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Immunohistochemical Study of Cyclin Dependent Kinase5 (CDK5) in Some Hyperproliferative Skin Disorders

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Abstract
Background: Hyperproliferative skin disorders (HSD) encompass a range of pathologies characterised by abnormal skin proliferation and differentiation. Cyclin-dependent kinases (CDKs) play a crucial role in regulating cell cycle progression. This study aimed to assess CDK5 and Ki-67 expression in psoriasis, verruca, and seborrheic keratosis, shedding light on their potential roles in the pathogenesis of hyperproliferative skin disorders. Methods: This case-control study included 80 participants, comprising patients with psoriasis, verruca, and seborrheic keratosis, as well as healthy controls. Dermoscopic, histopathological, and immunohistochemical evaluations of CDK5 and Ki-67 were conducted. Results: The study revealed a significant correlation between CDK5 expression and disease course in psoriasis, with progressive cases showing higher CDK5 levels. Additionally, a significant association was observed between CDK5 expression and the diffuse acanthosis pattern in psoriasis. In verruca, CDK5 expression was positively correlated with histopathological changes, particularly diffuse acanthosis and parakeratosis. CDK5 expression was highest in verruca compared to psoriasis, seborrheic keratosis, and controls. Conversely, Ki-67 expression was highest in psoriasis. Statistically significant differences were found in nuclear expression of both CDK5 and Ki-67 among the studied groups. Conclusions: CDK5 and Ki-67 may play distinct roles in the pathogenesis of hyperproliferative skin disorders. CDK5 appears to be more closely associated with verruca, while Ki-67 is highly expressed in psoriasis.

Keywords: Cyclin-Dependent Kinase 5 (CDK5), Ki-67, Hyperproliferative Skin Disorders, Psoriasis, Verruca, Seborrheic Keratosis, Immunohistochemistry.

1. Introduction

Cyclin-dependent kinases (CDKs), such as CDK5, are essential serine/threonine kinases that regulate cell cycle progression by forming heterodimeric complexes with cyclins. CDK5 is unique in its activation by non-cyclin activator proteins and plays diverse roles in various biological processes [1, 2].

Ki-67, a nuclear protein, serves as a marker for cellular proliferation during specific cell cycle phases, excluding G0 [3, 4].

Hyperproliferative skin disorders (HSD) are characterised by disrupted skin proliferation and differentiation (Quadri et al., 2022). (Quadri et al., 2022). Psoriasis, a chronic systemic disease, involves T cell-mediated keratinocyte hyperproliferation, leading to raised erythematous plaques with silvery scales [5-7].

Verrucae (warts), caused by the human papillomavirus (HPV), are benign skin lesions that may resolve spontaneously over time. Histopathologically, verrucae exhibit atypical keratinocytes (koilocytes), acanthosis, and vascular changes [8].

Seborrheic keratosis (SK) is a common benign skin neoplasm, typically presenting as well-demarcated, slightly raised, brownish to black. Microscopically, SK consists of sharply demarcated lesions with homogeneous basaloid cells and intraepidermal pseudocysts [9].

This study aimed to assess CDK5 and Ki-67 expression in psoriasis, verruca, and seborrheic keratosis, shedding light on their potential roles in the pathogenesis of hyperproliferative skin disorders.

2. Methods Patients

This case control study was carried out on a total of 80 individuals attending the Outpatient Clinic of Dermatology, Venereology and Andrology at Benha University Hospital, from July 2017 to September 2019. An informed consent was taken from all participants before starting the study.

They were divided into patients' group: included sixty patients, twenty patients with psoriasis (plaque type), twenty patients with verruca vulgaris and twenty patients with seborrheic keratosis (acanthotic or hyperkeratotic) and control group: included twenty apparently healthy volunteers who were age and sex matched with the patient group.

Inclusion criteria were clinically diagnosed patients with psoriasis, verruca and seborrheic keratoses, involving both genders, aged ≥ 18 years old patients

Exclusion criteria were any patients receiving any systemic therapy, phototherapy or applying topical therapy within one month prior to the study initiation, with auto immune, inflammatory cutaneous or systemic diseases,

with any known metabolic, endocrinal or neurological disease, that could influence cognitive functions (diabetes mellitus, hypertension, cardiovascular diseases, chronic liver or kidney diseases), pregnant female subjects or mental retardation.

Methodology:\sAll patients were subjected to the following: Full history taking including personal history, onset, course and duration of the disease, history of other skin diseases, History of systemic diseases, Family history of psoriasis, verruca and seborrheic keratoses, Drug history: previous forms of therapy either systemic or local.

Full clinical examination: general examination including involving Weight (in kilogrammes) and Height (in metres) (in meters), Local dermatological examination involving Complete cutaneous examination was done to determine the site, extent, morphology of the lesions.

The PASI score was calculated in psoriasis patients as described by Fredriksson and Pettersson, (1978). (1978). Psoriatic plaques are graded based on three criteria: redness (R), thickness (T), and scaliness (S) (S). Severity is rated for each index on an 0-4 scale (0 for no involvement up to 4 for severe involvement) (0 for no involvement up to 4 for severe involvement). The body is divided into four regions comprising the head (h), upper extremities (u), trunk (t), and lower extremities. In each of these areas, the fraction of total surface area affected is graded on a 0-6 scale (0 for no involvement; up to 6 for greater than 90 percent involvement) (0 for no involvement; up to 6 for greater than 90 percent involvement)

Skin biopsies: Skin biopsies were taken with 3-4 mm punch from each patient suffering of psoriasis, verruca or seborrheic keratoses. In addition, one site matched punch biopsy was taken from each control subject. These biopsies were taken under local anaesthesia. All biopsies were fixed in 10 percent neutral buffered formalin.

Interpretation of CDK5 immunohistochemistry: Positively stained cells displayed brown nuclear coloration via DAB reaction. CDK5 immunostaining scores combined positive cell rate and staining intensity. Scores ranged from 0 (0 percent) to 4 (76-100 percent) for positive rate and 0 (no staining) to 3 (strong staining) for intensity. Total scores exceeding two indicated positive CDK5 expression [10].

Statistical analysis:

Data were thoroughly reviewed for completeness and accuracy. Pre-coded data were entered into the SPSS software programme (version 20) for statistical analysis. Summary statistics included mean and standard deviation for normally distributed quantitative variables, while median, first quartile, and third quartile were used for non-normally distributed quantitative variables. Qualitative variables were presented as numbers and percentages. To compare qualitative variables, the chi-square test was employed, and for normally distributed quantitative variables, the independent T-test was utilised. Non-normally distributed quantitative variables were assessed using nonparametric tests such as the Kruskal-Wallis and Mann-Whitney tests. A significance level of $p < 0.05$ was considered statistically significan. Data were organised and presented in tables and graphs in accordance with Coggone's guidelines (2003). (2003).

3. Results

The present study included sixty patients divided into three equal groups having psoriasis, verruca vulgaris and seborrheic keratoses. In addition to twenty healthy volunteers. Psoriasis group included 20 patients with mean age 33.1±12.24 years; more than half of patients (60 percent) were males. 20 percent of patients had family history of psoriasis.

Histopathological examination of psoriatic biopsies revealed that 40 percent of tissues had hypogranulosis, thinning of epidermis over rete ridges, inflammatory cells (polymorphonuclear leukocytes and lymphocytes), and dermal angiogenesis. The pattern of acanthosis was diffuse in majority of tissues (80 percent), Munro micro-abscess was found in 40 percent of tissues. Parakeratosis was diffuse in 70 percent of tissues and the other 30 percent showed focal parakeratosis. The inflammatory infiltrate was moderate in more than half of tissues (70 percent) and vascular changes (angiogenesis) were present in almost (90 percent) of tissues. HE examination of verruca biopsies showed that 30 percent of tissues had hyperkeratosis, while 40 percent of tissues had papillomatosis, and hypergnulosis. The pattern of acanthosis was diffuse in about 60 percent of patients, Koliocytes presented in majority of patients (80 percent). (80 percent). Parakeratosis was found in 60 percent of patients. The inflammatory infiltrate was mild in more than half of patients (70 percent) and vascular changes (angiogenesis) were present in half of patients. Histopathological examination of seborrheic keratoses biopsies revealed that there was equal percentage of intradermal pseudocyst, and epidermal cell proliferation in

(40 percent) of patients. The pattern of acanthosis was focal in about 70 percent of patients, dyskeratosis and horn cyst were found in most patients, parakeratosis was found in 60 percent of patients, the inflammatory infiltrate was mild in more than half of patients (60 percent) and vascular changes (angiogenesis) were present in 40 percent of patients. Table 1\s

Table (1) Histopathological findings of the psoriasis, verruca, seborrheic keratoses patients

	Psoriasis group (N=20)	
Variables	**N**	**%**
HE:		
• Hyperkeratosis	20	100%
• Hypogranulosis	8	40%
• Thinning of epidermis over rete ridges	8	40%
• Inflammatory cells	8	40%
Pattern of acanthosis:		
• Diffuse	16	80%
• Focal	4	20%
Munro micro-abscess	8	40%
Parakeratosis:		
• Diffuse	14	70%
• Focal	6	30%
Inflammatory infiltrate:		
• Mild	6	30%
• Moderate	14	70%
Vascular changes(angiogenesis)	18	90%
	Verruca group (N=20)	
Variables	**N**	**%**
HE:		
• Hyperkeratosis	6	30%
• Papillomatosis	8	40%
• Hypergranulosis	8	40%
• Focal parakeratosis	4	20%
Pattern of acanthosis		
• Diffuse	12	60%
• Focal	4	20%
• No	4	20%
Koliocytes	16	80%
Parakeratosis	12	60%
Inflammatory infiltrate		
• Mild	14	70%
• Moderate	6	30%
Vascular changes(angiogenesis)	10	50%
	Seborrheic keratoses group (N=20)	
Variables	N	%
HE:		
• Hyperkeratosis	2	10%
• Epidermal basal cell proliferation	8	40%
• Intradermal Pseudocyst	8	40%
• Intradermal true hom cyst	18	90%
• Papillomatosis	6	30%
Pattern of acanthosis		
• Diffuse	6	30%
• Focal	14	70%
Dyskeratosis	20	100%
Parakeratosis	12	60%
Inflammatory infiltrate		

• Mild	12	60%
• Moderate	8	40%
Vascular changes(angiogenesis)	8	40%

The results of this study showed there was statistically significant relation between CDK5 and progressive course patients had (P =0.04). Regarding the relation between CDK5 and clinical characteristics within patients with verruca this study revealed that; there was no statistically significant relation between CDK5 and each of recurrency, course, and duration of disease. **Table 2**

Table (2) Relation between CDK5 and clinical characteristics within patients with psoriasis and verruca.

Variables			CDK5		Test	P value
			Negative	Positive		
Course	Progressive	N	8	8	3.333	0.04
		%	50.0%	50.0%		
	Stationary	N	4	0		
		%	100.0%	0.0%		
Duration	Mean ± SD		6±2.76	9.5±7.03	-	0.637
	Median (IQR)		6 (5-6)	7.5 (3.5-17.5)	0.472	
PASI score	Mean ± SD		21.15±8.85	18.80±8.36	-	0.536
	Median (IQR)		22.7 (14.6-28.3)	22 (9.4-25.1)	0.620	
Variables			CDK5		Test	P value
			Negative	Positive	X2/Z	
Recurrency	First time	N	8	6	0.087	0.769
		%	72.7%	66.7%		
	Recurrent	N	3	3		
		%	27.3%	33.3%		
Course	Progressive	N	8	8	0.808	0.369
		%	72.7%	88.9%		
	Stationary	N	3	1		
		%	27.3%	11.1%		
Duration	Mean ± SD		5.64±3.38	4.22±2.22	-0.387	0.699

As shown in this study, there was statistically significant relation between CDK5 and diffuse acanthosis pattern (P =0.04). This study revealed that when comparing between CDK5 and histopathological examination of verruca biopsies there was significant relation regarding the diffuse acanthosis pattern as 22.2 percent of diffuse acanthosis pattern biopsies had positive CDK5 expression (P= 0.006), also there was significant relation regarding parakeratosis as 22.2 percent of parakeratosis biopsies had CDK5 expression (P= 0.002). Table 3\sTable 3: Relation between CDK5 and histo-pathological examination within patients with psoriasis, verruca and seborrheic keratoses

Variables			**CDK5**		**Test**	**P**
			Negative	**Positive**	**X2**	**value**
Acanthosis pattern	Diffuse	N	8	8	3.333	0.04
		%	50.0%	50.0%		
	Focal	N	4	0		
		%	100.0%	0.0%		
Parakeratosis	No	N	8	6	0.159	0.690
		%	57.1%	42.9%		
	Yes	N	4	2		
		%	66.7%	33.3%		
Munro micro-abscess	No	N	8	4	0.556	0.456
		%	66.7%	33.3%		
	Yes	N	4	4		
		%	50.0%	50.0%		
Inflammatory infiltrate	Mild	N	4	2	0.159	0.690
		%	66.7%	33.3%		
	Moderate	N	8	6		

		%	57.1%	42.9%		
Vascular changes (angiogenesis)	No	N	2	0	1.481	0.224
		%	100.0%	0.0%		
	Yes	N	10	8		
		%	55.6%	44.4%		
Variables		CDK5			Test	P
		Negative		**Positive**	X2	value
Acanthosis pattern	Diffuse	N	10	2	10.236	0.006
		%	90.9%	22.2%		
	Focal	N	0	4		
		%	0.0%	44.4%		
	No	N	1	3		
		%	9.1%	33.3%		
Parakeratosis	No	N	1	7	9.731	0.002
		%	9.1%	77.8%		
	Yes	N	10	2		
		%	90.9%	22.2%		
Koliocytes	No	N	1	3	1.818	0.178
		%	9.1%	33.3%		
	Yes	N	10	6		
		%	90.9%	66.7%		
Inflammatory infiltrate	Mild	N	7	7	0.471	0.492
		%	63.6%	77.8%		
	Moderate	N	4	2		
		%	36.4%	22.2%		
Vascular changes(angiogenesis)	No	N	5	5	0.202	0.653
		%	45.5%	55.6%		
	Yes	N	6	4		
		%	54.5%	44.4%		
Variables			CDK5		Test X2	P
			Negative	Positive		value
Acanthosis pattern	Diffuse	N	4	2	0.010	0.919
		%	30.8%	28.6%		
	Focal	N	9	5		
		%	69.2%	71.4%		
Parakeratosis	No	N	6	2	0.586	0.444
		%	46.2%	28.6%		
	Yes	N	7	5		
		%	53.8%	71.4%		
Inflammatory infiltrate	Mild	N	7	5	0.586	0.444
		%	53.8%	71.4%		
	Moderate	N	6	2		
		%	46.2%	28.6%		
Vascular changes(angiogenesis)	No	N	7	5	0.586	0.444
		%	53.8%	71.4%		
	Yes	N	6	2		
		%	46.2%	28.6%		

The research found that there was no statistically significant association between Ki67and histological evaluations of psoriasis patients.

As per link between Ki67 expression and the histopathological findings in seborrheic keratoses patients this investigation found that there was no statistically significant association between Ki67 and histopathological findings inside seborrheic keratoses biopsies. Table 4

Table (4) Relation between Ki67 and histological investigation among individuals with psoriasis and seborrheic keratoses

Variables	KI67		Test	P value
	Negative	**Positive**	X2	

Acanthosis pattern	**Diffuse**	N	9	7	2.692	0.101
		%	69.2%	100.0%		
	Focal	N	4	0		
		%	30.8%	0.0%		
Parakeratosis	**Yes (diffuse)**	N	8	6	1.266	0.260
		%	61.5%	85.7%		
	Yes (focal)	N	5	1		
		%	38.5%	14.3%		
Munro micro-abscess	**No**	N	9	3	1.319	0.251
		%	69.2%	42.9%		
	Yes	N	4	4		
		%	30.8%	57.1%		
Inflammatory infiltrate	**Mild**	N	4	2	0.010	0.919
		%	30.8%	28.6%		
	Moderate	N	9	5		
		%	69.2%	71.4%		
Vascular changes (angiogenesis))	**No**	N	2	0	1.197	0.274
		%	15.4%	0.0%		
	Yes	N	11	7		
		%	84.6%	100.0%		
Variables			Ki67		Test	P value
			Negative	Positive	X2	
Acanthosis pattern	Diffuse	N	4	2	0.045	0.831
		%	28.6%	33.3%		
	Focal	N	10	4		
		%	71.4%	66.7%		
Parakeratosis	No	N	7	1	1.944	0.163
		%	50.0%	16.7%		
	Yes	N	7	5		
		%	50.0%	83.3%		
Inflammatory infiltrate	Mild	N	7	5	1.944	0.163
		%	50.0%	83.3%		
	Moderate	N	7	1		
		%	50.0%	16.7%		
Vascular changes (angiogenesis)	No	N	8	4	0.159	0.690
		%	57.1%	66.7%		
	Yes	N	6	2		
		%	42.9%	33.3%		

The research found that there was no statistically significant association between Ki67and histological evaluations of psoriasis patients.

As per link between Ki67 expression and the histopathological findings in seborrheic keratoses patients this investigation found that there was no statistically significant association between Ki67 and histopathological findings inside seborrheic keratoses biopsies. Table 4

Table 4: Relation between Ki67 and histological investigation among individuals with psoriasis and seborrheic keratoses

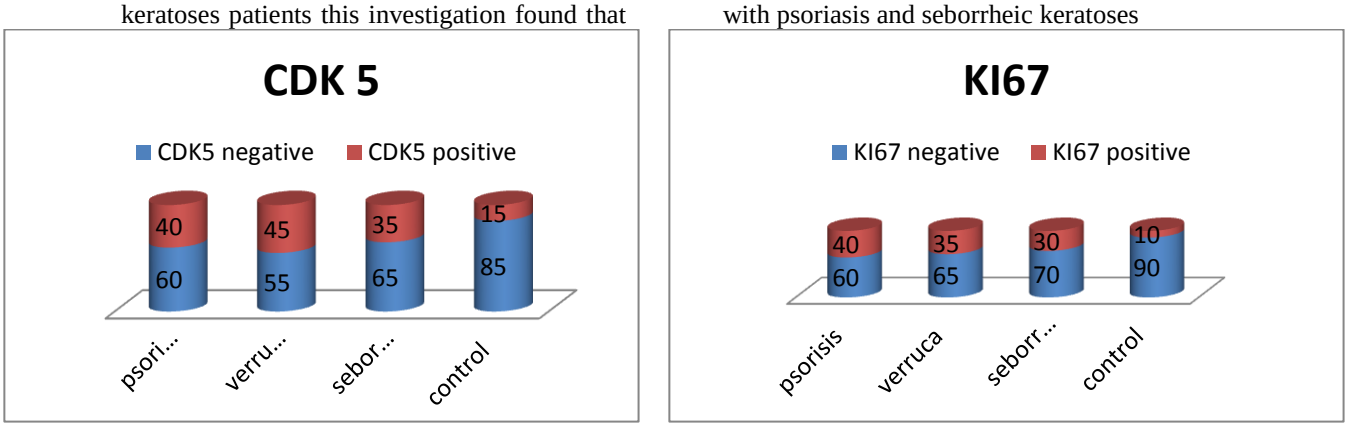

Fig. (1) Comparison between groups as regarding overall CDK5, Ki67 expression

4. Discussion

This research indicated that CDK5 expressed in 9 patients with verruca, 8 patients with psoriasis, 7 patients with seborrheic keratoses, and 3 volunteers. Ki67 expressed in 8 patients with psoriasis, 7 patients with verruca, 6 patients with seborrheic keratoses, and 2 volunteers. There is statistically significant link between the analysed groups for nuclear expression of either CDk5 (P= 0.04) or Ki67 (P= 0.032) expression. Figure 1

5. Conclusion

This research indicated that CDK5 expressed in 9 patients with verruca, 8 patients with psoriasis, 7 patients with seborrheic keratoses, and 3 volunteers. Ki67 expressed in 8 patients with psoriasis, 7 patients with verruca, 6 patients with seborrheic keratoses, and 2 volunteers. There is statistically significant link between the analysed groups for nuclear expression of either CDk5 (P= 0.04) or Ki67 (P= 0.032) expression. Figure 1.

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