under the receiver operating characteristic curve was 0.55 and non-significant ($P = 0.42$).

**Conclusion:** There is no association between plasma levels of 8-OHdG and PACG or its specific clinical markers. Further studies in a much larger cohort are needed to validate these findings.

**Keywords:** 8-OHdG; DNA damage; glaucoma; oxidative stress; Saudi Arabia.

1. **INTRODUCTION**

Among the different types of glaucoma, primary angle-closure glaucoma (PACG) is more common in the Asian population than Caucasians [1]. Clinically, PACG is characterized by a narrow iridocorneal angle that results in blockage of the aqueous outflow structures. A small cornea, shallow anterior chamber depth, and short axial length are considered anatomical risk factors for PACG [2]. Despite advances in understanding the genetic [3], epigenetic [4], environmental [5] and gene-environment interaction factors [6] contributing to the disease etiology, the exact mechanisms, and pathogenesis of glaucoma are still not wholly understood and remains an active area of research investigation.

Several experimental evidence suggests an active role of oxidative stress mechanisms in glaucomatous retinal ganglion cell death [7-9]. Oxidative stress is the consequence of an imbalance between excessive production of reactive oxygen species (ROS) and, conversely, due to impaired antioxidant defense mechanisms, mitochondrial abnormalities, or a combination of these systems [10, 11]. Increased oxidative stress due to excessive accumulation of ROS can induce oxidative damage/modification to deoxyribonucleic acid (DNA), proteins, and lipids. Treatment with hydrogen peroxide was reported to cause trabecular meshwork (TM) degeneration that can result in increased resistance to aqueous humor (AH) outflow and elevated intraocular pressure (IOP), supporting the role of oxidative damage in glaucoma pathogenesis [12]. Oxidative DNA damage, represented as 8-hydroxy-2'-deoxyguanosine (8-OHdG), has been shown to be significantly increased in glaucoma patients [7]. Furthermore, both IOP and visual field abnormalities are proportional to the amount of oxidative DNA damage in the TM cells [8].

Nuclear and mitochondrial DNA undergo continuous damage and oxidative modification during oxidative stress [13]. 8-OHdG, a predominant forms of ROS-induced DNA lesions', is the most frequently measured oxidatively modified biomarkers. 8-OHdG levels have been consistently proven to increase in the serum or urine of patients with the oxidative stress-associated disease [14]. Likewise, increased levels of 8-OHdG have been reported in different forms of glaucoma, including PACG [15-19]. Oxidative stress-related biomarkers can have pathophysiological and therapeutic relevance to glaucoma [20]. Despite the high prevalence of PACG, the role of oxidative stress in PACG has received limited attention compared to other types of glaucoma [16,21,22]. Previous studies have shown that plasma 8-OHdG are significantly increased in primary open-angle glaucoma (POAG) [19] and pseudoexfoliation glaucoma (PXG) [18]. Based on these findings, we investigated systemic oxidative stress-induced DNA damage in patients with PACG. We estimated plasma levels of 8-OHdG as a marker for oxidative DNA damage and evaluated their possible association with PACG and its specific clinical indices compared to glaucoma-free controls.

2. **METHODOLOGY**

2.1 **Study Design and Population**

We conducted a retrospective case-control study in participants of Saudi origin with an established clinical diagnosis of PACG (n = 43) and non-glaucomatous controls (n = 45) recruited between August 2018 to September 2019 at the King Abdulaziz University Hospital in Riyadh, Saudi Arabia. All patients underwent a standardized ophthalmic examination and diagnosis by glaucoma specialists. The inclusion/exclusion criteria for patients and controls have been described previously [19, 23]. Briefly, PACG patients showed (i) clinical evidence of anterior chamber angle closure, ii) optic disk or retinal nerve fiber layer defects, and (iii) visual field abnormalities. Subjects with secondary causes of glaucoma, history of ocular
trauma, steroid or antioxidant and vitamin usage, and refusing to participate were excluded. Participants ≥40 years old with normal IOP (< 21 mmHg) and optic disc, open angles, without any history of ocular disease(s) or eye surgery, and absence of any infectious or immunomodulating diseases (rheumatoid arthritis, lupus, Crohn's disease) were used as a controls. The study was approved by the institutional review board committee and adhered to the Declaration of Helsinki's principles involving human research.

### 2.2 Estimation of 8-hydroxy 2'-deoxyguanosine Levels

Plasma levels of 8-OHdG were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit based on competitive sandwich principle (Trevigen, Gaithersburg, MD, USA). The assay was performed as per the manufacturer's instructions on an automated ChemWell ELISA analyzer (Awareness Technology Inc., FL, USA). Each plasma sample was analyzed in duplicate, and the 8-OHdG concentrations were calculated using a standard curve and expressed in ng/mL [19].

### 2.3 Statistics

Normality testing was performed using the Kolmogorov–Smirnov test. Mean and median differences were analyzed by the Student t-test and Mann-Whitney U-test, respectively. Association and correlation of 8-OHdG with the disease was tested by Chi-square test, regression analysis and Spearman’s method as indicated. The area under the curve (AUC) value was determined using A receiver operating characteristic (ROC) curve and tested by Mann–Whitney U-test. A two-sided P-value of less than 0.05 was considered significant. Statistical analyses were performed with SPSS version 19.0 (IBM Corp., Armonk, New York, USA) and StatView software version 5.0 (SAS Institute, Cary, NC, USA).

### 3. RESULTS

#### 3.1 Population Characteristics and 8-OHdG Levels

Table 1 shows the participants' demographic and clinical characteristics included in this study. There were no significant differences between the two study groups regarding age, gender, systemic diseases, and smoking status. As shown in Table 1, the mean, median, range, and gender distribution of 8-OHdG levels showed no significant distribution between PACG cases and controls. The box-plot distribution of plasma levels of 8-OHdG according to study groups and gender is shown in Fig. 1.

### 3.2 Correlation of 8-OHdG with Demographic and Clinical Parameters

8-OHdG showed a moderate negative correlation with males but none with age, IOP, cup/disc ratio, and antiglaucoma medications in PACG subjects (Table 2).

### 3.3 Logistic Regression Analysis

Regression analysis showed that risk factors like age, gender, and 8-OHdG had no significant association with PACG outcome (Table 3).

### 3.4 ROC Curve Analysis

The area under the ROC curve value for plasma 8-OHdG was 0.55 (P = 0.42), indicating that plasma 8-OHdG was not a good marker to satisfactorily identify PACG cases and controls.

### 4. DISCUSSION

Oxidative DNA damage has been implicated in age-related degenerative diseases, including glaucoma [24]. In this study, we report a lack of association between a marker of systemic oxidative stress-induced DNA damage, plasma 8-OHdG, and PACG in a Saudi cohort. In contrast, many studies have reported a definite link between 8-OHdG levels and different types of glaucoma.

Initial investigations by Izzotti et al. demonstrated the involvement of oxidative DNA damage in glaucoma pathogenesis [7]. The study demonstrated higher than threefold elevation in TM 8-OHdG concentrations in glaucoma patients. In another study of 28 patients, AH and serum 8-OHdG levels were also reported to be higher in glaucoma patients [15]. Also, urinary 8-OHdG levels were shown to be related to visual field progression in normal-tension glaucoma [17, 25]. In another recent study, plasma and AH levels of 8-OHdG were also reported to be high in POAG as a result of defective DNA base excision repair pathway [26]. Due to a strong positive correlation between plasma and AH 8-
OHdG levels the authors suggested that systemic 8-OHdG levels may represent a marker for 8-OHdG concentrations in the eye [26]. Likewise, Chang and colleagues reported an increased level of serum 8-OHdG in PACG patients of Chinese origin [16]. Unlike Chang et al., however, we failed to observe any association between PACG patients and plasma 8-OHdG levels. Although the sample size in ours and the study conducted by Chang et al. [16] was comparable, the discrepancy in the outcome could be attributed to clinical variations in the patient population and other factors such as antioxidant status or ethnic variation.

![Fig. 1. Box plot showing the distribution of 8-hydroxydeoxyguanosine (8-Ohdg) levels (a) between primary angle-closure glaucoma (PACG) cases and controls and (b) according to gender in cases and controls](image)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls (n = 45)</th>
<th>PACG (n = 43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, Mean (SD)</td>
<td>59.9 (8.3)</td>
<td>62.4 (6.6)</td>
<td>0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male/Female, n</td>
<td>30/15</td>
<td>22/21</td>
<td>0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Systemic diseases, n %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (13.3)</td>
<td>11 (25.5)</td>
<td>0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypertension</td>
<td>5 (11.1)</td>
<td>8 (18.6)</td>
<td>0.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>2 (4.4)</td>
<td>2 (4.6)</td>
<td>0.96&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smokers</td>
<td>5 (11.1)</td>
<td>8 (18.6)</td>
<td>0.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8-OHdG concentration, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>16.95 (10.66)</td>
<td>13.59 (4.50)</td>
<td>0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median</td>
<td>13.78</td>
<td>12.93</td>
<td>0.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range</td>
<td>3.03 – 47.43</td>
<td>7.84 – 32.77</td>
<td>-</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>17.02 (11.02)</td>
<td>12.69 (5.27)</td>
<td>0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median</td>
<td>13.12</td>
<td>11.60</td>
<td>0.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range</td>
<td>3.03 – 47.43</td>
<td>7.84 – 32.77</td>
<td>-</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>16.80 (10.29)</td>
<td>14.54 (3.41)</td>
<td>0.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Median</td>
<td>17.97</td>
<td>14.83</td>
<td>0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Range</td>
<td>3.48 – 35.26</td>
<td>8.83 – 18.25</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Independent samples t-test (two-tailed); <sup>b</sup>Chi-square test; <sup>c</sup>Mann-Whitney U-test
Table 2. Correlation between 8-hydroxydeoxyguanosine levels and specific clinical indices of glaucoma in primary angle-closure glaucoma (PACG) patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.03</td>
<td>0.85</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.35</td>
<td>0.02</td>
</tr>
<tr>
<td>Intraocular pressure, mmHg</td>
<td>0.08</td>
<td>0.58</td>
</tr>
<tr>
<td>Cup/disc ratio</td>
<td>-0.13</td>
<td>0.39</td>
</tr>
<tr>
<td>Number of antiglaucoma medications</td>
<td>0.13</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*Females as reference

Abbreviation: R, Spearman’s rho correlation coefficient

Table 3. Binary logistic regression analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>SE</th>
<th>Odds ratio (95% confidence interval)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.04</td>
<td>0.03</td>
<td>1.04 (0.98–1.10)</td>
<td>0.15</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.68</td>
<td>0.46</td>
<td>0.50 (0.20–1.23)</td>
<td>0.82</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>-0.06</td>
<td>0.03</td>
<td>0.95 (0.89–1.00)</td>
<td>0.07</td>
</tr>
<tr>
<td>Constant</td>
<td>-1.39</td>
<td>1.90</td>
<td>0.25</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Females as the reference

Increased oxidative DNA damage affecting the TM cells has also been correlated with glaucoma-specific clinical parameters such as IOP and visual field loss [7, 8]. However, we did not observe any significant correlation between 8-OHdG levels and markers of clinical severity of the disease such as IOP, cup/disc ratio, and the number of antiglaucoma medications.

The mechanisms by which 8-OHdG and oxidative DNA damage can induce glaucomatous optic neuropathy is only exploratory. An increase in oxidative DNA damage in the TM might directly affect the regulation of the extracellular matrix structure and aqueous outflow, leading to clinical manifestations of glaucoma [12]. Furthermore, the existence of oxidized guanine can result in transversion mutations [27] induce telomere shortening [27, 28], or have an epigenetic effect on transcriptional regulation [27]. There are multiple mechanisms by which oxidative stress can affect TM function and cause glaucoma [29]. There is strong evidence for the role of oxidative stress and glaucoma in the Saudi population via mitochondrial dysfunction, DNA damage, and impaired antioxidant system. These have been consistently demonstrated in POAG [10, 19, 30], and PXG [18, 31, 32], but not in PACG [21, 33] (including this study). Thus suggesting a role of different oxidative mechanisms such as induction of growth arrest [34], regulation of cell-cycle progression [35], or rearrangement of cytoskeletal structure [36], among others [29], which might disrupt TM regulation and lead to the development of glaucoma.

The lack of association between 8-OHdG levels and PACG in this cohort requires careful interpretation due to its few limitations. First, only a single biomarker was investigated in this study, and the involvement of other oxidative biomarker(s) has not been ruled out [16]. Second, we must acknowledge that the systemic status of 8-OHdG might not precisely represent the actual oxidative status in the anterior segment of the eye, where free radical injury is much higher and better representative of the disease events [22]. Verifying such would require studying AH samples. Third, the study is purely descriptive in nature without any proof of mechanisms(s). Finally, the lack of association might be because of relatively small number of participants examined in this study. Nevertheless, with a difference (δ) of 4 ng/mL in the mean 8-OHdG levels, mean SD (σ) of 7.5, and a 2-sided type I error probability (α) equivalent to 0.05, the study exhibited a probability of 0.70 to identify any significant link between 8-OHdG and PACG.

5. CONCLUSION

In conclusion, we observed no association between 8-OHdG levels in plasma and PACG or glaucoma-related clinical markers like IOP and cup/disc ratio in a Saudi cohort. Furthermore, based on ROC curve analysis, 8-OHdG levels in plasma might not be a useful biomarker in PACG. However, further replication studies are needed to validate these observations in a much larger cohort. These findings also do not rule out
the role of other plausible mechanisms and biomarkers of oxidative stress pathways in PACG.

CONSENT AND ETHICAL APPROVAL

The study was approved by the institutional review board committee and adhered to the Declaration of Helsinki's principles involving human research. All participants signed a written informed consent.

ACKNOWLEDGMENTS

The author would like to thank the Vice Deanship of Scientific Research Chair and Glaucoma Research Chair in Ophthalmology, King Saud University; Mr. Taif A. Azad for technical assistance; and clinical coordinator Mr. Abdulrahman Al-Mosa.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

17. Yuki K, Murat D, Kimura I, Tsubota K. Increased serum total antioxidant status and decreased urinary 8-hydroxy-2'-

137


27. Markkanen E. Not breathing is not an option: How to deal with oxidative DNA damage. DNA Repair (Amst). 2017;59:82-105.


