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RESEARCH ARTICLE

The possible role of human parvovirus B19, Nuclear factor- kappa B p65 and interleukin- 6 in thyroid tumors

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Abstract

The present study was designed to detect parvovirus B19, NF-κB p65 and IL-6 by immunohistochemical technique and to investigate the correlation between them in Iraqi patients with different types of malignant and benign thyroid tumors. A total number of (94) formalin-fixed paraffine-embeded thyroid tumors tissue collected from archives department of histopathology laboratories during the period from July 2013 till November 2013. Tissue blocks were divided into three groups. First one included (53) tissue block of malignancy thyroid whereas the second group included (41) tissue blocks of benign thyroid tumors and the third group included (21) tissue blocks which were selected from the same benign cases which have normal tissue. By using immunohistochemistry, B19 virus was detected in (66%) of malignant cases compared to (48.8%) in benign tumors. NF-κB p65 was positively expressed in all cases of malignant tumors and (97.6%) of benign tumors compared with (23.8%) normal tissue, $p<0.001$. IL-6 expression was higher in benign than that in malignant tumors but the difference was not significant (90.2% versus 81.1%, p=0.22), while it was significantly higher in benign and malignant tumors compared with normal tissue (52.4%, p=0.01 and p=003) respectively. In conclusion the results suggest the possible noxious role of B19 virus in these tumors. The high rate of expression of NF-κB p65 in tissue of thyroid tumors may indicates it's important role in cancer signaling pathways and finally IL-6 could play a role in immunological microenvironment of thyroid tumors regardless of presence or absence of parvovirus B19.

Introduction

Thyroid cancer is the most common neoplasm of the endocrine system, which originates from follicular thyrocytes or parafollicular C-cells of the thyroid gland, represent a model of malignant transformation from benign adenomas and well differentiated carcinomas to poorly differentiated carcinoma (Ringgel, 2004; Xing, 2013) .Although relatively rare, thyroid cancer is the seventh most frequent human malignancy, and is increasing in incidence more rapidly than any other cancers. From 1995 to 2008, thyroid cancer age-adjusted incidence rates more than doubled in the U.S., whereas the incidence of most major cancers (lung, prostate, breast and colorectal) decreased during the same period (Sprague et al., 2008), Thyroid cancer occurs at ages from childhood to old age. Women are three times more likely than men to be afflicted (Wartofsky, 2010).

In Iraq thyroid cancer ranks the eighth among commonest ten cancers in female (Iraqi cancer registry, 2010). Therefore, thyroid cancer is a growing health problem. Depending on the cell origin and histological characteristics,

thyroid carcinomas are generally classified to papillary thyroid carcinoma, follicular thyroid carcinoma, anaplastic thyroid carcinoma (poorly differentiated), and medullary thyroid cancer. Most of these thyroid tumors arise from the follicular thyrocytes whereas medullary thyroid cancer is the only C-cell-originated tumor (Cohen et al., 2003).

Human parvovirus B19 (parvovirus B19, B19V) is a small, nonenveloped DNA virus belonging to the genus Erythrovirus (Parvoviridae family) (International Committee on Taxonomy of Viruses (ICTV)(2007)The viral genome of B19 is 5,596 nucleotides in length and encodes for a large non-structural protein (NS1), as well as two capsid proteins (VP1 and VP2), of which VP1 contains a unique region referred to as VPU. It has been shown to be a cause of several, wide-ranging human illnesses (Young and Brown, 2004), including aplastic crisis, erythema infectiosum (fifth disease), arthritis, thrombocytopenia, hydrops fetalis, and myocarditis. B19 has also been highly associated with neurological disorders (Douvoyiannis et al., 2009; Hammond and Hobbs, 2007) and autoimmune disorders with symptoms similar to rheumatoid arthritis and lupus erythematosus (Lunardi et al., 2008). Recently, B19V infection has been strongly associated with hashimotos s thyroiditis and thyroid cancer (Wang et al., 2008, 2010; Adamson et al., 2011 and Adamson, 2013).

At least 20% of all cancers arise from association with infection and chronic inflammation and even those cancers that don't develop as a consequence of chronic inflammation, exhibit extensive inflammatory infiltrates with high levels of cytokines expression in the tumor microenvironment. Several such cytokines were found to serve as were growth and survival factors that act on premalignant cells (Greten and Karin, 2005), stimulation of angiogenesis, tumor progression and metastasis, and maintain tumor-promoting inflammation (Grivennikov et al., 2010).

Tumor necrosis factor(TNF) and interleukine-6(IL-6) are the best characterized protumorigenic cytokine to be involved in cancer owing to their ability to activate the oncogenic transcription factors NF-κB,AP1(TNF) and STAT3(IL-6) in epithelial cells (Balkwill, 2009).

The NF- kappa B family of transcription factors regulates the expression of a wide spectrum of genes involved in inflammation, immune response, cellular stress, cancer, and apoptosis. In most cell types, NF-κB is present in a latent form in the cytoplasm and bound to NF-κB inhibitory proteins collectively termed I-κBs. A wide variety of extracellular signals, such as pro-inflammatory cytokines , ъbacterial and viral infections, oxidative stress, etc., initiate signaling cascade that culminates in the phosphorylation and subsequent degradation of I-κBs, through a proteasome-dependent pathway (Pacifico et al.,2004).

Samples, Materials and Methods

Samples:

A retrospective study included ninety four formalin-fixed, paraffin – embedded tissue blocks, which have been diagnosed as thyroid tumors. Fifty three of these were malignant thyroid tumors, forty one were benign thyroid tumors and twenty one case selected from benign tumors which have normal tissue as control. All these samples are related to period between period 2010 till 2013. The study samples were collected from the pathology archives of the histopathology unit in Teaching laboratories Department, Medical/ city, Al-Shaheed Ghazi hospital lab, Al-Kindy hospital lab, Al-karama hospital lab and central public health lab. All Hematoxylin and Eosin stained tissue sections were reviewed, the best sections and those representing the original tumor site from each specimen were selected. In period from August 2013 to April 2014 all the preparations for immunohistochemistry were performed in central public health lab. From each block, four sections of 4µm thickness were taken, one section was stained with Hematoxylin and Eosin (H&E) and the other three sections were immunohistochemically stained for human parvovirus B19, NF-κBp65 and IL-6.

Immunohistochemistry and scoring

Paraffin sections of 4µm thickiness were deparaffinised and treated with hydrogen peroxide to block endogenous peroxidase activity. Heat-induced antigen retrieval was performed in sodium citrate (pH 6.0) and protein block at room temperature to block the non specific antibody-antibody-binding site. Then the sections were incubated overnight at 4° C with mouse monoclonal antibody against the B19 proteins VP1/VP2 (1:30) dilution, Monoclonal rabbit Anti-human NF-kB p65, clone E379 (1:250) dilution, Monoclonal mouse Anti-human IL-6, clone ab9324 (1:70) dilution, (abcam company, UK). After washing in PBS they were incubated with secondary antibodies(Mouse specifying reagent) for 10 minutes, and then followed by goat anti-rabbit HRP conjugatefor 15 minutes, according to the instructions of the manufacturer (Universal Detection Kit, Abcam, UK). Finally the immune reaction was visualized as a brown color with 3, 3 – diaminobenzidine (DAB, Abcam ab80436 Kit) for 5 minutes, then washed in distilled water. Then the slides were counterstained with Mayer's hematoxylin for 1-3 minute before mounting. The entire procedures were performed at room temperature. Additionally, a negative control for both markers in which the primary antibody was omitted and replaced by phosphate buffered saline was used. In addition, Placenta tissue infected with human parvovirus B19 were used as positive control for human parvovirus B19, Breast carcinoma

tissue for NF-κB p65 and human cervix squamous carcinoma tissue IL-6 were added to process with the thyroid tissue sections in the same run for precision and standardization of the elaborated IHC results of all markers.

Scoring for human parvovirus B19

IHC was performed to detect and determine of B19 capsid protein in each tissue section. Each case was assigned a positive score based on the value by multiplying the average intensity of each positive area by the total positively of the whole tissue section. Cases with score less than five were considered negative (-), with those from 5.1-20 ranking (+), and greater than 20.1 ranking (++) (Adamson, 2013).

Scoring for NF- κB p65

Cells were considered to positive for NF-κB p65 when immunoreactivity was clearly sereved in cytoplasm or nucleic. Expresion of NF- κB p65 was evaluated based on percentage of stained cells and staining intensity. At least 5 representative areas were randomly selected 200 x magnifications under a light microscope. Points were allocated based on the percentage of positive cells as follows: Points were allocated based on the percentage of positive cells as follows: 0 point, ≤ 1% positive cells ; 1 point, 1-25% positive cells; 2 points, 25-50% positive cells; 3 points, 50-75% positive cells; 4 Points, >75% positive cells. The staining Intensity was classified as follows: 1 point, weak intensity; 2 points, moderate intensity; 3 points, strong intensity. Points for the percentage and intensity of positive cells were multiplied get the overall score (OS) for each specimen. According to the OS, specimens were defined as negative (OS<1) or positive (OS≥1) (Zhenxian et al., 2006).

Scoring for IL-6

Sections were scored by using the following method: the intensity of staining was scored from 0 to 3. 0, absent; 1, weak; 2, moderate; 3, strong and the proportion of malignant cells positively stained was scored from 0 to 4. 0, no positive cells; 1, <10 % positive cells; 2, 11-50%; 3, 51-7%; 4, 76-100%. The two scores were then added to yield the total score (0-7). Immunoreactivity was defined as negative when the score was 0, weak when it was 1-3, and strong when it was ≥4 (Basolo et al., 1998).

Statistical analysis: Data were translated into a computerized database structure. An expert statistical advice was sought for. Statistical analyses were done using SPSS version 21 computer software (Statistical Package for Social Sciences).Some of the quantitative outcome variables were measured on an ordinal scale (like intensity). Compliance of quantitative random variables with Gaussian curve (normal distribution) was analyzed using the Kolmogorov-Smirnov test. The percentage and score measures of selected markers were shown to be non-normally distributed quantitative variables. These variables in addition to those measured on an ordinal scale (like intensity) can be described by median and inter-quartile range. The difference in median between 2 groups was assessed by non-parameteric test (Mann-Whitney), while between more than 2 groups Kruskal-Wallis test was used. Associations between 2 categorical variables were explored by cross-tabulation. The statistical significance of such associations was assessed by Chi-square ($\chi 2$) test. The statistical significance, direction and strength of linear correlation between 2 quantitative normally variables, one of which being non-normally distributed was measured by Spearman's rank linear correlation coefficient. P value less than 0.05 level of significant were considered statistically significant.

Results

Immunohistochemical expression of human parvovirus B19 –capsid protein in patients with thyroid tumors.

The results of B19 protein detection in patients with thyroid tumors, using immunohistochemistry (IHC) technique revealed that 66% (35 out of 53) of malignant thyroid tumors, 48.8% (20 out of 41) of benign thyroid tumors and 4.8% (1 out of 21) of normal thyroid tissue blocks have positive reaction. Statistically, the positive rate of B19 VP1/VP2 antigen in thyroid malignancy cases was higher than benign but not significantly while cases of thyroid malignancy and benign cases were both significantly higher than normal thyroid tissue $p<0.001$ (Table 1).

The scoring system and cut-off value were used depending on (Adamson, 2013). Figure (1) shows the difference in median of human parvovirus B19 score categories, the score category that got the higher percentage (6-20) of expression of B19 was in malignant cases (19 cases, 35.8%) and in benign cases it was(12 cases, 29.3%). While lowest score categories were (11 cases, 20.8%), (6 cases, 14.6%) in malignant and benign cases respectively, which was among the score (>20) compared with healthy tissue which have (1 case only 4.8%) in score (>20).

Table 1: Human parvovirus B19 expression in study groups.

	Healthy control (normal thyroid tissue)	Diseased controls (Benign thyroid tumor)	Cases (Thyroid malignancy)	P value
Parvovirus B19	N (%)	N (%)	N (%)	<0.001
Negative	20 (95.2)	21 (51.2)	18(34)	
Positive	1 (4.8)	20(48.8)	35(66)	
Total	21 (100)	41(100)	53(100)	
P (Chi-square) for 2 groups comparison:				
Cases (Thyroid malignancy) x Positive controls (Benign thyroid tumor) = 0.09[NS]				
Cases (Thyroid malignancy) x Healthy control (normal thyroid tissue) <0.001				
Positive controls (Benign thyroid tumor) x Healthy control (normal thyroid tissue) = 0.001				

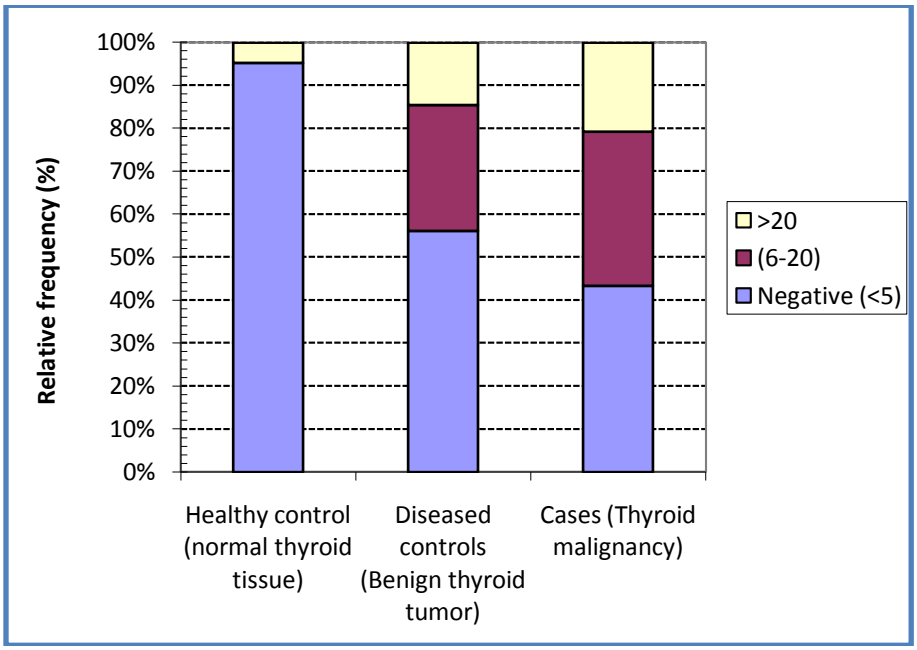

Figure 1: Component bar chart showing the difference in score categories of human Parvovirus B19 marker between the 3 study groups.

Immunohistochemical expression of NF-κB p65

A positive signal of NF-κB p65 was detected in 100% (53 out of 53) in thyroid malignancy cases, among which 46 were in cytoplasm (fig5 B) whereas only 7 cases were in nucleus (fig5 C). Likewise it was detected in 97.6% (40 out of 41) in benign cases among which 39 were in cytoplasm and only one was detected in the nucleus. Rate of expression in both malignant and benign cases , were differ significantly ($p< 0.001$) in comparison to the rate of expression in tissue blocks taken from normal tissues which was recorded in only 5 out of 21 (23%) of them (Table 2). Among the 35 cases of thyroid tissues that were positive for B19 VP1/VP2 protein, nuclear translocation of NF-κB was found in 3 cases only. This result showed that the nuclear translocation of NF-κB doesn't well correlate with VP1/VP2 antigen expression.

Figure (2) showing the significant difference (p< 0.001) in median score of NF-κBp65 between the 3 studied groups. The median score of NF- κB p65 in normal tissue, benign and malignant thyroid tumors were 0, 6, and 6, respectively.

Table 2: Immunohistochemical expression of NF-κB p65in study groups.

	Healthy control (normal thyroid tissue)	Diseased controls (Benign thyroid tumor)	Cases (Thyroid malignancy)	P. Value
NF-κB p65	N (%)	N (%)	N (%)	<0.001
Negative	16 (76.2)	1 (2.4)	0(0)	
Positive	5 (23.8)	40(97.6)	53(100)	
Total	21 (100)	41(100)	53(100)	
P (Chi-square) for 2 groups comparison:				
Cases (Thyroid malignancy) x Positive controls (Benign thyroid tumor) = 0.44[NS]				
Cases (Thyroid malignancy) x Healthy control (normal thyroid tissue) <0.001				
Positive controls (Benign thyroid tumor) x Healthy control (normal thyroid tissue) < 0.001				

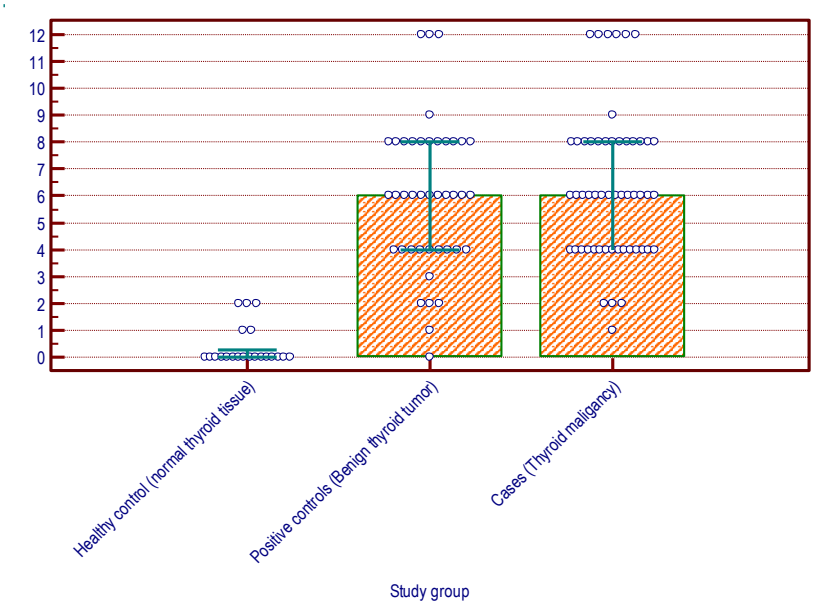

Figure 2: Bar chart showing the difference in median score of nuclear factor kappa P65 marker between the 3 study groups.

Immunohistochemical expression of IL-6

The result demonstrated in (table 3) shows that IL-6 reactivity was positive in 81.1% (43 out of 53) of malignant thyroid tumors, 90.2% (37 out of 41) of benign thyroid tumors and 52.4% (11 out of 21) of normal tissue. Even though the positive rate was higher in benign than that in malignant thyroid tumors but there was no significant difference, while there was a significant difference in median of score category between them (fig3). The difference

in the distribution of IL-6 expression among malignant, benign tumors compared to normal tissue is significant (P=0.012 and P=0.003), respectively.

Table 3: Immunohistochemical expression of IL-6 in study groups.

	Healthy control (normal thyroid tissue)	Diseased controls (Benign thyroid tumor)	Cases (Thyroid malignancy)	P.Value
IL-6	N (%)	N (%)	N (%)	0.002
Negative	10 (47.6)	4(9.8)	10(18.9)	
Positive	11 (52.4)	37(90.2)	43(81.1)	
Total	21 (100)	41(100)	53(100)	
P (Chi-square) for 2 groups comparison				
Cases (Thyroid malignancy) x Positive controls (Benign thyroid tumor) = 0.22[NS]				
Cases (Thyroid malignancy) x Healthy control (normal thyroid tissue) =0.012				
Positive controls (Benign thyroid tumor) x Healthy control (normal thyroid tissue) =0.003				

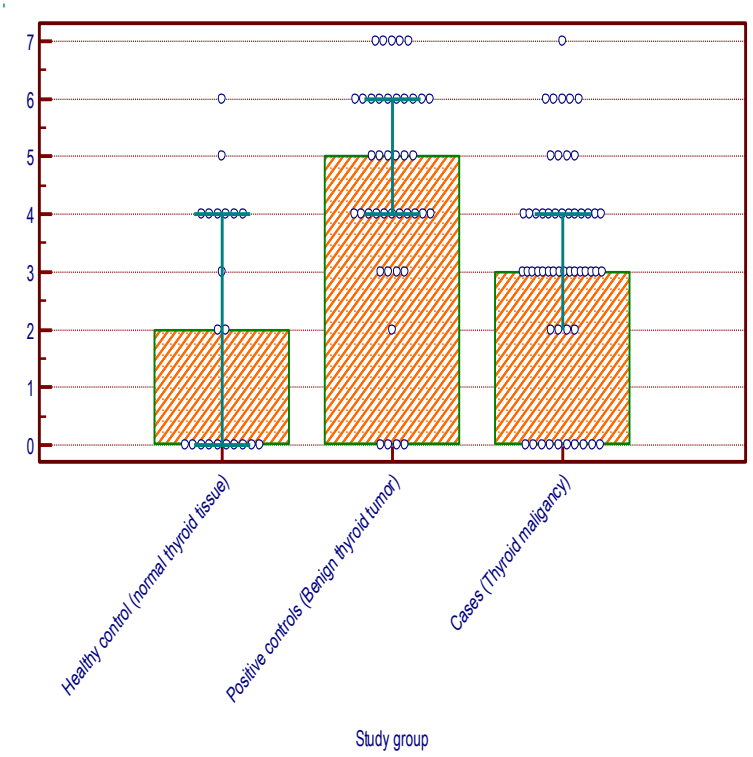

Figure 3: Bar chart showing the difference in median score of IL6 marker between the 3 study groups.

Assessment of the difference in expression of studied markers among histopathological types of malignant cases.

The current study observed that there are no significant differences P> 0.05 in expression of human parvovirus B19 and NF-κB between histopathological types of malignant type's papillary and follicular carcinoma as shown in (table 4).

Table 4: Difference in expression of human parvovirus B19 and NF-κB p65 among histopathological types of malignant cases.

	Follicular Thyroid Carcinoma (FTC)		Papillary Thyroid Carcinoma (PTC)		P
	N	%	N	%	
Human Parvovirus B19					0.41[NS]
Negative	1	12.5	10	30.3	
Positive	7	87.5	23	69.7	
Total	8	100.0	33	100.0	
Nuclear factor-Kappa B P65					**
Negative	0	0.0	0	0.0	
Positive	8	100.0	33	100.0	
Total	8	100.0	33	100.0	

Table 5: linear correlation between study markers.

Markers	Linear correlation (r}	P.value
Human parvovirus B19 score category & NF-κB p65 score	0.033	0.81[NS]
Human parvovirus B19 score category & IL-6 score	-0.109	0.44[NS]

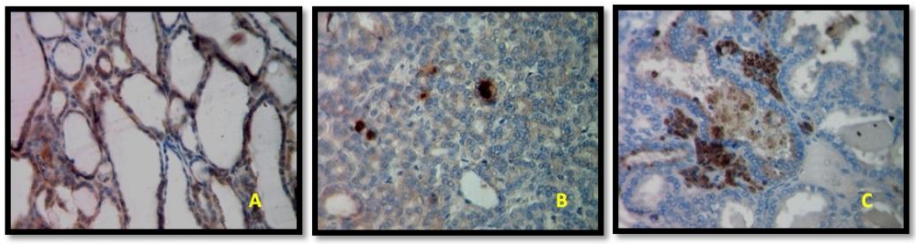

Figure.4 Immunohistochemical expression of B19 capsid protein in thyroid tumors, (A) Normal thyroid follicle with cytoplasm expression, (B)Adenoma which express nuclear staining (C) papillary carcinoma which express nuclear staining, thick arrow and cytoplasm expression, thin arrow. X40

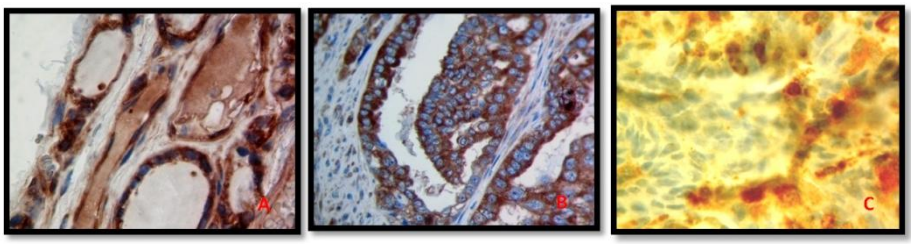

Figure5: Immunohistochemical expression of NF-kB P65 (A) Normal thyroid follicles with cytoplasm staining (B) papillary carcinoma with cytoplasm staining (C) nuclear staining X40.

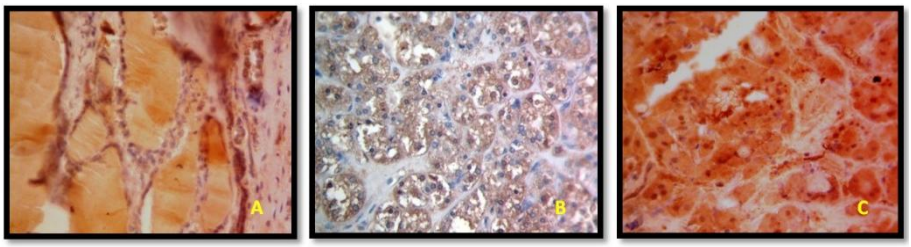

Figure 6: Immunohistochemical expression of IL-6 (A) Normal thyroid follicles with positive staining (B) follicular carcinoma with moderate cytoplasm staining (C) Folliclular carcinoma with strong cytoplasmic staining. X40

Discussion

In the present study, B19 capsid proteins were frequently detected in malignant thyroid tumors tissue 66% compared to benign 48.8% and normal tissue 4.8%. These results support that recorded by Wang et al., (2008)who found B19 infection in papillary thyroid cancer tissue samples using nested PCR even in 95%-97%, insitu hybridization (ISH) in 83.3% and immunohistochemistry (IHC) in 63% of their cases. The results also agreed with Adamson et al., (2011) who extend the data available on B19 detection in thyroid to show 21 of 24 (88%) PTC tumors and 3 of the 3 ATC undifferentiated tumors tissue samples were positive for B19 capsid protein by IHC. Adamson, (2013) results also showed that B19 infection in the thyroid tissue increased capsid protein detection in adenoma and tumor. These studies were the only three attempts which have been done in China and Florida trying to correlate B19 and thyroid tumors while the present study, according to our best knowledge was the first one in Middle East.

There was no significantly difference between papillary and follicular thyroid carcinoma in expression of B19 capsid proteins which were 69.7% and 87.5%, respectively as shown in table (2). These results were higher than that in benign and normal tissue, among the other finding in this respect, (1 out of 3) cases with anaplastic carcinoma revealed a positive expression of B19 while no one of the three cases with medullary carcinoma was with positive expression. These findings came in agreement with that of Wang et al., (2008) in respect to papillary and medullary carcinoma but not in follicular cases, who interpreted such findings depending on the well known observation that B19 can only infect the cells that have the proper receptors to which the virus can bind (Brown et al., 1994). Human thyroid follicle epithelia have been shown to have globoside (Bouchon et al., 1985). Therefore; the expression of the specific cellular receptor of B19 on these cells might interprets their susceptibility to B19 infection. While the absence of such receptors on medullary thyroid carcinoma cells may justify the insusceptibility to infection with this virus (Wang et al., 2008). However, in the last few years Norja et al., (2006) have found that the persistence of B19 genome in human tissues is ubiquitous and lifelong. Another publication has demonstrated short regions of sequence identity between B19 and human genes, and these sequence identity may be biologically relevant to the persistence of the viruses in human tissues (Kerr and Boschetti, 2006).

B19 DNA has been detected in sera and may persist in various tissues including skin, myocardial endothelium, tonsilis, liver, thyroid, testis, brain and synovial (Bultmanetal., 2003; Norja et al., 2006 ; Hobbs, 2006; Adamson et al., 2011 and Polcz et al., 2012).

The increased presence of B19 capsid could reflect the efficacy of B19 infection in these tumors or of B19 replication (Koduri, 1998), the persistence of B19 genome mainly denotes latent infection. While, usually persistence of viral genome with expressed viral proteins in the tumor cells indicates a productive infection of virus in pathways leading to the development/or progression of cancer (Pagano et al., 2004). Our findings hat B19 capsid protein expression in papillary and follicular thyroid carcinoma can be interpreted depending on the above mentioned which may indicate that the virus was active in papillary thyroid carcinoma and follicular thyroid carcinoma lesions, and the productive infection of the virus might play some role, directly or indirectly, as a cofactor in the development of the tumor. Keeping in mind that benign thyroid proliferative disease may become neoplastic (Pang et al., 1994; Bravo et al., 2003; Moore, 2006) and some benign thyroid diseases without histopathological evidence of papillary thyroid carcinoma harbor similar molecular genetic changes with papillary thyroid carcinoma (Wirtschafter et al., 1997; Rhoden et al., 2006).

Although the pathogenesis of thyroid tumors is not fully understood, it is known that activation of NF-κB, a mediator of viral –induced tumorigenesis (Mosialos, 1997), play a critical role in the process of thyroid cell transformation (Visconti et al., 1997). This study showed that NF-κB p65 was over expressed and activated in thyroid tumors (benign and malignant) without correlation with expression of viral proteins. These results were discordant with results of Wang et al., (2008) who found significant correlation between activation of NF-κB p65 and VP1/VP2 protein by IHC and double labeling of ISH, respectively. Such discordance may be due to many factors, including differences in antibody types, fixation, detection methods, and numbers of patients included, different staging systems in addition to racial and geographical factors. While Tasi et al., (2013) in a close study found significant increase of NF-κB p65 was detected in livers from NZB/WF1 mice receiving B19-NS1 but not VP1u or VP2 as compared to those receiving PBS.

On the other hand, the NF-κB among cancer signaling pathway has been reported in variety of malignant tumors (Counter and Gilmore, 2006).However, there are only few studies of NF-κB pathway in thyroid cancer. The results above were compatible with one result in the original study of Liu and Brown who showed both nuclear and cytoplasm immune reactivates with antibodies against NF-κB p65 in all 10 cases of follicular carcinoma that included in their study, including moderate to strong nuclear staining intensity (Liu and Brown, 2012). Moreover, the activated NF-κB which has shown to be associated with malignant and benign thyroid tumors in this study, was in concordance with results that have indicated in Chinese population by Zhenxian et al., (2006) who suggested that NF-κB p65 has important role in thyroid carcinoma and may be potential targets for gene therapy in thyroid patients. Another study which similar partially by Pacifico et al., reported no nuclear staining for p65 in normal thyroid follicular cells whereas few nuclei from papillary carcinoma cells were positive for p65. Follicular carcinoma cells showed 50% of their nuclei stained positively for NF-κB, whereas anaplastic carcinoma cells showed that almost 100% of their nuclei strongly stained for NF-κB. The study of Pacifico et al., showed that transcription is activity was constitutively elevated in primary human thyroid carcinomas and was correlated with malignant phenotype. In particular, anaplastic thyroid carcinoma cells displayed almost 100% of their nuclei positively stained for NF-κB. Also it activated was detected in an in vitro model of human thyroid cancer that resembles that in vivo differentiated and undifferentiated thyroid tumor. In these cell lines which demonstrate that persistent NF-κB activity was progressively detected in papillary thyroid carcinoma cells to follicular carcinoma cells until reaching the highest levels in anaplastic carcinoma cells suggesting that sustained activation of NF- κB confers an advantage for clonal selectivity (Pacifico et al., 2004). In contrast the present result showed that the cytoplasmic staining by the antibody against p65 was higher than that of nucleus in malignancy and benign thyroid tumors without significant difference between papillary and follicular carcinoma. The most interesting finding in the present study is the expression of NF- κB P65 in the cytoplasm which was significantly correlated to thyroid tumors (malignant and benign) compared with normal tissue independently of patients gender, age and histopathological type. Up to our knowledge, this is first study conducted in Iraq regarding the role of NF- κB p65 in thyroid tumors. During activation, NF- κB p65 is phosphorylated at several residues. What residues, when and where seem to be dependent on different stimuli that activate NF- κB.

Sakurai et al ., (1999) was first to report that upon tumor necrosis factor alpha induced activation, IKB kinase phosphorlates NF-κB p65 at serine -536 and that this occurs in the cytoplasm of Hela cells before activated p65 translocates into the nucleus.

A number of molecular studies by Kimura et al., (2003) and Xing, (2007) also suggest the reason for this activated NF- κB levels. The carcinoma comprises a group of different types of tumor cells with distinctive clinical and pathological characteristics that occur due to different genetic mutations involving specific oncogenes. The RET/PTC rearrangements, as well as BRAF and Ras mutations, are often seen in aggressive cancers, These genetic alterations potently activate the MAKP pathway, which can turn cause NF- κB activation and ongogene-mediated progression and aggressive behavior of papillary thyroid carcinoma. Neely et al., (2011) recently reported that the

RET/PTC-3 gene can also activate the canonical NF-κB pathway by the NF- κB-inducing kinase in both papillary thyroid carcinoma and in thyroid epithelial cells found in Hashimoto's thyroiditis. Loss of function of tumor suppressors, including PTEN down-regulation, P53 mutations, and B-CATENIN, also contributes to the progression of thyroid cancer (Yang et al., 2012). Also the inflammation process chronically activates NF-κB. This stimulates the expression of cytokines, favors initiation and progression of tumors, and provides a niche for malignant transformation (Li et al., 2013).

Data presented in table (5) showed that no significant correlation between IL-6 and B19 capsid proteins, a result which disagreed what reported by Adamoson, (2013) who referred to increasing detection of B19 capsid proteins within thyroid correlates significantly with both increased IL-6 expression locally and circulating in serum which may be contributed to increase inflammation. Such findings can be attributed to the cytokine levels which are also influenced by various factors, such inflammation, fibrosis, viral load, and occurrence of malignancy (Hyodo et al., 2003) and such factors relating to the change of individual cytokine levels differed with disease phases(Okumoto et al., 2004). Bluth et al., found that Th2 cytokine responses predominated early in B19 infection whereas late in B19 infection both Th1 and Th2 cytokine responses (Bluth et al., 2003).

The results of IL-6 expression obtained in this study were congruent to literatures which stated that IL-6 expression has been reported in normal thyroid epithelial cells, in colloid nodules, in follicular adenomas and in papillary thyroid carcinoma (Zheng et al., 1991; Watson et al., 1994; Bartalena et al., 1995 and Basolo et al., 1998). These results were consistent with Trovato et al., who reported that the expression of IL-6 was with moderate reactivity in 33% of normal thyroid and it was positive in cases of benign and papillary thyroid tumors. On the other hand, the result in current study which shown no significant difference between papillary and follicular thyroid carcinoma came inconsistent with a study done by Trovato who found all follicular and anaplastic thyroid carcinoma were with negative expression (Trovato et al., 2003). Other study by Basolo et al., reported IL-6 reactivity was slightly diminished in well differentiated carcinoma and severely reduced in undifferentiated histotypes ($p<0.001$) compared to normal tissue. IL6 antibodies produced a defined cytoplasmic staining in well differentiated carcinoma but failed to stain undifferentiated carcinoma (Basolo et al., 1998).This discrepancy in the results could be due to the choice of methods used, sensitivity and specificity of different antibodies used, sample size, and different modes of scoring systems and interpretation of the results.

IL-6 is constitutively produced by (TFCs) thyroid follicular cells (Watson et al., 1995; Zhen et al., 1991) and appears to influence differentiated thyrocyte functions (Bartalena et al., 1992).In cultures of human TFCs. IL-6 inhibits TSH induced peroxidase gene expression and reduces T3 secretion (Tominga et al., 1991), a finding confirmed in rats (Tyson and Mc Cann, 1991). These facts explained the positive expression of IL-6 in normal thyroid tissue in present work. While strong IL-6 staining was mainly seen in benign suggests that cytokines were found to serve as growth and survival factors that act on premalignant cells, (Greten and Karin, 2005). It stimulates angiogenesis, tumor progression and metastasis, also maintain tumor promoting inflammation. IL-6 is perhaps the best characteristed protumorigenic cytokine and it initially suspected to activate the oncogenic transcription factor NF-κB (Grivenniko and Karin, 2011).

In conclusion, the present study results of the detection of parvovirus B19 capsid protein, NF-κB p65 and IL-6 in malignant and benign thyroid tumors in Iraqi patients suggesting the noxious role of B19 virus in these tumors, the high rate of expression of NF-κB p65 among patients with thyroid tumors indicates it's important role in cancer signaling pathway. In addition to IL-6 could play role in immunological microenvironment of thyroid tumors regardless of presence or absence of parvovirus B19.

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