Thyroid Carcinoma Patients Correlated With Cytomegalovirus by using Insitu Hybridization

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Abstract

Worldwide, the most common cancer-related deaths and poses one of the top ten most risk cancers globally in many countries, is the thyroid carcinoma. In human cancers, there are important factor associated with it . The viral agent is one of them which is involved in the spread of tumors, including thyroid cancers. So, the present study was designed to determine the relationship between Cytomegalovirus infection with some Iraqi patients who suffer from the thyroid carcinoma in tissue cancer from different site (papillary, follicular and lymph node) by using In Situ Hybridization Technique, related to their age, gender, site distribution, histological grade and the tumor stage. The sixty biopsies were randomly collected during the period between June 2017 until October 2018,CMV was detected in(28.3%)(17 out of 60 patients, the mean age was 39 years old, ranged between (27-72) years old, female to male ratio was (1.3 : 1) with 34 women and 26 men. The most of these cases were papillary carcinoma 39, followed by follicular 13 and the rest 8 cases were at the lymph node. Histological grades that involved were 37 well differentiated, 18 were poorly differentiated, and 5 were moderately differentiated. Tumor staging included 43 patients who fall in stage I and II, while the rest 17 patients fall in stage III and IV. Regarding to the positive results, CMV correlates in a highly significant association with each of (age, gender, grading, tumor stage, and site distribution at $p<0.01$).

It could be concluded from that the in situ hybridization is useful in the clinical evaluation of patients with thyroid carcinoma. And suggested that the viral agent cytomegalovirus is involved in thyroid carcinogenesis and may play an important role in the malignant transformation of thyroid cancer.

Keyword: *Thyroid cancer, cytomegalovirus , Insitu hybridization.*

Introduction

Thyroid cancer is the most common cancer in the head and neck and the major tumor malignant of the endocrine gland[(1)]. thyroid cancer types which including the well differentiated papillary (80% of the cases), and follicular (15% of the cases),while the latter being divided into conventional and oncocytic type (Hürthle cell), anaplastic carcinoma as well as poorly differentiated carcinoma[(2)]. poorly differentiated and anaplastic carcinoma are both can arise de novo, or secondarily from papillary thyroid cancer and follicular thyroid cancer [(3)].

The early stage well differentiated papillary patients as well as the follicular carcinoma patients usually have an excellent prognosis ,whereas, the patients with either aggressive tumors or distant metastases, have a 5-years survival rate of 40% [(4)]. Unlike the thyroid cancer three aforementioned types , medullary cancer, which derives from the neural crest, more specifically, parafollicular , or C cells [(5)]. It accounts for about 3–4% of all cases of thyroid cancer[(6)], its clinical course varies from the indolent to rather aggressive, with high mortality rates association [(5)]. Primary thyroid lymphoma represents the rare non-Hodgkin lymphoma [(7)]which derives predominantly from the B-lymphocytes [(8)].

From 2000 until 2014, the thyroid cancer incidence for both individuals of both genders has significantly increased, particularly for females aged between 55 to 64 years [(9)]. The increase rates refers to tumors in all stages. The reasons for this increase have not been completely

elucidated yet [9].

Many risk factors associated with thyroid cancers, for instance ,viral agents are considered as a carcinogenic risk factor in more than 20% of human cancers [10,11,12] Human Cytomegalovirus have been implicated in the etiology of many human malignancies like breast cancers, cervical cancers, prostate cancers, colon cancer and thyroid cancer [13, 14].

Many molecular methods can be used to identify the nucleic acid of viruses like HCMV, the one of these is InSitu Hybridization (ISH) method which can be used the frozen tissues and preparation of cytology as well as the tissue fixing by using radioactive labeled probes and also with non-radioactive labels as fluorescent moieties, biotin and enzyme conjugated probe [15].

Materials and Methods

This study was designed as a retrospective study, involved thyroid tissue samples which were collected as archival tissue blocks, during the period from June 2017 until October 2018, the series of sixty formalin fixed paraffin embedded tissue samples which were enrolled in the current study had been biopsied with patients data age, gender and diagnosis of histopathology and the site distribution. sixty patients with thyroid cancer (mean age 39 years) ranged between (27-72) years, female to male ratio 1.3 :1 with 34 women and 26 men. They have collected randomly from Teaching laboratories\ Medical city in Baghdad. Whom already diagnosed as a (papillary , follicular and lymph node) of thyroid cancer by a specialist , They are compared with 10 healthy control where their data of ages and sex were matched to patients group.

preparation technique of the slides and tissue sectioning was done in the teaching laboratories/ Department of Histology/ Medical City/ Baghdad. The first tissue section mounted in ordinary slide to be stained with Hematoxylin and eosin, and the two subsequent tissue sections were mounted in the charged slides to be used for In Situ Hybridization for detection of DNA of the HCMV. In Situ Hybridization technique for molecular detection of CMV \ DNA in these tissues were performed by high sensitivity generation ISH as steps follow:

1- Pre-hybridization step : All the samples were deparaffinized and dewaxed by Xylene and with series of ethanol 100, 90 and 70% then in D.W. after that immersing in citrate buffer (pH: 6), deproteinization by placing in the proteins K solution, then dehydration by immersing these slides in D.W. and 70, 90 and 100% ethanol.

2- Hybridization step: By adding CMV probe to each slide, and placing them in an oven at 98 C° to denaturation of the DNA probe, then removing and incubation for overnight at room temperature to allow hybridization of the probe with the target nucleic acid.

3- Post hybridization step: By using protein block buffer to falling off all coverslips then conjugating onto all sections, using substrate, and counterstained with ethanol, xylene and mounted with DPX.

Statistical Analysis

The statistical analysis system – SAS (16) was used to effect of differences factors in study parameters. The chi-square $\chi 2$ test at the comparative between percentage in this study.

Results and Discussion

The differentiated thyroid carcinoma arising from a follicular epithelial is the commonest endocrine malignancy, while papillary thyroid carcinoma accounts for the majority of the differentiated thyroid cancer (17). That given the fact in which the prevalence of non-medullary familial thyroid cancer in about only 5% (18) , differentiated thyroid carcinoma is mostly sporadic and the only established epidemiological factor is associated with thyroid cancer are ionizing radiation as well as iodine deficiency (19).

The sixty paraffin embedded tissue blocks of thyroid carcinoma patients were enrolled and studied in the current study by using In Situ Hybridization method for detection of Cytomegalovirus infection and as shown in table(1). Cytomegalovirus detected in 17 out of 60 (28.3%)of patients and this disagreed with other finding research (20)who reported no relation between CMV and thyroid cancer by using PCR technique for CMV\ DNA. There is no longer research for thyroid cancer with HCMV in Iraq. But the virus cytomegalo is detected in many cancers type by using the Insitu hybridization

method like with head and neck OSCC in(36.6 %)of patients(21).Mean age of the patients was (39 years old), 38 out of 60 were under 40 years of age and 22 were above 40 years(figure 1)and there is a highly significant correlation between CMV and the age of patients at P<0.01. Some previous studies have shown that thyroid cancer percentage was noticed to increase by age, and the likely recurrence of cancer is lower in young patients . Also besides , age played a significant role in the survival disparity ,and the mortality rate is starting to climb at age of 45, and the rate of recurrence starting at age 60 years [22]. The mortality risk was 5.4 times higher in aged >45 years versus <45 years [23]. and the presence of distant metastases, the age remains as a strong prognostic indicator [24]. It could be suggested there is a correlation between age and cancer development, because the prolonged exposure to lots of factors that may promote cancers, same as viruses and radiation and some chemicals [25].According to the thyroid carcinoma site distribution, the most affected site was the papillary 39 out of

60 (65%), followed by the follicular 13 out of 60 (21.6%) then the lymph node 8 out of 60 (13.3%) as in (figure 2).Statically there is a significant association between CMV and the site distribution at P<0.01. The present study, in an agreement with [26] who found that papillary carcinoma accounts for 71% of the total thyroid malignancies in that studied. Even though confirmed with [27] and [28] who also observed the incidence of papillary carcinoma to be 76.6% and 78.56%, respectively. The major type of malignant tumor among all thyroid carcinomas worldwide, comprising an estimated 80% of thyroid cancers(29). As in (Figure 3) shows the female: male ratio is 1.3:1 and the gender distribution is 1.3: 1 with 34 females and 26 male and statically there is a significant association at P<0.01. Our findings confirmed with other studies [26,30] which observed that female predominance in their studies with the female : male ratio of 5:1 and 2.6:1, respectively. It suggested that thyroid cancer and thyroid disease have more incidence rates in females than in male, because the females is more exposure to hormonal changes than males. Regarding the grade of the tumor and as shown in (figure 4) , the well differentiated is the most predominant type , it was 37 out of 60 cases followed by poorly differentiated 18, then 5 cases were moderately differentiated with the significantly statistic association at P<0.01. In many previous studies of thyroid cancer related viruses the major samples were well differentiated because the main type which derives from follicular epithelial cells is well-differentiated papillary followed by well differentiated follicular carcinoma then the poorly and moderately differentiated. [3,30,31]. In (figure 5)the predominant stage of the tumor is I,II 43 out of 60, whereas, 17 out of 60 were fell in stage III, IV, statically there is a significant correlation at P<0.01.This finding was disagreed with (20) who found and reported that is More than one-third of their patients fell in stage III and IV and thyroiditis did not correlate with tumor stage.

In our study, it recognized some of the limitations , the patients number that studied was small, So as the results obtained from selected population maybe not extrapolated to the other population. Some previous study has shown that CMV could widely distribute in other organs [21,32].

Table 1: (Distribution of patients with thyroid cancer according to their age, gender, histological grade, site distribution and tumor stage correlation with Cytomegalovirus by using In Situ Hybridization technique)

Factors		Negative results	Positive results	Chi-square (P-value)
Sex	Female	22 (64.71%)	12 (35.29%)	9.36 ** (0.001)
	Male	21 (80.77%)	5 (19.23%)	12.47 ** (0.001)
Chi-square (P-value)		6.04 ** (0.0134)	6.04 ** (0.0134)	---
Age	27-40	27 (71.05%)	11(28.95%)	10. 51 ** (0.001)
	41-72	16 (72.73%)	6 (27.27%)	12.88 ** (0.001)

Cont... **Table 1: (Distribution of patients with thyroid cancer according to their age, gender, histological grade, site distribution and tumor stage correlation with Cytomegalovirus by using In Situ Hybridization technique)**

Chi-square (P-value)		0.224 NS (0.166)	0.224 NS (0.166)	---
Grade	Well	25 (67.57%)	12 (32.43%)	9.25 ** (0.001)
	Moderate	2 (40%)	3 (60%)	7.33 ** (0.0036)
	Poor	16 (88.89%)	2 (11.11%)	12.73 ** (0.001)
Chi-square (P-value)		9.67 ** (0.001)	9.67 ** (0.001)	---
Stage	I II	30 (69.76%)	13 (30.24%)	12.09 ** (0.001)
	III IV	13 (76.47%)	4 (23.53%)	13.66 ** (0.001)
Chi-square (P-value)		2.055 NS (0.274)	2.055 NS (0.274)	---
Site	Papillary	28 (71.80%)	11 (28.20%)	10.41 ** (0.001)
	Follicular	9 (69.23%)	4 (30.77%)	11.27 ** (0.001)
	Lymph node	6 (75%)	2 (25%)	13.77 ** (0.001)
Chi-square (P-value)		2.166 NS (0.108)	2.055 NS (0.274)	---
** (P<0.01), NS: Non-significant.				

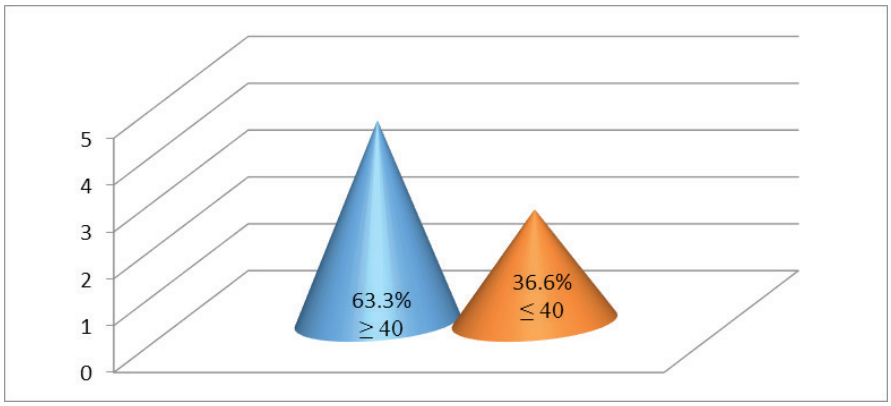

Figure 1: Distribution of thyroid cancer patients according to their age

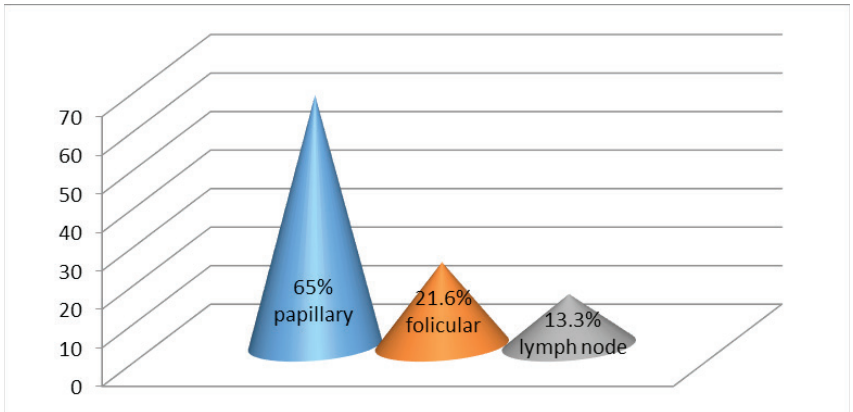

Figure 2: Thyroid cancer patients according to their site distribution

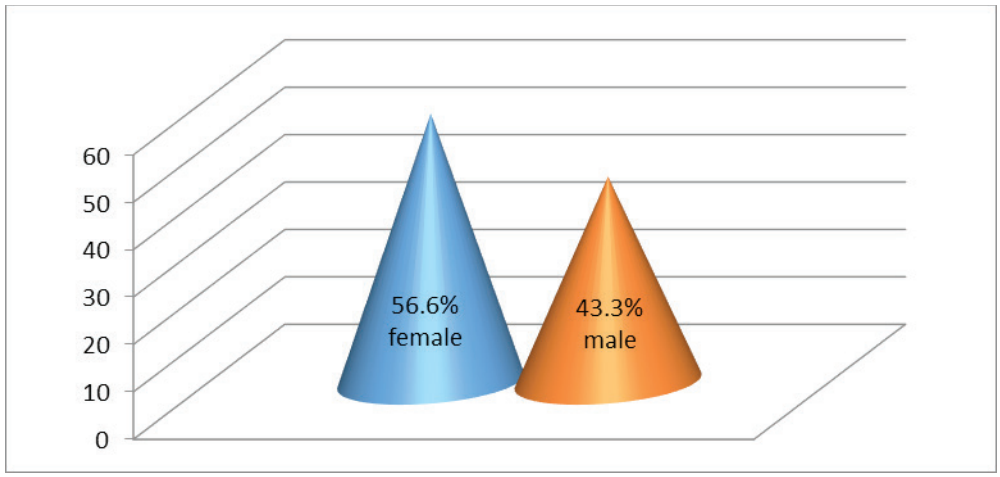

Figure 3: distribution of thyroid cancer patients according to their gender

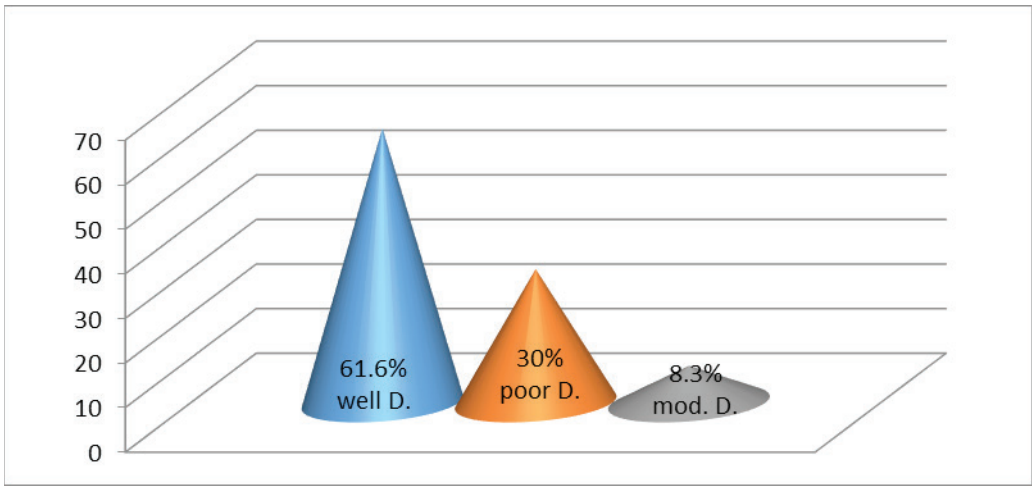

Figure 4: distribution of thyroid cancer patients according to the histological grade.

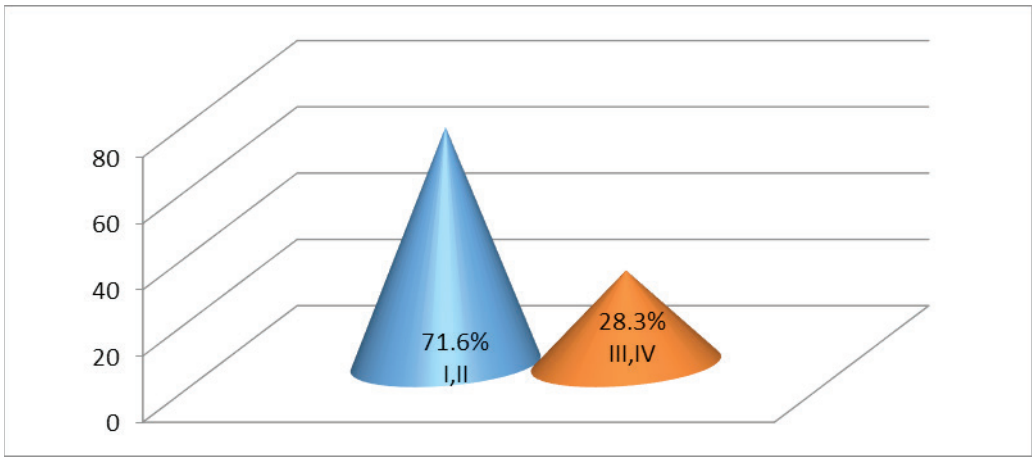

Figure 5: distribution of thyroid cancer patients according to their tumor stage

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science and all experiments were carried out in accordance with approved guidelines.

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