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Molecular Detection of Parvovirus B19 and Immunohistochemical Localization of Interleukin 6 of Tissues from Thyroid Cancer

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ABSTRACT

Background: Human parvovirus B19 has recently been detected in papillary thyroid carcinoma at a high frequency. An increased cytokines proinflammatory upregulation like interleukin-6 concentrations have been recently reported in the inflammation of thyroid tissues with possible role in thyroid cancer.

Objective: analyzing the compatible effect of IL-6 expression and Human parvovirus B19 infection on tissues out of several patients with benign and carcinomatous thyroid lesions and elucidate the associative role of Human B19 parvovirus in thyroid carcinogenesis.

Patients and methods: Ninety formelin-fixed, parraffin- embedded thyroid tissues registered within this study; 40 thyroid biopsies tissue out of papillary thyroid carcinoma (PTC) , 30 benign thyroid hyperplasia and 20 thyroid tissues with ordinary pathological variations, as an ostensibly tissues of healthy control. Detection of Human parvovirus B19 (hB19) was conducted through chromogenic in situ hybridization (CISH) method while the expressed protein of IL-6 gene through taking immunohisto-chemistry (IHC) system of recognition.

Results: In thyroid carcinoma, the Human parvovirus B19- positive CISH reactions and the IL-6- IHC positive reactions were investigated in 17.5% (7 out of 40) and 50% (20 out of 40) of tissues, respectively, while 6.7% (2 out of 30) and 26.7% (8 out of 30) of Human parvovirus B19- positivity and IL-6 -IHC reactions was detected in benign thyroid group. None of control thyroid tissues revealed CISH reaction while10% had positive IHC reaction. The correlation between Human parvovirus B19 and IL-6 was significantly important (P= 0.003).

Conclusions: The substantial IL-6 co-expression and Human parvovirus B19 could indicate their potential part played in either thyroid pathogenesis and / or carcinogenesis.

Keywords: Human parvovirus B19, Thyroid carcinoma, benign thyroid lesion, IL-6, CISH, IHC.

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INTRODUCTION

Thyroid carcinoma is a common endocrine malignance that increased rapidly during the last decades. many histological customs are recognized, together with papillary, medullary,follicular, and undifferentiated anaplastic thyroid carcinoma (1). Several factors are required in the threshold and development of autoimmune thyroid diseases (AITD), such as genetic, hormonal, environmental, and nutritional factors. Nevertheless, the AITD pathophysiologic variations are thought to be interceded through inflammatory cytokines (2). Such cytokines, as IL1β, IL6 and TNFα, are important facilitators of the autoimmune immunologic process, including thyroid reactive T cells, involved in (HT) Hashimoto's thyroiditis and Graves' disease (GD) (3). Parvoviruses are among the tiniest DNA viruses infecting an extensive species of animal and of humans, where some are pathogenic, causing infection as well as diseases range from asymptomatic through benign to life-threatening one (4). There is a growing literature on the association of human erythrovirus B19 (EVB19) with thyroid cancers. While the data at the present time are limited for a causative role of EVB19 in thyroid carcinogenesis, however, an indirect role for this virus was hypothesized in this issue (5). It has been reported an association of persistent Human parvovirus B19 infection with wide range of non-erythroid tissues, including liver, brain, kidneys, colon, lymphoid,lungs, thyroid, testis, and tonsil (6).

The IL-6 upregulation can affect the thyroid cellular microenvironment and may be significant to pathogenesis of B19 viral infection. In this respect, Parvovirus B19 express non-structural protein 1 which cellular promoters and, transactivates viral such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) through the NF-κB-binding site in the IL-6 promoter (7).

In addition, the resulted high levels of NF-κB have a key role in thyroid cancer through its controlling the anti-apoptotic and the proliferative signaling pathways (8).Several studies showed that IL-6 via its relation to the signaling pathways have been identified to contribute to proliferation, invasion and metastasis, as well as poor prognosis of many types of cancers (7-9).

The current research may be the first in Iraq to determine the percent of parvovirus B19 infection and IL-6 protein expression in tissues attained out of a group of patients from Iraq with thyroid tumors.

MATERIALS AND METHIDS

This study enrolled a total number of ninety (90), formalin-fixed, paraffin-embedded blocks from thyroid tissues. These tissue blocks were gathered out of the archives of histopathological laboratories of Ghazi Al-Hariri Teaching Hospital / Baghdad; Al-hilla teaching hosptals as well as al-sader Teaching Hospital. The tissues block were related to the archives of past 7 years (i.e. 2013, 2014, 2015, 2016,2017, 2018 and 2019) and

are including, 40 thyroid carcinomas, 30 benign thyroid tumors and 20 healthy thyroid tissue as a control group. The diagnoses had been centered on their accompanied the corresponding patients' pathological reports. Histopathological parts were created for biopsies and stained by hematoxylin and eosin for final definitive diagnosis.

The detection of parvovirus B19 was carried out on 4µm parraffin embedded tissue sections by chromogenic in situ hybridization (CISH) kit (purchased from ZytoVision GmbH. Fischkai, Bremerhaven. Germany). using digoxigenin-labeled oligonucleotides probe that targets parvovirus B19 DNA. Immunohistochemistry / Detection system (Abcam. England) was used to demonstrate the IL-6 gene expression. This method is centred on the investigation of the gene product expression (protein) in malignant, benign and normal cells taking particular monoclonal antibodies.

The detailed methods for performing CISH and IHC reactions were conducted according to the instructions of the manufacturing company, at the Molecular Virology Research Laboratories at Communicable Diseases Research Unit /Baghdad Medical College/ University of Baghdad and at the advanced Microbiology Laboratory, at College of Science, University of Babylon.

This study utilized SPSS program (version-23) for the statistical analysis, where Chi-Square test (χ2), Odd ratio and Spearman's rho have been used to evaluate the differences between variables.

RESULTS

The archival specimens had been interconnected to papillary thyroid carcinoma (PTC) patients whose ages ranging from 32 to 76 years and their mean age was (54.48) years. The patients' mean age with benign thyroid tumors (BT) and apparently healthy control tissues (AHC) were (47.40) and (53.87) years, separately. No significant difference between PTC, BT and AHC was found regarding their age distribution (Table 1). The gender frequency distribution showed 62.5% (25 out of 40) papillary thyroid carcinoma were males and 27.5% (15 out of 40) were females. In benign thyroid tumor group, 63.3% (19 out of 30) were males and 26.7% (11 out of 30) were women. Finally, 65% (13 out of 20) were males and 35% (7 out of 20) were females in those with wholesome grouping tissues. On distributing of papillary thyroid carcinoma group based on their rating ,the outcomes of well differentiated grade (I) papillary thyroid carcinoma constituted 55% (22 out of 40), whereas moderately papillary thyroid carcinoma (II) and poorly differentiated papillary thyroid carcinoma grades (III) constituted 25% (10 out of 40) and 20% (8 out of 40) , respectively.

Table 1. Patients characteristics

		Malignant Thyroid Group N= 40(%)	Benign Thyroid Group N = 30 (%)	Healthy tissue Group N=20(%)
Age	**Mean of Age (%)**	54.48	47.40	53.87
	Range of Age	32 – 76	17 -66	36 – 91
	≤ 50	16 (40)	17 (56.7)	12 (60)
	51 - 91	24 (60)	13 (43.3)	8 (40)
Gender	**Male**	25 (62.5)	19 (63.3)	13 (65)
	Female	15 (27.5)	11 (26.7)	7(35)
Grade	**Poorly**	8 (20)		
	Moderately	10 (25)		
	Well	22 (55)		

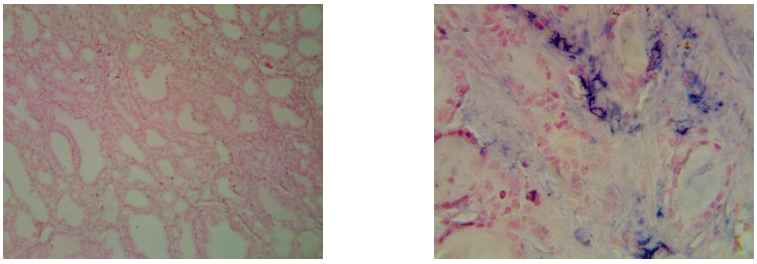
II. Results of Human parvovirus B19 (hB19) –CISH and IL-6 -IHC in Patients with Thyroid Tumors:

The investigation of hB19 and the IL-6 expression was made by taking CISH & IHC techniques into action, respectively. The expression of hB19 & IL-6 are recognized by its blue and brownish colors at nuclear and cytoplasmic localization, respectively. Based on the results (Table 2 & Figure 1 ,2), the hB19 expression was noticed in 7 tissues (17.5 %) malignant papillary thyroid tumor, 2 tissues (6.7%) of benign thyroid tumor, and none of control thyroid tissues group ,while the expression of IL-6was found in 20 tissues (50 %) of malignant papillary thyroid tumors, 8 tissues (26.7%) of benign thyroid tumors, and 2 tissues (10%) of control thyroid tissues group (Table 2)

Table 2. The results of Human parvovirus B19 (hB19) –CISH and IL-6 -IHC in Patients with Thyroid Tumors:

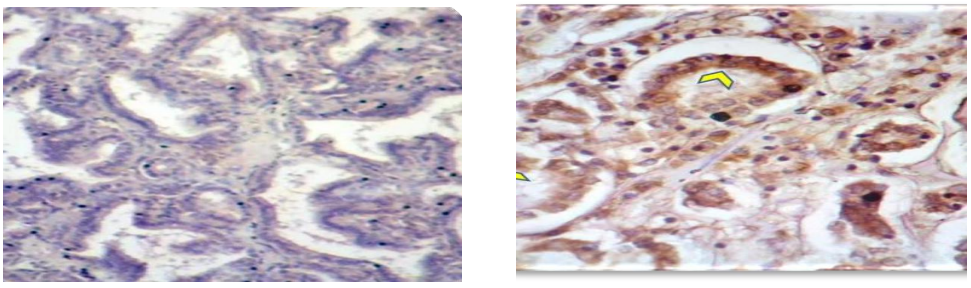
		hB19 (hB19) signals			signals of IL-6- IHC		
		+ (%)	-(%)	p-value	+(%)	-(%)	p-value
PTC n=40		7 (17.5)	33 (82.5)	<0.001	20 (50)	20 (50)	0.030
Benign Thyroid tumors n=30		2 (6.7)	28 (93.3)		8 (26.7)	22 (73.3)	
Control* * n= 20		0 (0.00)	20 (100)		2 (10)	18 (90)	
Age group	**≤50**	4 (45)	50 (55)	< 0.05	20 (66.7)	10 (33.3)	0.211
	51-91	5 (55)	31 (45)		10 (33.3)	20 (66.7)	

Gender	**male**	6 (10.5)	51 (89.5)	0.698	8 (14.1)	45 (85.9)	0.322
	Female	3 (9.1)	10 (91.1)		12 (32.4)	21 (67.6)	
Grade	**Poorly**	1 (12.5)	7 (87.5)	0.286	2 (28.6)	5 (71.4)	0.233
	Moderately	3 (30)	7 (70)		7 (70)	3 (30)	
	Well	4 (18.2)	18 (81.8)		11 (50)	11 (50)	



A **B**

Figure1: Chromogenic In situ hybridization results for parvovirus B19-DNA detection in thyroid tumors; BCIP/NBT stained and counter stained by nuclear fast red; A. Negative B19-DNA-CISH reaction (20X).B. Thyroid cancer tissue with positive B19-DNA -CISH reaction (40X).



A **B**

Figure 2: results of Immunohistochemical for expression ofIL-6 of thyroid cancers; brown color indicates DAB chromogen and Mayer's hematoxyline (blue) for counter stained A. thyroid cancers with negative IL-6- IHC reaction (20X).B. Thyroid cancers with positive IL-6- IHC reaction (40X).

III. Correlations between the grades, Human parvovirus B19 (hB19) –CISH and IL-6 -IHC in Patients with Thyroid Tumors patients:

A greatly substantial correspondence was noticed among hB19 and **IL-6** markers in PTC (*P*=0.003). Likewise, a strong positive interconnection among IL-6 and grade of PTC (*P*=0.006). However, there are no significant correlations among hB19 and other markers (Table 3).

Table 3 Spearman's rho statistical testing of age, grade, hB19-CISH and IL-6-IHC to evaluate the studied markers in PTC.

Spearman's rho		**Age groups** (years)	**Grade**	**hCMV**
Grade	r	-0.135		
	P	0.477		
hCMV	r	0.040	0.133	
	P	0.788	0.412	
IL6	r	0.176	0.419	0.483
	P	0.387	**0.006***	**0.003***

*Correlation is extremely substantial (*P*<0.01).

DISCUSSION

The prognosis of well differentiated thyroid cancers (WDTC) is an excellent prognosis disease (10), its prognosis is meticulously associated with age at

presentation, where young patients have an excellent prognosis and older patients, are at higher risk of disease-specific death, specifically those having advanced or distant disease (11).The American Joint Committee on Cancer/Union for International Cancer Control staging system for WDTC was allocating a cutoff at 45 years for disease stage dependent on age (12). Persistent viral infections, in general, might paves the way to the chronic disease's progression and eventually invasive cancer. Some researchers have been reported on the possible role of parvoviruses in a wide variety of thyroid diseases (13,14). The expression of B19 genes in thyroid cancerous is related with certain variations in inflammatory genes as well as their effects on thyroid cellular microenvironment which may finally lead to tumor development and progression (15).Nonetheless, few data regarding the B19 gene expression in thyroid cancers tissue was reported(16).

In thyroid carcinoma, the Human parvovirus B19-positive CISH reactions was detected in 17.5% of thyroid cancer tissues, while 6.7% of parvovirus B19- positivity was detected in benign thyroid group (table 2). The current study shade light for a possible key role for B19 infection in thyroid tumorigenesis as well as progression of the thyroid cancer through the associated inflammatory mechanism.

Etemadi *et al*., (15) found positive rate of 86.11% (31\36 cases) for B19 DNA in thyroid cancer tissues was detected in papillary 17 ccases;medullary 11 cases, and 3 cases in follicular thyroid cancer samples. Wang *et al*. (17) found 95%–97%, 83.3%, and 63% of B19 infection in papillary thyroid cancer tissue by using PCR, in situ hybridization, and immunohistochemistry, respectively. Adamson *et al*. (18) showed 88% of B19 capsid protein in papillary thyroid cancer tissue by IHC. B19 viral infection may lead to down regulation and then alteration in expression of, thyroidhormone receptor alpha (THRα) and retinoid X receptor alpha (RXRA), are required in tumorigenesis of thyroid (19). Furthermore, estimation a highly concetration of NF-κB in malignant thyroid tumors in comparison with non-cancerous thyroid tissues in patients infectious with hBV19 suggests the important role of hB19V in the carcinogenesis of thyroid carcinoma (20). In thyroid carcinoma, the IL-6-positive IHC reactions was detected in 50% (20 out of 40) of tissues, while 26.7% (8 cases) of IL-6 -IHC reactions was detected in benign thyroid group (table2). Provatopoulou *et al*., (21) have revealed considerably advanced levels of circulating IL-6 in benign as well as PTC patients as compared to the healthy individuals. Moreover, those observations could illustrate that circulating IL-6 could be probably derived partly from spillover of tumor produced IL-6 and might explain an association of high IL-6 levels with larger tumor size and f extra-thyroidal extension of these tumors (22). B19 NS1 transactivates cellular promoters of the inflammatory agents such as IL-6 and TNF-α through the NF-κB-binding site in the IL-6 promoter (23). Al-Gharrawi *et al*., (24) and Adamson (25) pointed out that IL-6 could be a key part in the thyroid tumor's immunological microenvironment and expression ofIL-6 could be augmented by B19 infection.

The significant correlation of IL-6 and Parvovirus B19 detection rates in thyroid tumor tissues might shade light for a possible role of this virus and IL6 in either thyroid pathogenesis or carcinogenesis.

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