



## Evaluation of Serum and Salivary IgG in Head and Neck Squamous Cell Carcinoma

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### Authors' contributions

This work was carried out in collaboration between all authors. Author TJL designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AAS and OAL managed the analyses of the study. Author EEUA managed the literature search. All authors read and approved the final manuscript.

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### ABSTRACT

**Aims:** To evaluate salivary and serum IgG levels in patients with head and neck squamous cell carcinoma (HNSCC) and healthy control subjects and to assess the effect of treatment on IgG levels.

**Study Design:** A cross sectional study.

**Place and Duration of Study:** Department of Oral Pathology and Department of Radiotherapy, University College Hospital, Ibadan, Nigeria between January 2010 and December 2010.

**Methodology:** Seventy eight subjects comprising 30 patients with untreated HNSCC, 18 patients with HNSCC receiving treatment and 30 healthy, age and gender-matched individuals were included. Serum and salivary samples from the participants were analysed for total IgG using ELISA technique.

**Results:** The mean serum IgG in untreated and treated HNSCC patients was significantly lower compared with healthy controls ( $P=.001$ ), while mean salivary IgG was

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significantly elevated ( $P=.001$ ) in untreated HNSCC patients compared with treated and healthy controls. There was no significant correlation between serum and salivary IgG levels.

**Conclusion:** In patients with head and neck squamous cell carcinoma, serum IgG was reduced while salivary IgG was elevated compared with healthy controls. Our finding suggests differential roles of immunoglobulin G in serum and saliva of patients with head and neck squamous cell carcinoma. Hence, salivary IgG may be a useful biomarker in patients with head and neck squamous cell carcinoma, while serum IgG levels may be useful in monitoring treatment in these individuals.

*Keywords: Immunoglobulin G; head and neck squamous cell carcinoma; serum; saliva.*

## 1. INTRODUCTION

Malignant tumours of the head and neck region are life threatening conditions representing a diverse group of malignancies originating in the oral cavity, oropharynx, larynx and hypopharynx [1]. They constitute 5-30% of all cancers worldwide [2]. Head and neck squamous cell carcinoma (HNSCC) is the most common biologic type of head and neck tumors constituting the sixth leading cause of cancer-related deaths worldwide [3]. These tumors are commonly diagnosed at advanced stages [4] with over 500,000 new cases worldwide in recent years [5]. The majority of patients present with advanced disease and incur significant morbidity and mortality secondary to limited screening tools and markers for adjuvant therapy. Similarly, the prognosis of these cancers has remained relatively unchanged for the past years despite advances in diagnosis and management [6]. The role of the body's humoral immune response is an important factor in the pathophysiology, prognosis and management of individuals with head and neck squamous cell carcinoma.

Cell-mediated immune responses have been considered the most important defence against tumour cells in both animal and human studies [7], whereas humoral immune response to carcinogenesis is poorly understood. The mucosal immune system, with its local mechanisms represents the first line of defence against pathogens in the gastrointestinal, respiratory and genitourinary systems. It appears to be independent of systemic immunity and its relation to the development and control of neoplasia is not well understood, despite the fact that most human malignancies of epithelial tissues origin affect sites where the secretory immune system is vigorously functioning [8].

Biomarkers of head and neck squamous cell carcinoma are needed to aid in early diagnosis, risk assessment and therapy response. The search for biomarkers includes evaluation of tumor tissues and surrogate materials such as blood and saliva. Ideal biomarkers should be accurate and easy to perform, highly specific, objective, quantitative, and cost effective [5]. Salivary analysis holds promise as a non-invasive approach to identify biomarkers for head and malignancies. Concentrations of IgG in saliva may have significant prognostic value in early cancer diagnostics. Our hypothesis is that the serum and salivary levels of IgG may be a reflection of the humoral immune changes in patients with head and neck squamous cell carcinoma. The present study aims at evaluating the salivary and serum IgG levels in patients with head and neck squamous cell carcinoma and healthy control participants and to find whether there is any correlation between IgG levels in the two body fluids.

## **2. METHODOLOGY**

This study received ethical approval from the University of Ibadan/University College Hospital Ethics Committee (UI/UCH/EC/09/014).

The study population included patients with head and neck squamous cell carcinomas attending the Dental and Radiotherapy clinics of the University College Hospital, Ibadan and age/gender matched healthy control subjects. Head and neck squamous cell carcinomas were histologically diagnosed squamous cell carcinoma involving oral cavity, nasopharynx and larynx.

Healthy control participants were volunteer healthy individuals that came with their cancer patients to the hospital. Healthy control participants had a head, neck and mouth examination by TJL (Principal Investigator) and OAL (Co Investigator) in order to exclude oral mucosal lesions, as well as acute and chronic periodontitis. All healthy control individuals had not received any medication one month prior to the study. None of the healthy controls had a history of any chronic disease, prior malignancy, immunodeficiency and autoimmune disorders. All patients and controls gave informed consent.

### **2.1 Screening and Diagnosis of HNSCC**

Clinical examination and diagnosis was carried out with the participants seated in the clinic using WHO classification of head and neck tumours [9] by TJL and AOL. Tumour staging was done using the American Joint Committee on Cancer (AJCC) staging system. Biopsy procedure and histological diagnosis were performed by TJL in the Oral diagnosis clinic and Oral Pathology laboratory respectively.

The inclusion criteria were histological diagnosis of squamous cell carcinoma involving the head and neck region and absence of co morbid conditions like diabetes, chronic kidney disease and hypertension.

### **2.2 Collection of Saliva Sample**

Whole saliva was collected by asking participants to spit into a graduated universal bottle for a period of 10 minutes. This was immediately transferred to sterilized tubes and frozen. The samples were stored at -20°C until the time for immunoglobulin measurement.

### **2.3 Collection of Blood Sample**

Blood was collected simultaneously from peripheral veins in the upper arm using a size 21G needle on a 5mls disposable syringe after cleaning the area with methylated spirit swab. Samples were collected in EDTA bottles.

The samples were centrifuged for 15 minutes at 2000 rpm and plasma was separated from the cellular components using a plastic Pasteur pipette and stored at -20°C prior to use. The plasma was analysed for quantitative level of IgG.

## 2.4 Quantification of IgG

The samples stored in the freezer were thawed in a refrigerator for 18 hours [to preserve the immunoglobulins] and then centrifuged at 8000 rpm for 15 minutes. Total IgG in the samples was quantified using the Enzyme Linked Immunosorbent Assay (ELISA) method [10].

### 2.4.1 Assay protocol

Following the manufacturers (IC Lab Inc. USA, E-80G Lot #13). Instructions, each test sample was diluted into 1/10,000, 100  $\mu$ L of 6 standards were dispensed in duplicate with pipette into pre designated wells. The micro titre plate was incubated at room temperature for thirty minutes. Following incubation, the contents of the wells were aspirated. Each well was completely filled with appropriately diluted Wash Solution and aspirate. This was repeated three times. The wells were completely filled with wash buffer. The plate was inverted and the contents were poured out in a waste container. This was followed by sharply striking the wells on absorbent paper to remove residual buffer. This was repeated 3 times for a total of four washes. Then 100  $\mu$ L of appropriately diluted Enzyme-Antibody Conjugate was transferred to each well using pipette. This was incubated at room temperature for thirty minutes. The plate was kept covered in the dark and levelled during incubation. The wells were washed and blotted as previously described and 100  $\mu$ L of TMB Substrate Solution was pipetted into each well. The wells were incubated in the dark at room temperature for 10 minutes. After 10 minutes, 100  $\mu$ L of Stop Solution was added to each well. The absorbance (450 nm) of the contents of each well was determined and the plate reader was calibrated.

## 2.5 Data Analysis

The main outcome variables were mean serum and salivary IgG values in patients with head and neck squamous cell carcinoma and healthy controls. For salivary IgG levels, logarithm values (means  $\pm$  SD) were calculated due to large innate variability of salivary parameters. Variables were further analysed by ANOVA, Dunnett's post hoc tests and Spearman correlation tests. The level of statistical significance was  $P < .05$  for all analyses.

## 3. RESULTS AND DISCUSSION

The participants comprised 38 males and 40 females between the ages of 28 years and 85 years (mean = 53.5  $\pm$  13.6 years). These included 30 subjects with HNSCC who had not commenced treatment, 18 subjects with HNSCC who were receiving treatment (radiotherapy and chemotherapy in fourth to sixth week of their treatment) and 30 healthy control subjects. The subjects and controls were comparable in age and sex (Table 1). Cancer patients in the untreated and treated groups had TNM stage III and IV.

**Table 1. Demographic distribution of subjects and control**

Variable	Untreated HNSCC (n = 30)	Healthy Controls (n = 30)	Treated HNSCC (n = 18)
Age (mean $\pm$ SD years)	56 $\pm$ 16.3	50 $\pm$ 10.9	49 $\pm$ 6.7
Male	13	13	12
Female	17	17	6
M:F ratio	1:1.3	1:1.3	2:1

The mean+ SD of the serum IgG levels in the untreated HNSCC patients, treated HNSCC patients and healthy controls were  $2175.2 \pm 845.2$  mg/dl,  $1397 \pm 1137.3$  mg/dl and  $3245.1 \pm 76.87$  mg/dl respectively (Table 2), while the mean salivary (logarithm values) IgG levels were  $1.82 \pm 0.58$  mg/dl,  $0.87 \pm 0.25$  and  $0.67 \pm 0.24$  mg/dl respectively (Table 3). Mean serum IgG level was significantly lower in untreated and treated HNSCC patients compared with healthy controls ( $P = .001$ ), while the mean salivary IgG level was significantly higher in untreated HNSCC patients compared with healthy controls ( $P = .001$ ). Comparing untreated HNSCC patients and treated HNSCC patients, the mean serum and salivary IgG were lower in the treated HNSCC patients.

**Table 2. Serum IgG levels in HNSCC patients and healthy controls**

Variable	Untreated HNSCC subjects	Treated HNSCC subjects	Healthy control subjects
<b>N</b>	30	18	30
<b>Mean <math>\pm</math> SD (mg/dl)</b>	$2175.2 \pm 845.2^a$	$1397.1 \pm 1137.3^b$	$3245 \pm 76.87^c$
<b>Range</b>	16-2680	28-2400	284-2500

*P = 0.001 (ANOVA), applies to a and b versus c (Dunnett's test)*

**Table 3. Salivary IgG levels\* in HNSCC patients and healthy controls**

Variable	Untreated HNSCC subjects	Treated HNSCC subjects	Healthy control subjects
<b>N</b>	30	18	30
<b>Mean <math>\pm</math> SD (mg/dl)</b>	$1.82 \pm 0.58^a$	$0.87 \pm 0.25$	$0.67 \pm 0.24^b$
<b>Range</b>	0.2 – 2.5	0.2 – 2.4	0.2 – 2.4

*\* Logarithm values, P = 0.001 (ANOVA), applies to a versus b (Dunnett's test)*

There was no significant correlation between serum and salivary IgG levels in head and neck squamous cell carcinoma patients and healthy controls ( $r=.3$ ,  $P=.09$  and  $r=.07$ ,  $P=.71$  respectively).

The main finding in this study is a lower serum IgG level in patients with head and neck squamous cell carcinoma compared with healthy controls and further reduction in patients receiving treatment. The reduction in the immunoglobulin levels in carcinoma subjects may be a reflection of malnutrition and immune suppression which may be a factor predisposing them to the development of malignancy. In addition, exposure to chemoradiation may further suppress white cell production which may further deplete immunoglobulin production; this may explain the lower immunoglobulin levels seen among the patients receiving treatment. Some studies however found no changes in the levels of serum IgG in oral cancer patients compared with healthy individuals [11,12]. On the other hand, a study [13] reported elevated level of serum IgG in oral carcinoma patients compared with healthy controls. Contrary to our findings, Khanna et al. [14] reported that the treatment modalities (surgery and chemotherapy) had no effect on serum immunoglobulin levels. The variations in findings may be explained by the differences in the forms of treatment received by the subjects.

Another finding in this study is the significantly elevated level of salivary IgG in untreated HNSCC patients compared with healthy controls. This is similar to the finding of Shpitzer et al. [15]. They reported a significant increase in the concentration of total salivary IgG in oral cancer patients compared with healthy individuals. It was suggested that the elevated salivary IgG level might be an indication that the cancerous process compromised oral

mucosa leading to exudation of proteins such as IgG from the serum into the saliva. Similarly, Brandtzaeg, [16] showed that surface epithelium acts as a passive molecular sieve favouring transmission of IgG whereas the secretory epithelium is in addition provided with some selective transport mechanisms specific for other immunoglobulin classes. Extraglandular transfer of immunoglobulin is highly dependent upon mucosal inflammation as well as upon the serum level of the respective immunoglobulin. The inflammatory exudates from mucosal inflammation may contribute to the elevated fraction of the IgG observed in the saliva of carcinoma subjects.

In this study we did not find any significant correlation between serum and salivary IgG among the subjects. This may suggest that extravascular transfer of IgG primarily depends on the mucosal status of the individual and not necessarily the serum level.

The limitation of this study is its inability to correlate levels of IgG in serum and saliva of patients with head and neck squamous cell carcinoma with clinical stage due to late presentation of the participants.

#### **4. CONCLUSION**

In patients with head and neck squamous cell carcinoma, serum IgG was reduced while salivary IgG was elevated compared with healthy controls. Our finding suggests differential roles of immunoglobulin G in serum and saliva of patients with head and neck squamous cell carcinoma. Hence, salivary IgG may be a useful biomarker in patients with head and neck squamous cell carcinoma, while serum IgG levels may be useful in monitoring treatment in these individuals.

#### **CONSENT**

A statement of patient consent is has been presented in the manuscript.

#### **ETHICAL APPROVAL**

This study received Institutional Review Board (IRB) or Ethical Committee Approval (UI/UCH/EC/09/014) as indicated in the manuscript under methodology.

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#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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