ORIGINAL ARTICLE

Expression of Squamous Cell Carcinoma Antigen 2 in Patients with Verruca Vulgaris: An Immunohistochemical Study

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ABSTRACT

Background: *Human papillomavirus (HPV) infections frequently result in benign hyperkeratotic proliferations of the epidermis known as verruca vulgaris. Squamous cell carcinoma antigens (SCCA1 and 2) belong to the ovalbumin-serpin family and have been widely used as markers for tumors and some inflammatory diseases.* ***Objectives:*** *to assess the expression of SCCA2 in verruca vulgaris patients and its relationship to verruca characteristics.* ***Methodology:*** *This cross-sectional study included 25 verruca vulgaris patients, as well as 25 age and sex matched healthy controls. After clinical evaluation; biopsies were taken from the controls and lesional and peri-lesional skin in patients with warts. Immunohistochemical (IHC) staining was done using Rabbit Anti-human SCCA2 Polyclonal Antibodies.* ***Results:*** *All biopsies from the controls showed weak IHC staining; while nearly half of the patients showed moderate/strong IHC staining. There was a non-statistically significant difference between warts and non-lesional skin of patients in IHC scores. Patients with moderate/strong IHC were significantly older than those with no/weak IHC. The duration of warts was significantly longer in patients with moderate/strong IHC scores.* ***Conclusions:*** *SCCA2 may be involved in the pathogenesis of warts via inhibition of keratinocytes apoptosis. Future studies are needed to confirm this postulation and to evaluate its link to different cytokines in patients with warts.*

INTRODUCTION

Common cutaneous warts (Verruca vulgaris) associated with HPV account for up to 7-12% of the population[1]. HPV is a circular, double-stranded, non-enveloped DNA virus. More than 180 HPV types have been identified[2]. The virus attacks epidermal cells and enters a latent state of slow reproduction. While the outer layer of the epidermis is growing superficially, the virus causes hyperplasia and hyperkeratosis, which collectively, result in a wart clinically[3].

The immune response is thought to be essential for HPV clearance depending on intact cellular immunity including Natural Killer cells and cytotoxic T cells. The T-helper 1 cytokines (IL-2, interferon-γ, and tumor necrosis factor-α) and IL-17 are involved in HPV clearance[4-7]. Patients with recurrent warts had higher serum levels of IL-22, suggesting that it may play a part in the immune response against HPV[8]. Moreover; IL-22 downregulates molecules involved in keratinocyte cornification including pro-filaggrin, keratins 1 and 10, and calmodulin-like 5[9].

Squamous cell carcinoma antigens 1 and 2 (SCCA1 and SCCA2) are members of the ovalbumin-serpin family. They have been widely used as tumor markers, particularly for different types of squamous cell carcinomas (SCC)[10]. The amino acid identicalness in SCCA1 and SCCA2 is 92%[10].

The SCCAs have diverse biological functions such as inhibition of cell apoptosis, stimulation of cell proliferation, defense against bacterial and parasitic proteases, and suppression of immune defense against tumors. In the normal squamous epithelium, the granular and prickle cell layers express the SCCAs[11].

Apart from their role in SCC; SCCA molecules may play a key role in the pathophysiology of some inflammatory disorders including asthma[12], psoriasis[13], and atopic dermatitis (AD)[14]. It has been demonstrated that SCCA2 is a valuable biomarker for determining the severity of AD and psoriasis[11].

IL-22 and, to a lesser degree, IL-17A increased the expression of SCCAs. When IL-17 was combined with IL-22, the expression of SCCA2 in keratinocytes was markedly increased[11]. Induction of both SCCA1 and SCCA2 by IL-22 in keratinocytes was also reported by Gudjonsson el al[15]. SCCA1/2 can be thought of as downstream molecules of IL-22 and IL-17[11].

To our knowledge, SCCA2 expression was not evaluated in patients with common warts. This study was designed to assess SCCA2 expression in wart patients' skin and identify any relationships between it and wart characteristics.

METHODOLOGY

Patients:

This cross-sectional study included 25 verruca vulgaris patients, attending Dermatology, Venereology and Andrology Outpatient Clinics at Sohag University Hospitals between May 2022 and August 2023 as well as 25 age and sex matched healthy volunteers as control. Patients with a history of SCC or other malignancies, or who presented with other dermatological diseases or allergic diseases were not included in this study.

Ethical considerations:

The Research Ethics Committee at the Faculty of Medicine, Sohag University reviewed the proposed protocol and approved the study based on the committee standard operating procedure guidelines on 11/4/2022 under IRB Registration number: **Soh-Med-22-04-07**. An informed consent was obtained from all participants. The study was in line with the Declaration of Helsinki.

Methods:

Clinical evaluation:

Participants were asked about their age, sex, residence, marital status, and smoking. Wart onset, course, and duration were reported. Previous and family history of warts or treatment of warts were documented. Patients were examined to evaluate the type of warts, number, site, and size of the warts.

Skin Biopsy:

Using 2-mm disposable punches and strict sterile procedures, a single skin biopsy was obtained from each healthy volunteer (control group). Two biopsies were obtained from patients with wart lesions: one from the verruca vulgaris using 5-mm disposable punches, and one from the unaffected skin using 2-mm disposable punches, all while maintaining strict sterility.

Biopsy handling:

Specimens were sent to the histopathology laboratory of Sohag University Hospitals and paraffin-embedded tissue blocks were prepared from each specimen. Each block was divided into serial sections, which were then stained with hematoxylin and eosin (H&E). To confirm the wart diagnosis, stained slides were analyzed.

Immunohistochemical (IHC) detection of SCCA2:

Five µm thick sections were taken from the paraffin blocks on positively charged slides. Before being treated with hydrogen peroxide block (0.3% H2O2) for 10 minutes, the sections on positively charged slides were de-deparaffinized in xylene for 30 minutes and then rehydrated in declining graded alcohol (absolute, 95%, and 70% alcohol for 5±1 minutes each). After that, the slides were gently cleaned in phosphate buffer solutions (PBS).

Antigen retrieval was done by immersion of slides in Coplin Jars filled with 10-mmol sodium citrate buffer solution, pH 6.0. For roughly fifteen minutes, the slides were put in the microwave. After being taken out of the microwave, the containers were left to cool at room temperature for half an hour. Slides were washed in PBS and placed in it for fifteen minutes. Rabbit Anti-human SCCA2 Polyclonal Antibody (SRB laboratories, Catalog No.201r-3489- conc. 0.1 ml) was added to each section and incubated in a moist chamber at 4º C overnight. Slides were then rinsed gently with PBS two times (5 minutes for each). Then at room temperature, a secondary antibody was added to each slide for half an hour. Slides were then rinsed in PBS three times. Streptavidin peroxidase was applied as 2 drops and the slides were incubated at room temperature for 30 minutes. Freshly prepared diaminobenzidine (DAB) chromogen was applied to each tissue section and incubated for 5-15 minutes at room temperature followed by rinsing with distilled water.

Counterstaining with Mayer's Hematoxylin for five dips was done; then rapidly washed gently in tap water to take out the extra dye. The sections were dehydrated with ascending graded alcohol (70%, 95%, and 100% alcohol). Finally, clearance with xylene and mounting by DPX. Sections of human SCC were used as positive controls **(Figure 1.B)**.

Evaluation of SCCA2 expression:

SCCA2 positivity was recognized as a brownish cytoplasmic and nuclear staining of cells. The range of IHC was as follows: negative expression (0); weak expression (1+); moderate expression (2+); and strong expression (3+)[16] **(Figure 1)**.

Statistical analysis:

Data were analyzed using IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA). Descriptive statistics: Means, standard deviations (SD), medians, ranges, frequency, and percentages were calculated. Test of significances: Chi-square/Fisher's exact or Monte Carlo exact test was used to compare the difference in the distribution of frequencies among different groups. The normality of continuous variables was tested using Kolmogorov-Smirnov test/ Shapiro-Wilk test as appropriate. Student t-test was carried out to compare the means/ medians of dichotomous data. P value <0.05 was considered significant.

RESULTS

Socio-demographic characteristics:

There was a non-significant difference between cases and controls as regards age, sex, marital status, occupations, and smoking, with the age mean ± SD being 29.88 ± 8.6 and 35.12 ± 8.5 years in patients and controls respectively **(Table 1).**

Table 1: Socio- demographic characteristics of the studied populations (n=50).

Parameter		Control (n = 25)	Patients with warts (n = 25)	P-value
Age (years)	**Mean ± SD**	35.12 ± 8.5	29.88 ± 6.6	= 0.114*
Sex	Male	11 (44%)	12 (48%)	= 0.777**
	Female	14 (56%)	13 (52%)	
Marital Status	Single	11 (44%)	12 (48%)	= 0.777**
	Married	14 (56%)	13 (52%)	
Occupation	Unemployed	14 (56%)	16 (64%)	= 0.564**
	Employed	11 (44%)	9 (36%)	
Smoking	Smokers	2 (8%)	3 (12%)	= 0.633**

SD: Standard deviation
*Independent Sample t-test was used to compare the differences in mean between groups
**Chi-square test was used to compare the differences in frequency between groups

Clinical characteristics of the studied patients:

The median warts duration was 12 months, with a 1.5 mm median size. Multiple warts were discovered in 14 patients (56%) with the upper limb being the most affected site in 18 patients (72%). Only 4 patients (16%) had a positive family history **(Table 2)**.

Table 2: Disease characteristics of the studied cases (n=25).

Variable	Category	n = 25
Lesion Site	Upper Limb	18 (72%)
	Lower Limb	6 (16%)
	Both	3 (12%)
Onset	Acute	25 (100%)
Course	Progressive	25 (100%)
Disease Duration/months	Median (Range)	12 (1 - 24)
Previous Treatment	No	20 (80%)
	Topical Treatment	5 (20%)
History of Wart	No	25 (100%)
Family History of Wart	Negative	21 (84%)
	Positive	4 (16%)
Number of Warts	Single	11 (44%)
	Multiple	14 (56%)
Wart Size (cm)	Median (Range)	1.5 (0.5 – 2.5)

Immunohistochemistry scoring of SCCA2:

All sections of the control group showed mild IHC scores (Figure 1.A). There were highly statically significant differences between the control group and both perilesional and verruca vulgaris lesions, with non-significant differences between lesional and perilesional IHC scores (**Table 3**).

Table 3: Comparison of warts, peri-lesional, and control groups regarding IHC score

	Warts (n=25)	Peri-lesional (n=25)	Control (n=25)	P-value*
Immunohistochemistry score of squamous cell antigen 2				
No	1 (4%)	3 (12%)	0 (0%)	P1= 0.256 **P2< 0.001** **P3< 0.001**
Weak	11 (44%)	5 (20%)	25 (100%)	
Moderate	9 (36%)	11 (44%)	0 (0%)	
Strong	4 (16%)	6 (24%)	0 (0%)	

*Monte Carlo exact test was used to compare the differences in frequency between groups.
P1: Comparison between verruca vulgaris lesion and perilesional skin.
P2: Comparison between verruca vulgaris lesion and control.
P3: Comparison between perilesional skin and control.

Predictors of moderate /strong IHC score in warts:

Only age, and duration of warts were statistically significant. Patients with moderate/strong IHC were significantly older than those with no/weak IHC. The warts duration was significantly longer in patients with moderate/strong IHC scores (**Table 4**).

Table 4: Predictors of moderate/strong IHC among patients with warts (n=25).

	No/Weak IHC (n = 12)	Moderate/Strong IHC (n = 13)	P-value
Age/years (Mean ± SD)	26.69 ± 4.2	32.76 ± 5.1	**= 0.044***
Sex - Male	5 (41.7%)	7 (53.8%)	= 0.543**
- Female	7 (58.3%)	6 (46.2%)	
Occupation: - Unemployed	9 (75%)	7 (53.8%)	= 0.185**
- Employed	3 (25%)	6 (46.2%)	
Smoking	2 (16.7%)	1 (7.7%)	= 0.469***
Lesion Site - Upper Limb	9 (75%)	9 (69.2%)	= 0.438**
- Lower Limb	2 (16.7%)	2 (15.4%)	
- Both	1 (8.3%)	2 (15.4%)	
Disease Duration/month	4.77 ± 1.2	15.89 ± 2.5	**= 0.001***
Topical Treatment	3 (25%)	2 (15.4%)	= 0.495**
Positive Family History	1 (8.3%)	3 (23.1%)	= 0.168**
Wart Number: - Single	4 (33.3%)	7 (53.8%)	= 0.265**
- Multiple	8 (66.7%)	6 (46.2%)	
Wart Size (cm)	1.18 ± 0.6	1.44 ± 0.6	= 0.346*

SD: Standard deviation; IHC: Immunohistochemistry
*Independent Sample t-test was used to compare the differences in mean between groups
**Chi-square test was used to compare the differences in frequency between groups
***Fisher's exact test was used to compare the differences in frequency between groups

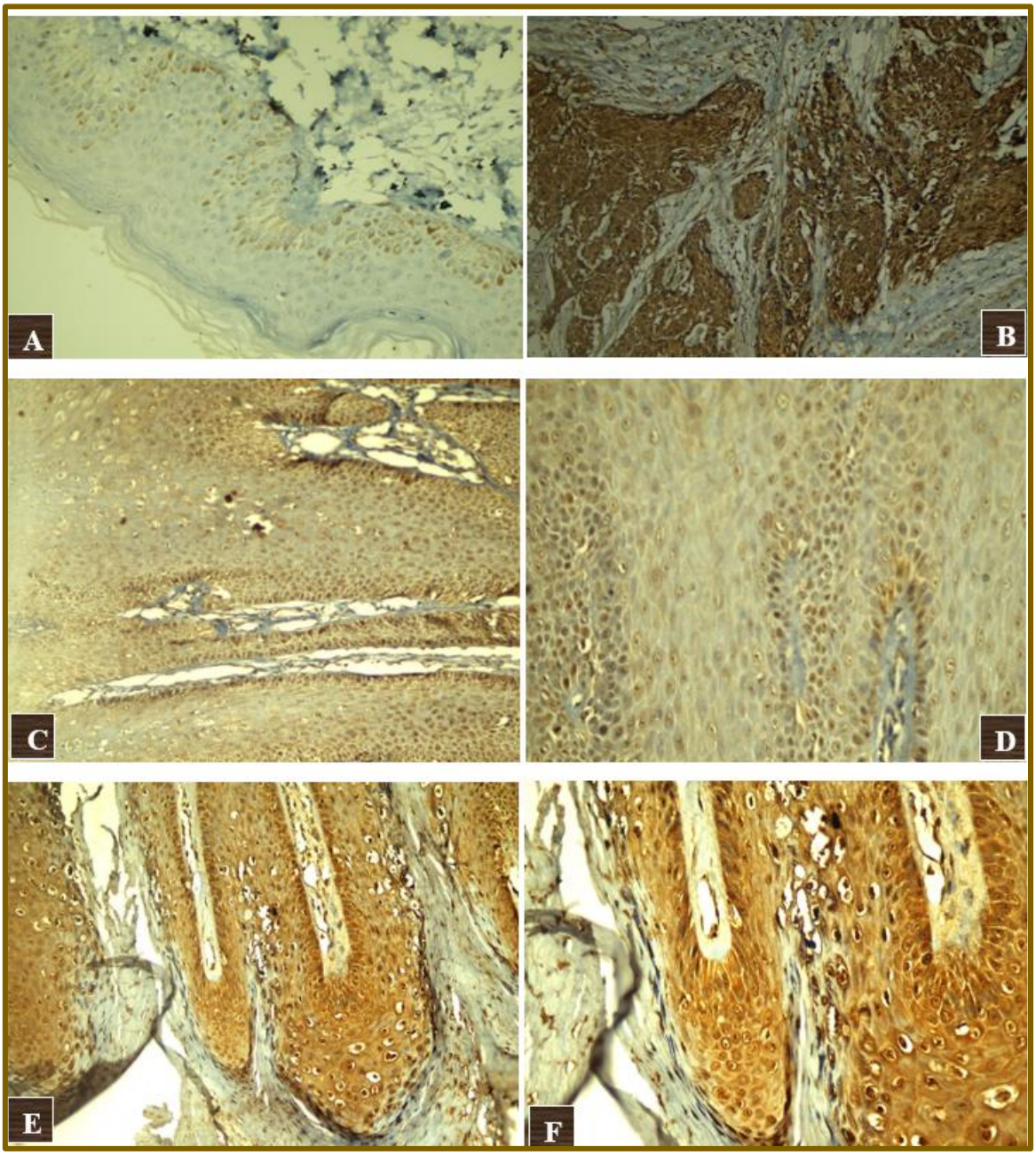

Fig. 1: Expression of squamous cell carcinoma antigen 2. A: Weak expression in normal skin (Control, x200), B: Strong expression in squamous cell carcinoma (Positive control, x200), C-D: Moderate expression in common warts (C: x200, D: x400), E-F: Strong expression in common warts (E: x200, F: x400).

DISCUSSION

Verrucae vulgaris are benign epidermal proliferations caused by HPV infection[17]. They can cause several problems owing to their location. For instance, they can occasionally itch or bleed[18]. Cytokines are essential for coordinating the immune system's defense against infections, including those caused by viruses like HPV. IL-22 and IL-17 are cytokines associated with type 17 inflammation. Concerning warts, IL-22 promotes epithelial cell proliferation and tissue repair, while IL-17 enhances immune responses against pathogens, including HPV. These cytokines aid in the immune surveillance and clearance of HPV-infected cells[19].

Being members of the ovalbumin-serpin family; SCCA1 and SCCA2 contribute to the pathophysiology of squamo-proliferative disorders such as inverted

papilloma[20, 21], SCC and verrucous carcinoma of several anatomical sites[22-24]. Also, SCCA2 can participate in many inflammatory diseases[11].

Taking into consideration that levels of SCCA2 level were not evaluated in patients with warts before; the current study aimed at assessing the SCCA2 expression in the skin of patients with warts and comparing the outcomes with those of patients' non-lesional skin and healthy controls' skin.

A highly statistically significant difference in the IHC scores was found between control and non-lesional skin in patients with warts. Absent or weak expression in both control and non-lesional skin was previously reported in psoriasis[13], and AD[25]. The SCCA immunoreactivity in normal skin was documented in several studies[26-28]. By radioimmunoassay, it was found that the concentration of SCCA was comparable in healthy skin to its concentration in the cervical SCCs and the squamous epithelia of the esophagus and vagina[26, 29].

A highly statistically significant difference in the IHC scores was found between control and warts lesions with over 50% of the patients having moderate and strong staining. Compared to healthy controls; strong expression of SCCAs was previously reported in individuals with psoriasis[13, 16, 30], and AD[14, 25, 31].

It is conceivable to speculate that the higher expression values of SCCA in warts suggest its possible role in the prevention of keratinocytes apoptosis allowing continuous keratinocytes proliferation and hence the growth of warty lesions which are in essence represent squamous proliferation[13].

The cytokines may play key roles in mediating the effects of SCCA in the keratinocytes. SCCA2 is most dominantly induced by IL-22, while IL-17 is a less inducer to SCCA2[11]. Since IL-22 was higher in wart patients, it might be involved in the immune system's antiviral response[8]. Moustafa et al[7] reported that found that patients with HPV infection had significantly higher serum IL-17 levels than healthy controls and that the length of the lesion was significantly positively correlated with serum IL-17 levels. The elevated expression of SCCA2 in warts may be explained by these findings.

On the other hand; other studies reported lower levels of IL-17[5, 6] and IL-22[6] in patients with warts than control. This may explain the discrepancy in the expression of SCCA2 in patients with verruca vulgaris.

In the present study; there was a non-significant difference between SCCA2 expression in warts and non-lesional skin. This could be connected to the genetic predisposition, recurrences, and multiplicity natures of warts[32].

In the current study; patients with moderate/ strong IHC scores were significantly older than those with no/weak IHC. On the contrary; levels of SCCA2 were not directly affected by age in patients with psoriasis[16] and in pediatric patients with AD[31].

In this study; the duration of warty lesions was significantly longer in patients with moderate/strong IHC scores. The connection between SCCA2 and the duration of skin disease is still poorly understood. While SCCA2 is primarily associated with SCCs, its role in skin diseases like psoriasis and AD suggests that it may be influenced by disease duration[11].

Despite the interesting findings of this study, it has some limitations. The relatively small number of participants limits the generalization of results. The cross-sectional nature restricts causal conclusions. Further longitudinal multicenter studies with larger cohorts with warts are recommended. Also; it is worthwhile to explore the correlations between SCCA (both serum levels and tissue expression) in warts and cytokines such as IL-17 and IL-22.

CONCLUSIONS

Both the wart skin and the peri-lesional skin of patients with warts expressed more SCCA2 than controls, with no significant difference in SCCA2 between warts and perilesional skin. These findings suggest a possible role of SCCA2 in the pathogenesis of cutaneous warts. However; future studies should be directed to explore the precise mechanisms that may contribute to the development of warts.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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