

## Human Papilloma Virus as Risk Factor for Non-melanoma Skin Cancer in Khartoum State-Sudan

MOHAMMED SIDDIG ABDELAZIZ<sup>1</sup>

Associate Professor

Department of Histopathology and Cytopathology  
College of Medical Laboratory Science  
Sudan University of Science and Technology, Sudan

MANHAL OSMAN AHMED ELBARAKA

MSc student

Department of Histopathology and Cytopathology  
College of Medical Laboratory Science  
Sudan University of Science and Technology, Sudan

### Abstract:

*This descriptive retrospective cross sectional study was carried out in Khartoum state, during the period from March to October 2015, to detect the association between human papilloma virus (HPV) infection and non-melanoma skin cancers using immunohistochemistry. 40 formalin fixed paraffin embedded blocks were collected from patients previously diagnosed as skin cancers, 20 samples were squamous cell carcinoma (SSC) and the remaining 20 samples were basal cell carcinoma (BCC). From each block only one section with 3µm in diameter were cut, to detect HPV using modified indirect immunohistochemical method (Thermo manufacture). The patient data were collected from patient file`s. Data was analyzed using SPSS computer program, frequencies, mean, chi square test were calculated. Among study group 26(65%) were males and 14(35%) were females, their age ranged between 20-90 year with mean age 63year. in SCC15 patients were more than 60 year and remaining 5 were less*

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<sup>1</sup> Corresponding author: mohamedsiddig88@gmail.com

*than 60 year, while in BCC 10 patients were more than 60 year and other 10 were less than 60 year. HPV were positive in 14 (35%) case, 11 of them were SCC and the remaining 3(7.5%) were BCC, while negative in 26 (65%) case, 17 (65%) of them were BCC and the remaining 9(22.5%) were SCC, with a significant association between HPV infection and type of skin cancer (P.value 0.008). The study concludes there is a significant association between HPV infection and type of skin cancers and no relation between age and sex with type of skin cancer.*

**Key words:** HPV, Non-melanoma Skin Cancer, Immunohistochemistry

## **INTRODUCTION:**

Skin cancer is abnormal growth of the cells, that capable to invade and destroy the underlying tissue structure<sup>1</sup>. Often subdivided into melanoma and non-melanoma skin cancer. Non-melanoma originates from the external skin surface; include squamous cell carcinoma and basal cell carcinoma<sup>2</sup>. Melanoma and non-melanoma skin cancer are the most common types of cancer in white population<sup>3</sup>. Both tumors entities show an increase incidence rate worldwide but are stable or have decreasing mortality rate<sup>4</sup>. Also noted in about 35-45% of all malignancies in caucasians<sup>5</sup>. Skin cancer is a common malignant neoplasm comprising a large heterogeneous group of cancers with variable histological, biological and clinical characteristics in the world<sup>6</sup>. Squamous cell carcinoma (SCC) more frequent skin malignancy accounting for 42.6% followed by basal cell carcinoma (BCC) accounting 32% of skin cancer<sup>7</sup>. In Sudan skin cancer represent 18.5% comparing to all malignancies in the earliest reports<sup>7</sup>. Ultraviolet radiation (UVR) is the major environmental factor that influences the induction of skin tumors<sup>8</sup>. Clinical behavior and epidemiology of SCC suggest a viral etiology such as HPV, also organ

transplantation increase risk for SCC<sup>9</sup>. HPV are more than 100 different types have been identified, each is known by a number, that subdivided into low-risk such as (6, 11, 42); usually causes benign proliferative lesions (warts), while the other type high risk oncogenic types such (16, 18, 31) have etiological role in epithelial cancer<sup>10</sup>. Diagnosis of skin cancer started with physical examination, skin biopsy via microscopic examination, the diagnosis confirmed with advance techniques; such as PCR and immunohistochemistry<sup>11</sup>. Advance treatments of skin tumors include systemic therapies such as epidermal growth factor receptor inhibitor, and topical immunomodulating drugs such as imiquimod<sup>12</sup>. The aim of this study was to detect the association of HPV with non-melanoma skin cancers using immunohistochemistry.

## **MATERIALS AND METHODS**

This is a descriptive retrospective cross sectional study, conducted in Khartoum state, Sudan. The study was conducted during the period from March to October 2015. 40 formalin fixed paraffin embedded blocks were collected from laboratory archive previously diagnosed as skin cancers, 20 samples were squamous cell carcinoma (SSC) and the remaining 20 samples were basal cell carcinoma (BCC). From each block only one section with 3 $\mu$ m in diameter were cut and attached to salinized slides (Thermo manufacture), to detect HPV using modified indirect immunohistochemical method using monoclonal mouse anti human HPV clone MIB-1(Thermo manufacture). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and placed in running water. Samples steamed for antigen retrieval for HPV using PT, slides were placed in sodium citrate buffer (pH 9.0), then were boiled at 97°C for 10 minutes, then sections were cooled at RT. Endogenous peroxidase activity was blocked

with 3% hydrogen peroxidase and methanol for 10 min, then sections were incubated with 100µl of primary antibodies for 20 min at room temperature in a moisture chamber, and then rinsed in phosphate buffer saline. Then the linker was added for 10 minutes and washed in three changes of PBS, followed by addition of 3, 3 diaminobenzidine tetra hydrochloride (DAB) chromogen for 5 minutes. Sections were counterstained with haematoxylin. Positive HPV staining was identified in form of dark brown color around the nucleus. The patient data were collected from patient file`s. Data was analyzed using SPSS computer program, frequencies, mean, chi square test were calculated.

## **RESULTS**

In this study, the expression of HPV marker were detected in 40 skin paraffin wax embedded blocks, of which 20 (50%) were squamous cell carcinoma, 20(50%) were basal cell carcinoma.

As shown in table 1, the age of the involved patients range between 20 to 90 years old; the majority of the patients (more than 60%) were between the age group 60 to 90 years old, with mean age 63. The majority of study group were male 26 (65%) while female were 14(35%) as shown in table 2. In table 3 the result of HPV were found as follow 14 (35%) were positive, while 25(65%) were negative.

The association between skin cancer (squamous and basal cell carcinoma) and result of HPV, was summarized in tables 4, as follow: in SCC 11(27.5%) cases were positive, while 9 (22.5%) cases were negative. In contrast in BCC, 3(7.5%) cases were positive and 17(65%) were negative, with significant association of HPV immunohistochemical result and types of skin cancer (P value 0.008). Distribution of sex and age among study population were showed as follow: out of 20 cases of SCC, 12 were male and 8 were female, 15 of them were >60 year and

5 were <60 year. While in 20 cases of BCC, 14 were male and 6 were female, of them 10 were >60years and 10 were <60 years.

**Table 1: Frequency of the age group among study population**

Age group	Frequency	percent
≤ 60	15	37.5
> 60	25	62.5
Total	40	100

**Table 2: Frequency of the sex among study population**

Sex	Frequency	percent
Male	26	65
Female	14	35
Total	40	100

**Table 3: Frequency of Immunohistochemical result of HPV**

Immunohistochemical result	Frequency	percent
Positive	14	35
Negative	26	65
Total	40	100

**Table 4: Relation of HPV immunohistochemical result, sex and age group with type of skin cancer**

Type of skin cancer	HPV immunohistochemical result		Sex		Age group	
	Positive	Negative	Male	Female	≤ 60	> 60
SCC	11	9	12	8	15	5
BCC	3	17	14	6	10	10
Total	14	26	26	14	25	15
<b>P value</b>	<b>0.008</b>		<b>0.741</b>		<b>0.740</b>	

## DISCUSSION

Skin cancer has been reported to be one of the most frequent malignancy in white populations . Also was noted to be one of common types of malignancy in Sudanese<sup>7</sup>.

The present study revealed that; the majority of the diagnosed patients were above 60 years old (elder age), this findings supported by Abbas., *et al*<sup>13</sup>, who found that; the

incidence rate of skin cancer has slowed down and decreased in younger age. The majority of cases of squamous and basal cell carcinoma were male, this result supported by Abbas., *et al*<sup>13</sup>, who found that; Male had higher incidence rate in non melanoma skin cancer than female , also supported by Liter. ,*et al*<sup>14</sup> ,who reported the highest incidence rate of non-melanoma skin cancer has been reported in Queensland , Astralia in male rather than in female, in contrast disagreed with Chuang, *et al*<sup>15</sup> ,who found that: Japanese female had non-melanoma skin cancer more than male.

The result of HPV was positive in 14 (35%) samples, this result is nearly to Aubin, *et al*<sup>16</sup> , who found ; cutaneous HPV have been detected in about 60 to 90 % of patient ; these result support the role of HPV infection in skin carcinogenesis , and also Harwood , *et al*<sup>17</sup>, who found; HPV was positive in 84% of squamous cell carcinoma and 75% of basal cell carcinoma , but disagreed with study of Park , *et al*<sup>18</sup>, who reported low percentage of HPV in Korean patient with skin cancer.

The present study found the presence of HPV in SCC is high, this result is match with study of Schmidt., *et al*<sup>19</sup>, who found cutaneous SCC provide evidence of general susceptibility to oncogenic HPV but didn't match with study of Accardi *et al.*,<sup>20</sup>, who found; HPV prevalence and viral load decrease during skin carcinogenesis and being significantly higher in actinic Keratosis than skin cancer. While the presence of HPV in BCC is low, this result is agreed with study of Birch-Johansen *et al*<sup>21</sup>, who found; HPV tended to be more prevalent in NMSC especially in squamous cell carcinoma compared to basal cell carcinoma, indicating a potential link between HPV and SCC. While disagreed with study of Mina *et al*<sup>22</sup>, who found no association between HPV and BCC).

Regarding the association of HPV expression with non melanoma skin cancer, our findings showed statistical association between HPV and skin cancer which is supported

by Aubin *et al*<sup>16</sup>; who reported the HPV infection have a role in skin carcinogenesis as co-factor with UV rays, while disagreed with study of Ally., *et al* <sup>23</sup>, who found that; there is no association between the presence of HPV and type of skin cancer.

## **CONCLUSION:**

Skin cancer is more frequent among Sudanese males than females. The majority of cases were in elder age. There were differences between the skin cancer types regarding their presence of HPV markers; there is association between the HPV infection and the type of skin cancer. There is no association between the type of skin cancer with age and sex.

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