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Association of human papillomavirus type 11, 16 and 18 among female patients with breast cancer in Baghdad.

A Thesis

Submitted to the Council of the College of Medicine - University of Diyala in Partial Fulfillment of the Requirements of the Degree of Master's of Sciences in Medical microbiology

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Supervisor Certification

I, certify that this thesis entitled **(Association of human papillomavirus type 11,16 and 18 among female patients with breast cancer in Baghdad) .** has been conducted under my supervision at the College of Medicine, University of Diyala, as partial fulfilment of the requirements for the Master Degree of Science in Medical Microbiology.

Professor Dr. Abdulrazak Shafiq Hasan

Given the available recommendation, I forward this thesis for debate by the examining committee.

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Committee Certification

We, as the examining committee, certify that we have read this thesis and examined the student (Alaa Wael Izzat) in and its contents, found it adequate as a thesis for the Master Degree of Science in Medical Microbiology.

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Dedication

To the soul of my brother, Ahmed Wa'el Al-Bekri, the Immaculate Martyr.

My dear mother, thank you for your love, support and everything you have gone through for me. I'm blessed because of you.

My dear father, Dr Wa'el Izzat Al-Bekri; my pride and happiness come from you.

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SUMMARY

Breast carcinoma is the commonest malignancy among women in developed and developing countries including Iraq. It ranked as the number one cancer documented in all Iraqi provinces. Breast cancer is one of the main causes of death in postmenopausal women, accounting for %23 of all cancer fatalities. According to Globocan registry data in 2020, there are 7515 persons, estimated 22.2% breast cancer registry from both genders from all ages with mortality of 3019 of Iraqi people. Previous studies have mentioned the carcinogenic role of HPV in transformed HPV virus-infected cells into a malignant phenotype, which is an important cause of cancer in humans.

This is a case-control study, which was conducted from August 2020 to July 2021 in an Oncology Teaching Hospital-Medical City and the Blood bank of the Private Nursing Home Hospital. This study enrolled 90 participants: 29 women apparently healthy as a control group and 61 women with clinically and laboratory diagnosed breast cancer as patients' group. Age ranges from 30 to 78 years old. A questionnaire form was made for this purpose. Blood samples were collected from each participant, Complete Blood Count was determined and a blood group was identified for each participant. Sera were separated and kept at -30° C till use. ELISA kits from SUNLONG company- China were used to detect the presence of HPV type 11,16,18 antigens and to determine overexpression of P53 tumour suppressor protein. Human privacy was respected by obtaining the verbal consent of the participants. Statistical analysis was done via SPSS version 27 and P -values less than 0.05 were considered significant.

All breast cancers were histologically diagnosed with almost advanced stages. The three types of HPV were identified in sera of examined Iraqi women with BC and healthy control under study. The positivity rate of HPV-11 among studied women with BC was 14.8% compared to 6.9% among the healthy control with an insignificant difference between the two groups (P=0.288). The most affected age among BC women with

positive HPV-11 was less than 50 years with the highest positivity rate and significant association (P=0.007).

Additionally, Human papillomavirus type 16 was identified with the highest positivity rate of 55.7% versus 3.4% the positivity rate of healthy control with a highly significant difference between the two groups (P=0.0001). While the most affected age of BC women with positive HPV-16 was ≥ 60 years.

Moreover, the highest positivity rate of BC women with positive HPV-18 was 47.5% compared to 6.9% of the healthy control with a highly significant difference between the two groups (P=0.0001). Additionally, the most affected age was less than 40 years with the highest positivity rate. High-risk Human papillomaviruses type 16 and type 18 were identified with the highest positivity rate among unmarried women. Human papillomaviruses under study were frequently found with the highest positivity rate among women with BC within the normal weight category with a significant association with HPV-16 only.

Additionally, the positivity rate of P53 overexpression was 65.6% among breast cancer women compared to the 6.9% positivity rate among the healthy control with highly significant differences (P=0.0001). The serological levels of mutant P53 overexpression were found to be age-dependent (P= 0.029). The Mean ± SD was higher in the age range < 40 years which was (633.1 ± 208.0) with significant association (P=0.029). A higher, but insignificant association was found between P53 overexpression and oral contraceptives consumption (P=0.090). Furthermore, the P53 was significantly associated with HPV-18 (P=0.001), but not with HPV-11 or HPV-16.

In conclusion, the association of infectious rate of HPV types was generally high among Iraqi women with breast cancer. The highest infectious rate in order of frequency was HPV- 16, then HPV-18 and HPV-11. The most affected age of women with breast cancer was less than 50 years. P53 overexpression has a highly significant association with breast cancer, as well as with HPV-18 infection only

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List of Abbreviations

Abbreviations	Meaning
BC	Breast Cancer BC
BCC	Basal Cell Carcinoma
BD	Bowen´s Disease
CBC	Complete Blood Count
cSCC	cutaneous Squamous Cell Carcinoma
DC	Dendritic Cells
DCIS	Ductal Carcinoma In Situ
E1, E2, E4, E5, E6 and E7	Early region proteins
EDTA	Ethylenediaminetetraacetic Acid
EGFRs	Epidermal Growth Factor (EGF) Receptors
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Endoplasmic Reticulum
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin-Embedded tissue
FNA	Fine Needle Aspiration
HNCs	Head and Neck Cancers
HPV	Human Papillomavirus
HPV11	Human Papillomavirus type11
HPV16	Human Papillomavirus type16
HPV18	Human Papillomavirus type18
HR	High - Risk
HRA	High-Resolution Anoscopy
HRP	Horseradish Peroxidase
HSPGs	Heparin Sulphate Proteoglycans Receptor

IDC	Invasive Ductal Carcinoma IDC
IL-1	Interleukin 1
ILC	Invasive Lobular Carcinoma
IMPC	Invasive Micropapillary Carcinoma
L1 and L2	Late region proteins
LC	Langerhans Cells
LCIS	Lobular Carcinoma In Situ
LNM	Lymph Node Metastasis
LR	Low-Risk
LVI	Lymph Vascular Invasion
M	Metastasis
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MRI	Magnetic Resonance Imaging
N	Lymph Node
NK	Natural Killer Cells
NKT	Natural Killer T Cells
NMSC	Non-Melanoma Skin Cancer
OD	Optical Density
P53	Tumour suppressor protein
Pap	Papanicolaou test
PCR	Polymerase Chain Reaction
PML	Promyelocytic Leukaemia
RDW	Red Blood Cell Distribution Width
RONS	Reactive Oxygen-Nitrogen Species
RRP	Recurrent Respiratory Papillomatosis RRP

T	Tumour
TGF-β	Transforming Growth Factors - Beta
Th1	T-helper cells type 1
TNM	Histopathological type, grade, stage (staging system)
VLPs	Purified Virus-Like Particles
RB	Retinoblastoma

1. Introduction

Human papillomaviruses (HPVs) represent a large collection of viral types. *Papillomaviridae* is a family of non- envelope viruses whose members are called Human papillomavirus. More than 200 different types of human papillomavirus (HPV) have been identified to date. The papillomavirus was first identified at the beginning of the 20th century when it became clear that skin warts or papillomas could be transmitted between individuals by a filterable infectious agent (Christensen, 2016). In 1935, Francis Peyton Rous, who had previously demonstrated the existence of carcinogenic sarcomas in chickens, further showed that the papillomavirus can cause skin cancer in infected rabbits (Rous *et al*., 2021). This was the first demonstration that the virus could cause cancer in mammals. Human papillomavirus is small, non-enveloped of 52-55 nm diameter with icosahedral shape. The viral capsid is not covered by a lipid membrane. The capsid protein is composed of 22 pentameric capsomers. The viral genome containing a single double-stranded DNA molecule with approximately 8000 base pairs (bp) which are bounded to cellular histones. The genome encodes eight major proteins, 6 located in the "early" region, proteins are E1, E2, E4, E5, E6 and E7 are regulatory in function. Two proteins are located in the "late" region L1 and L2 comprise the virus capsid required for virus transmission, spread and survival in the environment (Dcis *et al.*, 2019). Human papillomavirus can transmit by two routes, sexual route and non-sexual route, Two types of HPV (types 6 and 11) which induce benign genital warts or condylomata acuminate, they're considered low-risk HPV because they don't lead to cancer or other serious health problems. At least a dozen types of HPV can sometimes lead to cancer, though two (types 16 and 18) lead to the majority of cancer cases.

These are called high-risk HPV. Cervical cancer is most linked to HPV, but HPV can also cause cancer in the vulva, vagina, penis, anus, mouth, throat as well as breast

cancer. It can also cause cancer in the back of the throat, including the base of the tonques and tonsils (called oropharyngeal cancer) (Bucchi, 2016).

Human papillomaviruses infection occurs at the basal cell layer of stratified squamous epithelial cells. Infection stimulates cellular proliferation in the epithelium and infected cells display a broad spectrum of changes, ranging from benign hyperplasia to dysplasia to invasive carcinoma. To effectively replicate, HPV must utilize the host cellular machinery. During the process, the viral protein product encoded by E6 binds to the p53 tumour suppressor gene product, which results in the premature degradation of the p53 protein (Mantovani and Banks, 2001). The E7 protein binds to a tumour suppressor protein—the retinoblastoma protein—and inhibits its function(Pang and Thierry, 2013). These protein products mediate much of the virus' oncogenic potential and their production represents a key difference between the low- and high-risk strains of HPV (Reed and Zazove, 2013).

Human papillomavirus infection may be latent, subclinical, or clinical. It may take the pathway of low viral-load infection without clinical disease, or high viral-load infection with clinical disease(Juckett and Hartman-Adams, 2010). HPV can cause a wide range of infections ranged from benign lesions to malignant infections.

Human Papillomaviruses (HPVs) are recognized as carcinogenic agents in breast cancer in humans (Salman *et al.*, 2017a). For many reasons, the relationship between HPV and breast cancer is imperative. The anatomy of the breast duct exposes the open duct to the external environment, which increases the risk of HPV infection. Most cases of breast cancer originate from the epithelium of the breast ducts, where hyperproliferation of ducts is followed by subsequent tumor progression to invasive ductal carcinoma in situ. Therefore, it is speculated that HPV virus particles can be transported from the original infection site in the genital area, enter through the nipple and infect the mammary duct. Later, this may be the cause of certain types of breast cancer (Sher *et al.*, 2020).

1.2. Aim of the study

The present study was conducted to achieve the following goals:

1. Find an association between HPV and breast cancer among women who were histologically diagnosed as breast cancer among patients from Baghdad and different cities in Iraq by using an Enzyme-linked Immunosorbent Assay (ELISA kit)
2. Finding an association with tumour suppressor proteins P53 by using ELISA technique.