Evaluation of Humoral Immunity in Oral Cancer Patients from a Nigerian Referral Centre

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Abstract The immune system is central in the body's defense against non-self. Immunoglobulins and acute phase proteins have been reported to play active roles in carcinogenesis. This prospective longitudinal study was carried out to determine the state of humoral immunity in Nigerian oral cancer patients relative to controls. Twenty newly diagnosed untreated cases of oral squamous cell carcinoma recruited from our centre were included in the study. The controls included 20 apparently healthy and HIV negative volunteers. Serum immunoglobulin classes and acute phase proteins were measured using immunoplates. IgA and ceruloplasmin showed statistically significantly elevated levels in the patients compared with controls, while increases in IgM and IgG were insignificant. The raised levels suggest a role for immunoglobulin A and ceruloplasmin in the mechanisms involved in oral cancers. Findings from this study are similar to that reported elsewhere in the literature. Further work is needed to ascertain the role and usefulness of immunoglobulins and acute

phase proteins in staging, disease monitoring, therapy and prognostication.

Keywords Immunoglobulins · Acute phase proteins · Oral cancers

Introduction

Generally, head and neck cancers constitute between 5 and 50 % of all cancers globally, while in Nigeria, prevalence ranging from 20 to 44 cases per year has been reported in several centres [1–5]. While tobacco and alcohol are independent risk factors and etiologies for this condition, the immune system plays a significant role in the development.

Theories of immunosurveillance and immunostimulation illustrate the fact that an appropriate immune reaction results in protection and an inappropriate one results in tumour promotion [6]. Acute phase response is a reaction of organisms to disturbances, including cancer, in its homeostasis [7, 8]. Pattern of response may correlate with clinical stage of disease. An elevated level of IgG, IgA, or IgM antibodies is frequently observed in cancers of epithelial origin [9, 10]. This has been interpreted as responses to cancer growth [11]. The aim of this study was to evaluate humoral immunity in oral squamous cell carcinoma (OSCC).

Patients and Methods

Twenty consecutive, new, histologically diagnosed cases of OSCC who presented (within the study period and consented) at our hospital were recruited for the study. They were, 14 males and 6 females, aged between 20 and 73 years (mean 45.70, SD $\pm$ 20.83). Twenty (14 males and 6 females)

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apparently healthy volunteers aged between 20 and 77 years (mean 46.20, SD ± 18.44) were also enrolled as controls. Ongoing infections, clinically evident rheumatoid arthritis and steroid use were exclusion criteria. Patients and controls were screened for HIV after counseling before being recruited into the study. Blood samples were allowed to clot and retract after which the serum was separated into plain bottles and stored at -20°C until time of analysis. Ethical approval for the study was obtained from the institution's ethics review board before commencement.

Determination of Immunoglobulin Classes and Acute Phase Proteins

Immunoglobulin classes and acute phase proteins were quantified using prepared immunoplates based on the antigen-antibody precipitation reaction in agar gel. The plates were incubated at room temperature and the diameters of precipitin rings were measured using an illuminated Hyland viewer with a micrometer eyepiece. The values of immunoglobulin classes and acute phase proteins were extrapolated from standard curves. Mann-Whitney test was used to test the significance of difference between mean values. The probability, equal or less than 0.05, was considered significant. The statistical analysis was done using SPSS version 17 for Windows.

Results

Figure 1 shows site distribution of lesions in the patients; the most frequently involved site in this study was the palatal mucosa, accounting for 8(40 %) of all recorded sites. The mean value of ceruloplasmin (0.36 g/l) was significantly higher in the patients than the controls ($p = 0.01$), while mean values of alpha-1-antitrypsin (AAT) and transferrin concentrations were not significantly higher in the control group than the patients (Table 1). The mean serum levels of IgG, IgA and IgM were higher in the patients compared with controls. However, only IgA was statistically significant (Table 2).

Discussion

Epidemiological and experimental evidence in the past two decades have implicated the immune system in the etio-pathogenesis of cancers [12, 13]. While cellular immunity is anti-tumour, persistent humoral immune responses are known to exacerbate recruitment and activation of innate immune cells in neoplastic microenvironments, where they regulate tissue remodeling, pro-angiogenic and pro-survival

Fig. 1 Site distribution of cancers

Table 1 The mean values of serum acute phase proteins (ceruloplasmin, AAT and transferrin) in OSCC and controls

Index	Mean	Standard deviation	z value	p value
Ceruloplasmin (g/l)			4.57	0.01*
OSCC	0.36	0.08		
Control	0.20	0.06		
AAT (g/l)			1.36	0.17
OSCC	3.51	1.77		
Control	4.21	1.63		
Transferrin (g/l)			0.26	0.79
OSCC	2.01	1.22		
Control	2.17	1.37		

Significant at * $p \leq 0.05$

z Mann-Whitney test, p probability

Table 2 Mean values of immunoglobulins (IgA, IgG and IgM) in OSCC and controls

Index	Mean	Standard deviation	z value	p value
IgG (g/l)			0.45	0.64
OSCC	921.00	291.54		
Control	896.65	307.23		
IgA (g/l)			4.55	0.00*
OSCC	2218.42	1517.05		
Control	564.01	389.36		
IgM (g/l)			0.45	0.64
OSCC	367.42	510.77		
Control	241.5	184.94		

Significant at * $p \leq 0.05$

z Mann-Whitney test, p probability

pathways that together potentiate cancer development [14]. Elevated levels of immunoglobulins had been previously reported in various types of cancer including those of epithelial origin [9, 10]. In the present study, the mean serum level of IgA was significantly elevated ($p = 0.01$) in OSCC

compared with control. The mean serum concentration of IgG and IgM were also raised in OSCC group, but the increases were not statistically significant ($p = 0.64$, 0.64 respectively). Earlier studies reporting similar increases in immunoglobulins in cancer patients have explained their findings as being antitumour response by the immune system [9, 10], however current evidence of immunoglobulin synthesis by some tumour cells suggest otherwise [11, 12]. Increased levels of IgA in head and neck patients had been previously reported [15, 16]. The locoregional production of IgA in the glandular mucosal surfaces of the head and neck region perhaps partly explains this phenomenon. Though the exact role of IgA in these cancers is not fully elucidated, perhaps because of the heterogeneity characteristic of malignant cells and the relationship between immunity and cancer, the reported increased serum level was supposedly immunoreactive [13]. However, with current evidence, production of antibodies does not confer protection, but, paradoxically, correlates with poor prognosis and decreased survival for several human cancer types [17]. These anti-tumour antibodies are thought to enhance tumour growth by promoting pro-tumour immune responses and in general protecting tumour cells from cytotoxic T lymphocyte-mediated killing [18]. Increased levels of immunoglobulins in neoplastic microenvironments also result in accumulation of immune complexes that engender tumour-promoting inflammatory responses [19, 20] and are associated with increased tumour burden and poor prognosis in patients with breast, genitourinary, and head and neck malignancies [21–23].

In addition, studies in transgenic mouse models of de novo organ-specific cancer development have revealed that inflammation mediated by immunoglobulins and immune complexes might be functionally significant parameters of tumour promotion and progression [24].

In this study, the increased mean ceruloplasmin level compared with controls was found to be statistically significant ($p < 0.01$). Increased serum levels of ceruloplasmin had been previously reported in various malignancies [25–27]; the reasons for the increase have not been fully understood. While it may just be due to the fact that it is an acute phase reactant, increase may also be a direct response to increase in copper level, which had also been similarly reported to occur in malignancies. Another possible explanation is that the increase may also be due to a compensatory mechanism keeping iron in Fe^{3+} state, thus preventing Fe from undergoing the redox cycles necessary to initiate toxic effects.

In the present study, reduced level of AAT was recorded in OSCC when compared with controls, though this was not statistically significant ($p = 0.17$). This however is not in agreement with previously reported increased levels than have been found in malignancies, including head and neck

squamous cell carcinoma. The reason for this difference cannot be immediately explained.

Previous studies have shown that AAT can inhibit the cellular growth of cultured fibroblasts [28] and the growth of some human tumour cell lines [29, 30]. It is likely that AAT plays a role in controlling tissue destruction and tumour invasion by regulating specific trypsin-like enzymes that are being expressed by the neoplastic cells. Moreover, AAT has also been demonstrated in several neoplasms including carcinomas, mesenchymal tumours, haemopoietic and brain tumours [31]. The presence of AAT in tumour cells is now considered to be due to its production by tumour cells themselves [32, 33]. However, its significance in neoplastic tissues vis-a-vis the unpredictable pattern remains unknown.

Several reports have shown that cases with AAT expression in tumour cells had worse prognosis than those without AAT expression, suggesting that AAT production in tumours cells may correlate with more aggressive behaviour as reported in some gastrointestinal cancers [33].

Transferrin is a plasma protein that functions as the major iron transport protein. In vitro studies have demonstrated that plasma transferrin, which is synthesized in the liver, is an essential requirement for cellular proliferation. Though transferrin is reportedly secreted by malignant cells as an autocrine growth factor by permitting tumour growth in poorly vascularised area, this is not unequivocally so in all tumours [34]. The mean serum value of transferrin in the OSCC group was lower than the control, though difference is statistically insignificant ($p = 0.79$). This finding is in agreement with what was earlier reported by Yildirim et al. [34]. The fact that all tumours may not secrete this substance is a possible reason for varied reports. The heterogenic characteristics of cancer cells, the complexity of cancer tissue micro-environment and the relationship between immune system and carcinogenesis, make specific mechanism and function of secreted substances a major challenge. Further efforts are required at unraveling this connection as it could provide insights into therapeutic targets for certain cancers.

Conclusion

The results from this study suggest an active involvement of IgA and ceruloplasmin in the mechanisms of oral cancers. The locoregional production of IgA in the glandular mucosal surfaces of the head and neck region perhaps partly explains this phenomenon, it could however be due to a yet to be understood immunological response of tumour cells. Also the involvement of ceruloplasmin, an angiogenic acute phase protein, in OSCC suggests a role in tumour proliferation.

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