

Evaluation of EBV serum and salivary IgA antibodies level in Head and Neck Cancer Patients

Ahmed Sahib Abdulmir *, Nidhal Abdul Muhaimen*

ABSTRACT:

BACKGROUND:

Many previous studies revealed that salivary and serum IgA response to infectious agents such as EBV-coded antigens in patients with nasopharyngeal carcinoma (NPC) had a vital role in the combat against tumorigenesis. Moreover, it could be considered as a reliable index for the humoral limb of anti-cancer immunological action.

METHOD:

One hundred twenty two head and neck cancer (HNCA) patients were selected randomly from two main hospitals, Alkadhimya hospital and radiotherapy center in Baghdad. Also 100 apparently healthy control subjects (HC) underwent the same examinations and tests. Enzyme-linked immunosorbent assay (ELISA) test was applied on all HNCA patients sera and saliva, in order to measure anti-EBV IgA antibodies .

RESULTS:

Revealed that NPC patients were the only group that showed a sero-positive ELISA readings of anti-EBV serum IgA. No saliva EBV antibodies were detected in the studied groups .

CONCLUSIONS:

S.IgA level seems to be an applicable index for evaluating EBV burden originated from nasopharyngeal cancerous cells. Such index might help in diagnosing early and specifically the carcinogenesis of NPC, moreover, might help in evaluating the progress of the disease.

KEY WORDS: Head and neck cancer, HNCA, nasopharyngeal carcinoma, NPC, immunosuppression.

INTRODUCTION:

In context of humoral immunity role in HNCA, several early reports on the salivary IgA response locally to infectious agents such as viruses provided a basis for investigations of the specific IgA local immunological response to EBV-coded antigens in patients with NPC who has become a good patient sample for the humoral limb of anti-cancer action (1, 2). Serum IgA levels were elevated during pre- and post- therapy periods and serum immune complexes are elevated in the post therapy periods (3, 4). Some reports revealed that there was little correlation between the degree of elevation of IgA level and clinical course of disease in HNCA while in NPC, however levels of IgA specific to EBV have shown a reliable correlation with tumor load (5). Some reports revealed that the time of elevation of serum IgA and immune complexes is correlated with the concomitant drop in CMI activity (1, 6). While other study showed that IgA levels were elevated constantly in HNCA mainly in younger adults more than older ones,

and this elevation is associated with an enhanced immunologic helper state (7). In NPC IgA, IgG, IgE serum levels against EBV antigens were elevated, indicating the virion antigens are triggering components of humoral immunity, and serve as a tumor-associated antigens , which could be external or internal, and such antibodies may clarify the HNCA etiology (1, 3). In such cases it is important to determine the external etiological agent of cancer that confirmed by serology, for example a virion is serving as a primary cause or a co factorial one, is reactivated from a latent state. Additional support for an immune response to HNCA is derived from observations of antibody-secreting plasma cells either in or bordering the tumor, especially observed in high number in leukoplekias with dysplasia and much fewer such cells are seen in more advanced tumor (8). The relationship between elevated serum antiviral antibodies and local infiltrating antibody-producing cells in the tumor site, focuses our attention on the possible existence of specific relationship between the tumor antigens and the humoral immune response in HNCA,

Microbiology Department, College Of Medicine, Alnahrain University.

whether this response is beneficial or not (9). Soluble immune complexes were found to be also elevated in about 75% of HNCA patients.

Basler demonstrated the principle of high levels of IgA immune complexes in NPC and HNCA due to the close relation of this tumor to mucosal tissues, and mucosal tissues associated lymphocytic tissue (MALT) which are the primary sites for IgA production (10).

One of the most important diagnostic tools in the diagnosis of EBV-related HNCA is the measurement of anti-EBV serum IgA (S. IgA). Serum level of anti-EBV IgA has been considered as an unique feature of NPC in many regions in the world (11). But in Iraq such lightening on the importance of S. IgA against EBV has not yet been achieved. So, we did a full comparison of anti-EBV S. IgA levels among HNCA patients and with HC subjects. In our study we intended to evaluate the relationship between the level of serum and saliva IgA EBV antibodies and the HNCA disease and NPC in particular, in attempt to find out a reliable diagnostic and/or prognostic tool for HNCA patients.

PATIENTS AND METHODS:

Patients were selected from those with HNCA and at different stages of the disease progression from two main centers; Alkadhimya hospital and radiotherapy center in Baghdad. 122 head and neck cancer (HNCA) patients were involved, composed of 66 patients of CA larynx, 42 patients of NPC, 14 patients of Hypopharyngeal CA. While other types of HNCA including 4 patients of tonsillar carcinoma, 2 patients of the rest which include post-pharyngea, tongue, epiglottic and retromalar carcinomas, were all neglected for statistical inconvenience. The age of HNCA patients was ranging between 16 to 74 years old (median, 53 years) and (mean, 51.8 years).

Control group consisted of 100 apparently healthy people in good general health, ranging age from 21 years old to 66 years old and non of them was taking medicine regularly. Blood was drawn into glass tubes of 10 ml in size for serum separation. Serum stored at -20°C until used in ELISA (12). Saliva samples were taken from patients and control group put in glass universals cup and directly were frozen at -20°C till their use later on by ELISA, to detect the presence of secretory IgA in saliva, which is specific for EBV antigens in patients who proved sero-positive against EBV antigens and also in sero-positive control population.

ELISA assay : ELISA assay was designed to detect quantitatively the serum and salivary IgA against EBV antigens. We prepared our local

ELISA kit by coating 96 flat bottom plates at concentration of 10 ug/ml of the crude EBV antigen (Wellcome, UK).

Duplicate wells were made for every patient and proceeded the assay as recommended by (13).

Calculation of cut-off value for *S. gallolyticus* seropositivity :

Cut off value is considered as the upper limit above which all readings were considered as positive. Therefore, ELISA readings of control subjects (n=100) were used to calculate the cut-off value according to (14): 99% Confidence interval (cut-off value) = mean + 2.626 × Standard error mean. (2.626): taken from the table of student's t-test under the $p=0.01$ for the 99 degrees of freedom.

Statistical analysis:

Data analysis was performed by the following computer statistical programs: Microsoft EXCEL 97 and Statistica.

RESULTS AND DISCUSSION:

Out of 122 patients of HNCA we can notice the predominance of squamous cell carcinoma (SCC) over all types of HNCA (74% in CA larynx and 68% in hypopharyngeal CA), except for NPC patients that elicited a different feature of having 93% of highly undifferentiated carcinoma, namely Lymphoepithelioma (LE).

Statistical analysis showed that only NPC group was significantly of higher S. IgA level (mean ELISA reading = 1.34) than other groups of HNCA (mean ELISA reading for CA larynx = 0.98; for hypopharyngeal CA = 1.02) ($p < 0.01$), while there was no real difference between Hypopharyngeal CA and CA larynx ($p > 0.05$).

The cut-off point calculated was 1.22. Depending on this cut-off point, it was found that only 9 patients within NPC group were sero-negative for anti-EBV S. IgA, while in CA larynx only 6 patients were sero-positive and finally in Hypopharyngeal CA only 3 patients was sero-positive and the rest of patients were sero-negative (Table 1). This important serological feature reflects a fact that there is a strict relationship between EBV and etiology of NPC in Iraqi patients. Very rare those reports that revealed a positive detection of salivary anti-EBV IgA of HNCA, especially in NPC patients.

The majority of reports tell us that there is no positive results regarding this field (1, 3, 15).

We applied ELISA technique on saliva as well as on sera of HNCA patients. After completion of assay we did not detect any positive ELISA reading for the salivary IgA (mean ELISA reading = 0.099) when compared to HC group (mean ELISA reading = 0.089).

Therefore, we inferred that immunoassay on saliva anti-EBV IgA is not sufficiently sensitive for the diagnosis of EBV-related tumors.

The detection of anti-EBV S.IgA is still more specific than the detection of anti-EBV S.IgG, because anti-EBV S.IgA is only found in NPC patients, while anti-EBV S.IgG could be found in low levels in a large proportion of the population due to the childhood IM infection and in high levels in immune deficiency conditions, and few other cancers in addition to NPC (1, 3).

Because of the fact that 78.5 % of the sera of NPC patients in this study were positive to EBV S. IgA antibodies, thus, using serological kits for the detection of EBV antibodies seems to be a useful, cheap and non-invasive method to diagnose about 78.5% of NPC cases in Iraq, which is a very high percentage of cases that make the use of these serological tests seems really worthy.

In contrast to serum EBV antibodies, saliva antibodies against EBV were not detected at all. This has a very important impact on our imagination on what is happening inside the nasopharyngeal tissue.

According to many reports, it has been postulated that the real reservoir for EBV latent infection within the nasopharynx is the tissue infiltrating lymphocytes (TIL) which are in continuous recycling with PBL, and the transfer of EBV infection is Bi-directional between the source TIL and the recipient cells, the nasopharyngeal cells. But not all the nasopharyngeal tissue layers are susceptible to get EBV infection, it has found that only the deep layers have this criteria because they have CD21 receptors, the gate for EBV entry (16, 17). Therefore, S.IgA are triggered far more than salivary IgA which depends on the availability of EBV-encoded antigens on the superficial layers of nasopharyngeal tissue cells.

Hence, we conclude that S.IgA levels might be applicable index for evaluating the EBV burden that originated from nasopharyngeal cancerous cells. Such index might help in diagnosing early and specifically the carcinogenesis of NPC, moreover, might help in evaluating the progress of the disease and give a glimpse to the humeral limb of immunity among NPC patients.

Table 1 : Correlation of different HNCA types with each other depending on sero-positive / negative ELISA readings for anti-EBV S.IgA

NPC		Hypopharyngeal CA		p value
Sero +	Sero -	Sero +	Sero -	
33	9	3	11	0.009chi 0.018 F.E
NPC		CA larynx		0.00001 chi 0.0001 F.E
Sero +	Sero -	Sero +	Sero -	
33	9	6	60	
Hypopharyngeal CA		CA larynx		0.28 chi 0.52 F.E
Sero +	Sero -	Sero +	Sero -	
3	11	6	60	

REFERENCES:

1. Mun-Hon Ng., Chan Wwog-Hung, Sze Ng., Zong Yong. Epstein Barr virus serology in early detection and screening of nasopharyngeal carcinoma. *hinsess Journal of Cancer* 2006; 25: 250-256.
2. Order S.E., Klein J.L., Leichner P.K. Antiferritin IgG antibody for isotopic cancer chemotherapy. *Oncology* 1981; 38:154-160.
3. Tsuchiya S: Diagnosis of Epstein-Bar virus-associated diseases. *Crit Rev Oncol Hematol* 2002; 44: 227-238.
4. Katz A.E., Nysather J.O., and Harker L.A. Major immunoglobulin ratios in carcinoma of the head and neck. *Ann. Otol. Rhinol. Laryngol.* 1978; 87:412-416.
5. Neel H.B., Pearson G.R., Weiland L.H., Taylor W.F, Geopfert H.H., Pilch B.Z., Lanier A.P., Huang A.T., Hyams V.J. & Levine P.H. Anti-EBV, serologic tests for nasopharyngeal carcinoma. *Laryngoscope* 1980; 90:1981-1990.
6. Veltri K.W., Rodman S.M. , Maxim P.E. , Baseler M.W. and Sprinkle P.M. Immune complexes, serum proteins , cell-mediated immunity and immune regulation in patients with squamous cell carcinoma of the head and neck. *Cancer* 1986; 57:2295-2308.
7. Schantz S.P., Liu F.J., Taylor D., Beddingfield N., Weber R.S. The relationship of circulating IgA to cellular immunity in head & neck cancer patients. *Laryngoscope* 1988; 98:671-678.

8. Loning T., Burkhardt A. Plasma cells and immunoglobulin synthesis in oral pre-cancer and cancer. *Virchows Arch. Pathol. Anat. Histol.* 1988; 384:109-120.
9. Kieff, E., and A. Rickinson. Epstein-Barr Virus and its replication. *Fields Virology*. D.M. Knipe and P.M. Howley, editors. Lippincott-Raven, Philadelphia, PA. 2001; 2511–2573.
10. Baseler M.W., Maxim P.E. and Veltri R.W. Circulating IgA immune complexes in head and neck cancer, nasopharyngeal carcinoma, lung carcinoma, lung cancer and colon cancer. *Cancer* 1987; 59 : 1727-1731.
11. De The G., Desgranges C., Zeng Y., Wang P.C., Bornkamm G.W., Zhu J.S., and Shang M. Search for pre-cancerous lesions and EBV markers in the nasopharynx of IgA positive individuals. In: cancer campaign, nasopharyngeal carcinoma (vol. 5), E. Grundmann, G. R. F. Krueger and D.V. Ablashi (eds.), Gustav Fischer Verlag, Berlin 1981; pp.111-117.
12. Johnstone A. and Thorp R. *Immunochemistry in practice*. Blacwell scientific publications. Oxford. 1988; pp (30-31). Plasma separ.
13. Voller A., Bidwell D., Bartlett A. Enzyme-linked immunosorbent assay In: *Manual of clinical immunology*. 2nd edition. Rose N.R., Friedman H., eds. Washington DC: American society of Microbiology, 1980 pp 359-371.
14. Al-Murrani W. K., Al-Shummari A., Al-Obaidi A. and Mustafa A. M. New approach for the calculation of the cut-off point (value) in immunological and diagnostic tests. *Iraqi J Microbiol* 2000; 12: 1-9.
15. Ho H.C., Nag M.H., Kwan H.C., Chau J.C.W. Epstein Barr virus specific IgA and IgG specific antibodies in nasopharyngeal carcinoma. *Br. J. Cancer* 1999 ;34:656-59.
16. Keiji I., Takenobu Y., Kazuhide T., Daisuke S., Kazuyasu F., Hironori M., et al. A Spectrum of Clinical Manifestations Caused by Host Immune Responses against Epstein-Barr Virus Infections. *Acta Med. Okayama* .2004. 58: 169 -180
17. Gerald N. and Laurence Y. Epstein-Barr virus persistence and virus-associated tumors. *Lancet* 1994; 343:333-335.