

The Prevalence of Human Papilloma Virus among Breast Cancer Women in Relation with IL-8 Level in Kirkuk Province

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ABSTRACT

The study aimed at evaluating the frequency of HPV in breast cancer women in relation with serum IL-8 level. The study that carried out in Kirkuk city from 15th of January 2017 to 15th of June 2017, a total of 150 breast cancer women who admitted to Kirkuk oncology cancer and 96 healthy control women whose ages were between 30-75 years. Patients and control were investigated for detection of human papilloma virus (HPV) 16 E7 protein and interleukin-8 (IL-8) by ELISA technique.

The rate of breast cancer women with HPV 16 E7 (30.67%) was higher than that in control women (3.125%) with highly significant relation. The highest rate of breast cancer women was within the age group 40-49 years with no significant relation between breast cancer and age. The study showed that the highest means of IL-8 level (36.92 pg/ml) were found in breast cancer women without HPV, 29.09 pg/ml in HPV positive and 6.45 pg/ml in the control group with highly significant relation. There was a highly significant relation of WBCs, lymphocytes and neutrophils count and Hb level among the study groups concerning HPV. WBCs count and hemoglobin level were higher in the control group than in breast cancer women. The mean absolute lymphocytes count was higher in breast cancer women with HPV infection than those without HPV and the control group (4.13, 1.80 and 1.89 respectively), while the least mean of neutrophils count was reported in breast cancer women with HPV infection. Conclusions: It was concluded that there was a highly significant relation between HPV and EBV infection with breast cancer.

Keywords: Breast cancer, HPV, IL-8, Kirkuk.

الانتشار المصلي لفيروس الورم الحليمي البشري في النساء المصابات بسرطان الثدي وعلاقته مع IL-8 في مدينة كركوك

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الملخص

تهدف الدراسة الى تقييم الانتشار المصلي لفيروس الورم الحليمي البشري في النساء المصابات بسرطان الثدي مع علاقته به مستوى IL-8. أجريت الدراسة الحالية في مدينة كركوك (مركز كركوك للأمراض السرطانية) في الفترة الممتدة من 15 كانون الثاني 2017 ولغاية 15 حزيران 2017 وشملت الدراسة 150 امرأة مصابة بسرطان الثدي و96 امرأة غير مصابة كمجموعة سيطرة في الدراسة حيث تراوحت اعمار المجموعتين من 30 الى 75 سنة ، وتم اجراء فحوصات الـ ELISA للكشف عن فايروس الورم الحليمي البشري في المجموعتين فضلا عن الكشف عن مستوى IL-8. اظهرت النتائج ان نسبة إصابة مريضات سرطان الثدي بفيروس البشري الحليمي (30,67%) والتي كانت اعلى مما هو الحال في مجموعة السيطرة (3,125%). كانت أعلى نسبة للمريضات المصابات بسرطان الثدي ضمن الفترة العمرية 40-49 سنة ولم يكن هنالك أي تأثير مع سرطان الثدي. قد أظهرت الدراسة ان اعلى مستوى لـ IL-8 (36,92 بيكوغرام/ملييلتر) وجد في مريضات سرطان الثدي مقارنة بالمريضات المصابات بفايروس الورم الحليمي البشري (29,09 ، 28.74 و 18,74 بيكوغرام/ملييلتر على التوالي) ومجموعة السيطرة (6,45) مع وجود علاقة إحصائية بهذا الصدد. وان هنالك علاقة إحصائية قوية بين تعداد خلايا الدم البيضاء والخلايا العذلة والخلايا اللمفية ومستوى الهيموغلوبين في مجاميع البحث بما يخص الإصابات بفايروس الورم الحليمي. كان تعداد خلايا الدم البيضاء ومستوى الهيموغلوبين اعلى في مجموعة السيطرة مقارنة بمريضات سرطان الثدي بينما كان تعداد الخلايا اللمفية عاليا في مريضات سرطان الثدي اللواتي يحملن الإصابة بفايروس الورم الحليمي البشري مقارنة بغير المصابات ومجموعة السيطرة (4,13 ، 1,80 و 1,89 على التوالي). كان اقل تعداداً للخلايا العذلة قد سُجّل في مريضات سرطان الثدي المصابات بفايروس الورم الحليمي البشري. من هذا يُستنتج ان هنالك علاقة قوية بين سرطان الثدي والاصابة بفايروس الورم الحليمي البشري ومستوى IL-8.

الكلمات الدالة: Breast cancer ، HPV ، IL-8 ، Kirkuk.

1. Introduction

Breast cancer is a public malignancy and a major cause of morbidity in women. About 1,150,000 new cases/year are reported worldwide, according to the statistical data of the WHO in 2008. Many epidemiological studies show a family history of breast cancer is a predictor of breast cancer threat [1]. Surveillance of the increased risk of breast cancer for women with positive family histories of breast cancer leads to further sub-classifications into inherited or sporadic cases. Risk factors including obesity have been linked to breast cancer [2]. The involvement of viruses in definite breast tumors and cells lines has been described in different revisions. Viruses, such as specific types of human papillomaviruses (HPV) has appeared as major causal factors of some human cancers. The occurrence of either human papillomavirus (HPV)-16 or HPV-18 in the breast has been proposed to be related to the development of a malignant phenol type [3].

One of the interesting cytokine families is the Interleukins, its reported to promote *in vitro* growth of many type of cancer such as ovarian, cervical, prostate, lung, kidney cancer and melanoma cells [4]. Furthermore, their assistances to the tumor angiogenesis have been reported [5]. Interleukin-8 is a part of the CXC chemokine family of related proinflammatory cytokines, which was formerly identified as a chemoattractant for neutrophils [6]. Raised IL-8 levels were associated with disease progression and recurrence in human prostate, lung, gastric, and breast cancers [7].

The study aimed at evaluating the prevalence of HPV in breast cancer women in relation with serum IL-8a level in Kirkuk province.

2. Material and methods

A crosssectional study was carried out in Kirkuk city from 15th of January 2017 to 15th of June 2017. The number of breast cancer women under study were 150 women whose ages were between 30-75 years old. These patients admitted to Kirkuk oncology center. The control group who were matched to the breast cancer patients studied, included 96 healthy women and their ages were between 30-75 years old. These control group presented Kirkuk General Hospital.

3. Statistical Analysis

Seven and half ml of blood was collected by vein puncture using Vacutainer tubes from each patient enrolled in this study. Blood samples were placed into two sterile test tubes, in one of them 2.5 ml of blood was put in test tube containing anticoagulant EDTA and used for assessment of complete blood count (CBC) test using Ruby auto-analyzer. The second part of sample was 5ml for serological detecting of HPV 16-E7 protein using ELISA kit (SHANGHAI YEHUA Biological Technology Co., Ltd.) and, IL8 using ELISA (Quantikine® ELISA, USA), Computerized statistically analysis was performed using Anova version 11 statistic program. Comparison was carried out using; Chi-square (X²), T-Test probability and F-ratio (P value).

4. Results

A total of 150 breast cancer women and 96 healthy women (control group) were examined, their age ranged between 30-75 years old were investigated for prevalence of HPV 16 E7 protein and IL-8. As shown in [Table \(1\)](#). The rate of breast cancer women with HPV 16 E7 (30.67%) was higher than that of the control women (3.125%). The result was statistically highly significant. As presented in [Fig. \(1\)](#). the age group 40-49 years characterize the highest rate of breast cancer among the study women followed by 26% which characterize the age group 50-59 years and the lowest rate was 7.33% which belong to the age group 70-79% with mean age of 49.5 years and the highest rate of HPV infection (34.48%) was occurred in breast cancer women who belonged to the age 40-49 years, followed by 30.76% of the age group 50-59 years, [Fig. \(2\)](#). The study showed that the means of IL-8 level were higher in breast cancer women with and without HPV 16 E7 protein (36.92 and 29.09 respectively) as compared with the control group (6.45). However, the result was highly significant. [Table \(2\)](#). [Table \(3\)](#) shows highly significant relation of WBCs, lymphocytes, and neutrophils count and hemoglobin level among the study groups concerning HPV infection, the mean of WBCs count and hemoglobin level were higher in the control group than in breast cancer women. The mean absolute lymphocytes count was higher in breast cancer women with HPV infection than those without HPV infection and the control group (4.13, 1.80 and 1.89 respectively), while the least mean of neutrophils count was reported in breast cancer women with HPV infection, respectively, While the

least mean of neutrophil count were reported in breast cancer women with positive HPV result

Table (1): Frequency of HPV 16 E7 protein in breast cancer women and the control group.

HPV 16 E7 protein	Breast		Control	
	No.	%	No.	%
Positive	46	30.67	3	3.125
Negative	104	69.33	93	96.857
Total	150	100	96	100
$X^2 = 26.015$ $P=0.00001$ $P < 0.01$ Highly Significant(HS)				

Table (2): Relation of HPV infection with IL-8 among breast cancer women and the control group.

IL8 level	breast cancer women		Control group
	HPV-	HPV+	
No.	30	46	30
Mean	36.92	29.09	6.45
Std. Dev.	21.94	26.54	6.41
$F = 16.873$ $p= 0.0043$ $P < 0.0001$ Highly Significant (HS)			

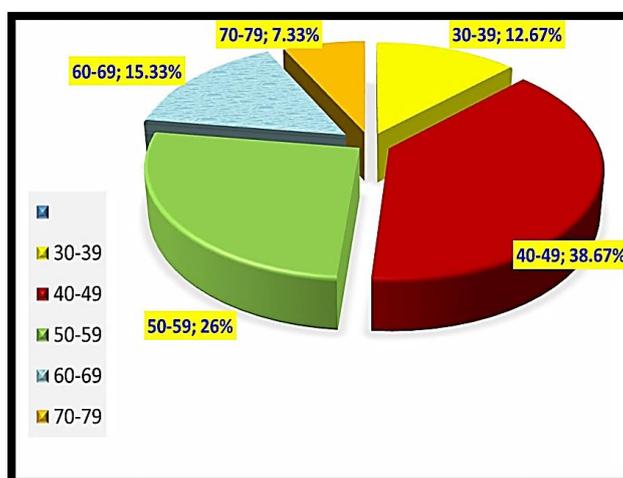


Fig. (1): Distribution of breast cancer women according to age

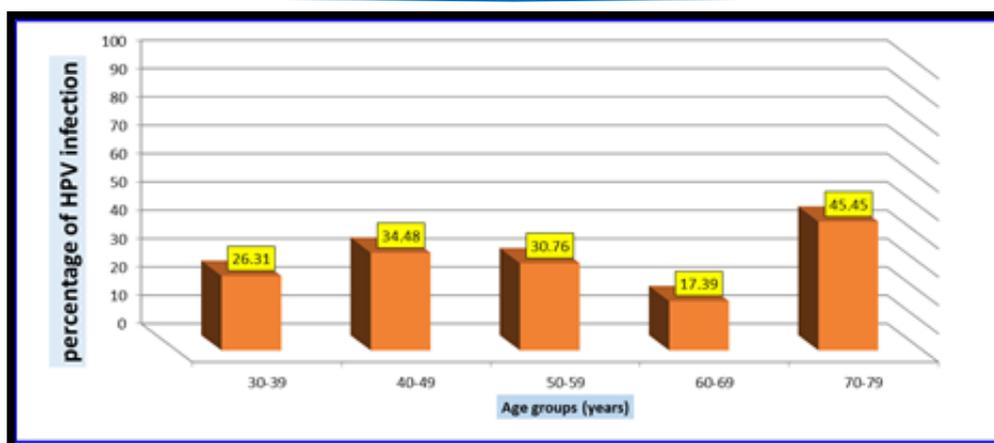


Fig. (2): Distribution of HPV 16 E7 protein in breast cancer women according to their

Table (3): Means and standard deviation of WBCs, lymphocytes, and neutrophils count and Hb level in relation with HPV infection among the study groups.

parameters	Study groups		N	Mean	St.Dv	F Ratio	P.value	Relation
W.B.C	Breast Cancer	HPV -	104	5.11	2.23	22.40	0.00001	HS
		HPV +	46	4.85	1.82			
	control	96	6.68	1.51				
HGB	Breast Cancer	HPV -	104	11.32	2.7875	6.058	0.0027	HS
		HPV +	46	11.27	3.1689			
	control	96	12.43	1.5261				
Lymphocyte	Breast Cancer	HPV -	104	1.80	0.5986	142.78	0.00001	HS
		HPV +	46	4.13	1.4789			
	control	96	1.89	0.5731				
Neutrophil	Breast Cancer	HPV -	104	3.80	1.7534	34.352	0.00001	HS
		HPV +	46	1.76	0.8223			
	control	96	3.4	1.1716				

5. Discussion

Breast cancer is a public malignancy and a major cause of morbidity in women. As shown in [Table \(1\)](#), the rate of HPV 16 E7 infection in breast cancer women (30.67%) was higher than that in control women (3.125%). The result was highly significant.

Numerous studies have reported a relationship between HPV and breast cancer with the prevalence ranging from 4–86% (8-22). Conversely other studies have not noticed HPV in breast cancers (23-26). Our study detected HPV-E7 protein in 46 of 150 (30.67%) cases. These results agreed with a number of other breast cancer studies that have been conducted universally: In Iraq, AL-Mansour A, et al. [8], showed a significant relation between HPV and breast cancer (16.9% in breast cancer vs. 5% in controls) Brazil 24.8% [15], Greece 15.9% [22], Iran 25.9% [16], Italy 29.4% [19], Japan 21% [18], Mexico 29.4% [18] and in U.S.A. 35% [17]. HPV DNA in normal breast tissue in breast cancer women, this was also reported in Turkey [21], and they proposed that if HPV has a contributory role to play in the carcinogenesis of breast cancer, it is rational to find the presence of HPV in neighboring normal breast tissue [21]. Kroupis C et al. [22] in his study also shown the existence of HPV DNA in breast carcinoma. This has made conflicting and controversial information which may be attributed to geographic location in either high or low HPV occurrence areas [27]. The dissimilarities in prevalence of HPV associated breast cancers are may be due to differences in laboratory procedures [28] and may be influenced by the technical limits, the epidemiology of HPV in different geographical area, different sexual behavior patterns, differing incidences of ano-genital HPV infection and perhaps different genetics in people could play variety roles in these differing outcomes [29].

Fenwick Sh [30] showed in his thesis that the age of patients with HPV+ve results ranged from 29–69 years with the mean age of 49 years. Richardson AK, et al. [31], presented in their study that the mean age of breast cancer women was 48 years with greatest in the 40–54 year age-group. As stated by American cancer society, age of the patient is an important factor both for the occurrence and management of the disease with 95% of all new breast cancer cases developed in women aged 40 years or older [32], which was along with our results. Also, our result was in relation to Al-Khafaji AH [33] which detailed that the peak frequency was recorded in age period 50 years. According to our results, breast cancer patients showed significantly higher serum concentrations of IL-8 compared to control group. Raised expression of IL-8 and /or its receptors has been characterized in cancer cells;

endothelial cell, infiltrating neutrophils and tumor –associated macrophages suggesting that IL-8 may act as a regulatory factor within the tumor microenvironment [34]. IL-8 expressed in all breast cancer however only 50% of the normal breast tissue samples expressed them [35].

It was reported that interleukins provoke cancer cell growth and contribute to loco-regional relapse as well as metastasis [36]. Higher serum levels of IL-8 in breast cancer patients have been associated with poor prognosis, increased tumor burden and declined post relapse

survival [37]. The current result was in agreement with Mohammed Z A [38] who stated that serum IL-8 was higher in breast cancer women than in controls and the variances in serum IL-8 between the controls and patients with breast cancer is significant ($P < 0.05$). Serum IL-8 measurement in breast cancer was seldom documented [39-41].

There was a highly significant relation of W.B.C, Hb, lymphocytes and neutrophil counts among the study groups concerning HPV. W. B. C count and hemoglobin were higher in control group than in breast cancer women. Hasan F F, et al. [42] reported that there were a negative effect of cancer treatment on the blood count which cause decrease the mean count of WBCs , RBCs and Hb level which adversely lead to other side effects like infection, anemia. The mean count of white blood cells decreased next completion of radiotherapy in all kinds of cancer involved in this study that fall in with a result done by Goff et al. [43]. In other study done by Papeet al. [44] they established that the count of lymphocytes and neutrophil decline after radiotherapy. Radiation affect mainly on bone marrow as a major site of radiation injury. The extreme sensitivity of bone marrow cells to genotoxic stress largely limits the adverse side effects of radiation [45]. In this study the hemoglobin level decreased after radiotherapy in all types of cancer and this frequently owing to the decrease the RBC and also to the other sideeffects of radiotherapy like nausea, vomiting and diarrhea with loss of appetite [45]. The study was in agreement with the study of Akinbami A, et al. [46] who found that WBC and Hb were decreased in breast cancer women while lymphocyte and neutrophil counts were raised matching with control group. This can be due to the point that neoplasms of all forms were associated with neutrophilia.

6. Conclusions

There was a highly significant relation between HPV 16 E7 protein and breast cancer women comparing with controls (30.67% vs. 3.125%). There was a highly significant relation among the study groups concerning serum IL8 level.

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