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Estimation the relation between Epstein-Barr virus in nasopharyngeal carcinoma with interleukin-10 in Kirkuk city

ABSTRACT:

Background Rickets is a condition associated with bone-deformity due to inadequate mineralization in growing bones. rickets of prematurity is a well-known condition that predisposes to pathological fractures in .

The aim of the study was evaluation the frequency of EBV antibodies in nasopharyngeal carcinoma patients and their relation with serum IL-10 level.

Patients & Methods: A cross sectional study was carried out from 15th of January 2018 to 15th of June 2018. The number of nasopharyngeal cancer patients under study were 68 patients whose ages were between 7-75 years old. These patients admitted to Kirkuk oncology center, Baghdad oncology center and Al-Sulaimaniya oncology center, while 22 healthy individuals control group, their age ranged between 30-75 years old. All patients and control group were investigated for detection of EBV IgG antibodies and the level IL-10 by using ELISA technique..

The Results: The study showed that the highest rate of EBV-IgG Abs occurred in patients with nasopharyngeal carcinoma (51.74%) comparing with the control group (4.55%). This result was highly significant. The highest rate of patients with nasopharyngeal carcinoma was (23.53%) patient within the age group 57-66 years. The least rate of patients with nasopharyngeal carcinoma was (10.29%) patient within the age group 7-16 years. The study showed that (58.82%) of patients enrolled in the study were males and (41.17%) were females. The highest rate (66.18%) of patients with nasopharyngeal carcinoma were in the stage of metastasis comparing with (33.82%) of patients without metastasis. The highest rate (71.43%) of EBV infection was found in patients with metastasis. The highest mean of IL-10 (42.31 pg/ml) in patients with nasopharyngeal carcinoma who were infected with EBV, followed by those without EBV infection (37.38 pg/ml), while the control group recorded a mean of (2.50 pg/ml), the relation was highly significant ($P = 0.00001$).

Conclusions: study was concluded that there is a highly significant relation between EBV and IL-10 among patients nasopharyngeal carcinoma.

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Introduction

Nasopharyngeal carcinoma is a disease with a remarkable geographic and racial distribution worldwide, the distinct difference in the incidence among geographic and population area implies that both environmental factors and genetic susceptibility play roles in the development of NPC [1]. Nasopharyngeal carcinoma (NPC) presents as a complex disease caused by an interaction of the oncogenic Epstein-Barr virus (EBV) chronic infection, environmental, and genetic factors, in a multistep carcinogenic process[2]. Approximately 90% of the adult population undifferentiated nasopharyngeal carcinomas (UNPC) all over the world are EBV positive by serology[3]. It is more than 50 years since EBV, the first human tumor virus, was discovered. Epstein-Barr virus has subsequently been found to be associated with a diverse range of tumors of both lymphoid and epithelial origin[1]. Interleukin -10, is a cytokine which exerts immunosuppressive functions by downregulating cytokine secretion, reducing antigen-presentation and inhibiting macrophage activation and T-cell proliferation[4]. It has been observed that IL-10 expression in NPC cells predicts clinical outcome, it has also been reported that IL-10 is not expressed by NPC cells [5]. The aim of the study was evaluation the

frequency of EBV antibodies in nasopharyngeal carcinoma patients and their relation with serum IL-10 level.

Patients and methods

A cross sectional study was carried out from 15th of January 2018 to 15th of June 2018. The number of patients with nasopharyngeal carcinoma were (68) patients whose ages were between 7-75 years old. These patients admitted to Kirkuk oncology center, Baghdad oncology center and Al-Sulaimaniya oncology center and (22) healthy individuals (control group), their age ranged between 30-75 years old, were investigated for detection of EBV IgG antibodies and level of I IL-10.

Five ml of blood was collected by vein puncture using disposable syringes from each patient and healthy control enrolled in this study. Blood samples were placed in plane tubes left for 30 minutes at 37 °C then were centrifuged at 3000 rpm for 15 minutes then the clot was removed and the remain re- centrifuged at 3000 rpm for 10 min and the obtained sera were then aspirated using automatic micropipette and transferred into clean test tubes for serological tests for detection of specific EBV IgG antibodies and determination the level of IL-10 by using ELISA technique.

Results

Regarding the relation of EBV infection with nasopharyngeal carcinoma, Table (1) shows that the highest rate (51.74%) of EBV-IgG Abs was occurred in patients with nasopharyngeal carcinoma comparing with the control group (4.55%). The result is highly significant. Figure (1) shows that the highest rate (23.53%) of patients with nasopharyngeal carcinoma was within the age group 57-66 years, followed by those within the age group 67-76 years (16.18%). The least rate (10.29%) of patients with nasopharyngeal carcinoma within the age group 7-16 years. The study showed that (58.82%) of

patients enrolled in the study are males while 41.17% are females. The study shows that the highest rate (66.18%) of patients with NPC are in the stage of metastasis comparing with (33.82%) of patients without metastasis, the highest rate (71.43%) of EBV infection were found in patients with metastasis, as shown Table (2). The highest mean of IL-10 (42.31 pg/ml) is records in patients with nasopharyngeal carcinoma who infected with EBV followed by those without EBV infection (37.38 pg/ml), while the control group records a mean of (2.50 pg/ml), the relation is highly significant ($P = 0.00001$), Table (3).

Table 1: Frequency of EBV-IgG antibodies in patients with nasopharyngeal carcinoma and control group.

EBV IgG	patients with nasopharyngeal carcinoma		Control	
	No.	%	No.	%
Positive	35	51.74	1	4.55
Negative	33	48.53	21	95.45
Total	68	100	22	100
<i>P. value = 0.00009</i> Highly Significant(HS)				

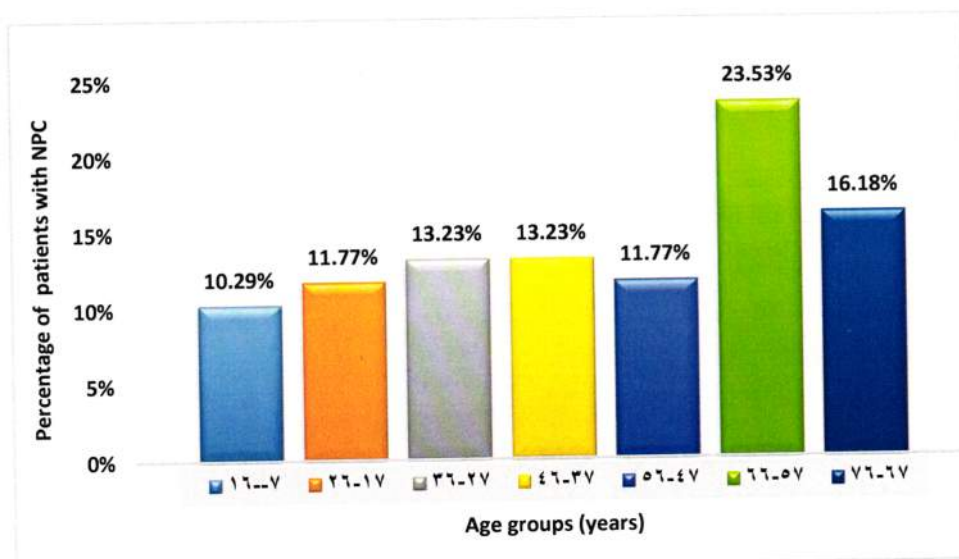


Figure 1: Distribution of patients with nasopharyngeal carcinoma according to age.

Table 2: Distribution of patients with NPC infected with EBV and HPV according to metastasis.

Stages of metastasis	Total No.	%	EBV +	
			No	%
No metastasis	23	33.82	10	28.57
metastasis	45	66.18	25	71.43
Total	68	100	35	100
<i>P. value from X^2: 0.588 (N.S)</i>				

Table 3: Levels of IL-10 in patients with nasopharyngeal carcinoma (with and without EBV infection) and the control group.

IL-10 level (pg/ml)	patients with nasopharyngeal carcinoma		Control group (n:22)
	EBV- (n:33)	EBV+ (n:35)	
Mean	37.38	42.31	2.50
SD.	25.67	25.35	0.95
<i>P. value = 0.00001 Highly Significant(S)</i>			

Discussion

The association between EBV and NPC has been well documented. Some studies were from Southeast Asia (4,5). Doğan *et al* [6] studied 30 specimens from patients with NPC, 29 of which were positive by Epstein-Barr encoding region (EBER) *in situ* hybridization, but only 13 were positive by latent membrane proteins by immunohistochemistry. The Italian study found that 16 of 24 samples of non-keratinising (types 2 and 3) NPC were positive by EBER *in situ* hybridization [7]. Previous studies demonstrated a strong association of EBV with risk of NPC [8,9]. Additionally, it has been demonstrated that EBV within intact oropharyngeal epithelium was derived from EBV-infected salivary cells through cell-to-cell contact (10). Multiple etiological factors are believed to be involved in NPC development, including genetic susceptibility, EBV infection, and diet (11,12). Although EBV antibody and DNA levels in serum are considered sufficiently sensitive and specific for NPC screening, the EBV antibody level depends on the host immune response to EBV infection and changes over time [13]. Zeng *et al* [14] wrote that approximately 90% of the adult population with undifferentiated nasopharyngeal carcinomas (UNPC) all over the

world are EBV positive by serology. Patients with NPC from various countries are described with ages ranging from 4 to 91 years. Generally, NPC is uncommon in individuals under the age of 20 years (less than 1%), whereas a bimodal age distribution has been described in northern Africa, with 20% of patients being below age 30 [11,15]. Adham *et al* [16] found that the age distribution of NPC patients had a peak at 40 to 49 years, and more than 80% of patients were diagnosed between 30 and 59 years of age and 20% of juvenile NPC cases, aged under 30 years. Devi *et al* [17] reported that risk in the NPC people was 60% males and 40% females. In high risk areas, such as Hong Kong, the NPC incidence in each sex rises sharply from the age of 20 onward and also reaches a plateau between 40 and 60 years of age. Some found that most patients presented with advanced stage of metastasis was 76% compared to 24% of those without metastasis (13-15). In the current study, patients have metastasis because of the fact that the nasopharynx is a very rich area with lymphatics, and it is considered as the main lymphatic drainage station to the upper deep cervical group as it was seen in many studies (15-17). Interleukin-10 is a cytokine mainly produced by macrophages, T-helper-2 cells and B lymphocytes, which can

both stimulate and suppress the immune responses, such as cytokine production, antigen presentation, macrophage activation and antigen-specific T-cell proliferation (18). In recent years, IL-10 has been reported to play a critical role in cancer development and metastasis [19]. Increased circulating IL-10 has been reported in patients with different types of cancer, including NPC [20]. Interleukin-10 can be secreted at a higher rate by metastatic cancer cells than by lymphocytes. It down regulates the inflammatory response of cell-mediated immunity. Interleukin-10 also inhibits antigen presentation (21). Ma *et al* (22) showed that serum interleukin-10 levels were significantly increased in patients with nasopharyngeal carcinoma and interleukin-10 have a crucial role in the progression of nasopharyngeal carcinoma. The relationship between IL-10 and cancer has been studied extensively, the ultimate role of IL-10 in tumor biology remains enigmatic [21]. Bhattacharyya *et al* [23] demonstrated that the significance of IL-10 production within the tumor microenvironment, which can be sustained by malignant cells and tumor-infiltrating macrophages and lymphocytes (including natural killer (NK) and T cells), is debated. Epstein Barr virus infection is known to

induce endogenous secretion of IL-10 as a mechanism of parasitism because IL-10 seems to be responsible for inhibition synthesis of IFN-gamma, the main macrophage –stimulating cytokine involved in the defense against EBV which facilitate the intracellular survival of virus by down-regulating the oxidative and inflammatory response (21). In fact in human severity of EBV has been closely associated with increased levels of IL-10 and the use of anti-IL-10 antibody to block the IL-10 activity or blocking IL-10 receptor can be effective approach for the treatment of infectious mononucleosis (24,25).

Conclusion:

The conclusion of this study was concluded that there is a highly significant relation between EBV and IL-10 among NPC patients.

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