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Immunohistochemical Expression of Involucrin in Verruca Vulgaris before and after Intralesional Injection of Vitamin D_3

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Abstract

Common warts (verruca vulgaris) are caused by human papilloma virus (HPV). They are one of the most common problems in dermatology but no particular therapy has demonstrated complete efficacy. Treatment options include cryotherapy, surgical excision, bleomycin and various lasers. Each mode of therapy has its own complications ,failure rate and may also develop a new lesion in anew area after successful treatment of previous one. The point of this work was to assess the security and viability of intralesional Vitamin D3 for the treatment of verruca vulgaris, distinguish histopathological changes in verruca vulgaris after infusion of nutrient D3 and look at between immunohistochemical articulation of involucrin when the infusion. This examination was performed on 20 patients with different verruca vulgaris infused with nutrient D3 and ten obvious sound people as control gathering. 40% and 20% of the rewarded patients demonstrated total reaction in the first and the second infused moles, individually. Incomplete reaction in 35% and 55% of the first and the second infused moles, separately. As respects the reaction of the non-infused mole, 66.7% demonstrated fractional reaction. The histopathological changes in moles after infusion of nutrient D3 were diminishing in the thickness of epidermis and decrease in incendiary cells in dermis. There are histopathological changes after infusion of nutrient D3 in verruca vulgaris incorporating stamped diminishing in epidermal thickness and diminishing of incendiary cells in the dermis. In verruca vulgaris, Involucrin articulation is higher after infusion of nutrient D3.

Keywords: Involucrin, Verruca Vulgaris, Vitamin D3.

1. Introduction

Verrucae are benevolent multiplications of the skin and mucosa that outcome from contamination with human papillomavirus (HPV). Verrucae are brought about by disease with a sort of human papillomavirus (HPV). Components that expansion the hazard incorporate utilization of open showers, working with meat, skin inflammation and a powerless safe framework. The infection is accepted to enter the body through skin that has been harmed marginally .They are commonly little, harsh, hard developments that are comparative in shading to the remainder of the skin. They typically don't bring about different manifestations, with the exception of when on the base of the feet, where they might be excruciating [1].

Involucrin protein is a 68 kDa, bar molded particle, glutamate-rich and contains an interior district made out of 39 rehashes of 10 amino acids .Its quality is mapped to 1q21, among calpactin I light chain, trichohyalin, profillaggrin, loricrin, and calcyclinIt is found in the cytoplasm and cross-connected to layer proteins by keratinocyte transglutaminases in separating keratinocytes [2].

Aside from its physiological cytoplasmic articulation in keratinocytes, modifications in involucrin articulation likewise reflect an absence of typical cell coordination in preterminal separation in hyperplastic, amiable, and threatening expansions of squamous epithelium/epidermis [3].

Nutrient D inadequacy is implicated in a not insignificant rundown of cutaneous issue including psoriasis, ichthyosis, immune system skin issue, atopic dermatitis, skin break out, male pattern baldness, and diseases as tuberculosis [4].

Intralesional nutrient D3 infusion might be another treatment alternative for moles that are inert to ordinary medicines. It is a straightforward, very much endured treatment technique that is anything but difficult to manage in outpatient facilities. Along these lines, Intralesional immunotherapy has picked up significance because of its viability in clearing both rewarded and far off moles by invigorating the phone interceded invulnerability against human papillomavirus [5].

Nutrient D is a fat-soluble steroid prohormone that has endocrine, paracrine, and intracrine capacities. Nutrient D has a wide scope of organic activities (e.g., restraint of cell multiplication, acceptance of terminal separation, and hindrance of angiogenesis). It likewise invigorates macrophages and cathelicidin creation [6].

Its belongings are interceded by means of the nutrient D receptor (VDR), which is available in keratinocytes, melanocytes, fibroblasts, and invulnerable framework cells of the skin, That restrain the outflow of interleukin-6 (IL-6), IL-8, tumor corruption factor (TNF)- α and TNF-γ intervened through VDR-subordinate pathway [7]. What's more, Toll-like receptor initiation of human macrophages upregulates the statement of (VDR)which is available in keratinocytes, melanocytes, fibroblasts, and invulnerable framework cells of the skin and nutrient D1-hydroxylase qualities, prompting articulation and emission of antimicrobial peptides [8]

The point of this investigation was to assess the viability of intralesional infusion of nutrient D3 in the treatment of verruca vulgaris and was to identify the immunohistochemical articulation of Involucrin marker in verrucae when intralesional infusion with nutrient D3.

2. Patients and methods

This Quasi interventional study, included twenty subjects of both genders experiencing verruca vulgaris selected from the Outpatient Clinic of Dermatology, Venereology and Andrology Department of Benha University Hospital in the period between January 2018 to January 2019. Furthermore, ten obviously sound people wanted Plastic Surgery division, Benha University were incorporated as a benchmark group.

A composed educated assent was gotten from all members. This examination was endorsed by the neighborhood Ethics Committee on Research including human subjects of Benha Faculty of Medicine.

Inclusion criteria

- Patients who were suffering from multiple verruca vulgaris (common warts) more than two lesions.
- Above the age of 18 years.
- No topical or systemic treatments for warts had been applied 4 weeks before injection of vitamin D3.
- The size of the wart was sufficient for the injection and for taking the biopsy.

Exclusion criteria

- Patients with a past history of allergic response to vitamin D.
- Patients with a past history of allergic skin disorder.
- Patients with bleeding disorders.
- Pregnant and lactating females.
- Patients with vascular diseases.
- Hepatic and renal patients.

All patients in this investigation were exposed to full history taking, total clinical assessment, Four mm Punch biopsy was taken when treatment with intralesional infusion of nutrient D3 under neighborhood sedation of depacaine.

Nutrient D3: 0.6 ml of Devarol-S of Memphis ® (200,000 IU, 5 mg/ml) was infused to the base of every mole utilizing a 31-measure syringe. The infusions were rehashed fourteen days separated for a limit of 4 meetings or until leeway of the injury was accomplished. A limit of 2 moles have been dealt with per meeting and patients were followed up for a half year after the last infusion concurring to [9].

The degrees of clinical reaction to treatment were isolated into ("no reaction," "incomplete reaction," and "complete response"). Of note, "no reaction" was characterized as definitely no improvement with infusions, "fractional reaction" as a recognizable improvement however not full freedom, and "complete reaction" as absolute leeway of mole [10].

Another biopsy was taken from clearly solid individual as control bunch under neighborhood sedation. The biopsies had been sent to Pathology division, Faculty of Medicine, Menoufia University fixed in impartial formalin 10%, and submitted to routine tissue handling finishing with paraffin inserted squares arrangement. Two 5 micron cut segments have been finished. One segment was recolored with hematoxylin and eosin for assessment of histopathological changes. Other area was cut on poly L lysine covered slides for immunohistochemicalrecoloring utilizing monoclonal neutralizer raised against involucrin.

Haematoxylin and eosin recolored areas were inspected by light magnifying lens to: Confirm the clinical analysis of moles which included: acanthosis, papillomatosis, hyperkeratosis, parakeratosis, stretched rete edges and nearness of koliocytopathic changes which are portrayed by huge keratinocytes with a capricious, pyknotic core encompassed by a perinuclear corona. Assessment of histopathological changes after infusion of nutrient D3 in moles as epidermal thickness and thickness of incendiary cells.

The technique usesd for immunostaining was a streptavidin-biotin-intensified framework. The counter acting agent utilized was mouse monoclonal neutralizer .every vial 200ul contain 200mg/ml partiality sanitized antibodies ,0.01 m pbs,30% glycerol. Involucrin is accessible at(Chongqing biospes, Chongqing,china) .the weakening was 1:5 as indicated by provided information sheet. Slides were exposed to deparaffinization and rehydration. Antigen recovery was performed by bubbling in citrate support saline (pH 6), trailed by cooling at room temperature. Endogenous perioxidase was obstructed by brooding with H2O2, 3%. The essential neutralizer was hatched for the time being at room temperature, and afterward the auxiliary immune response (prepared to-utilize) was applied with DAB as a chromogenic substrate and Mayer's hematoxylin as a counter stain. Typical skin was utilized as a positive control for involucrin.

Interpretation of involucrin expression

Positive expression was assigned when cytoplasmic or membranous expression was seen in any numbers of cells. Staining pattern of distribution: diffuse (when stained all epidermal layers) or focal (when stained some but not all layers of epidermis).**Intensity of expression was assessed subjectively according to depth of immunostaining into:** Mild (+), Moderate (++), Strong (+++)**.**

2.1 Statistical methods

The gathered information were introduced in reasonable tables and showed in an appropriate structure. Quantitative information were summed up as mean and standard deviation, while subjective information were summed up as recurrence and rate. Examinations between the diverse investigation bunches were completed utilizing the Chi-square test (χ 2) and Fisher's Exact Test (FET) to think about extents as fitting. The autonomous t-test (t) was utilized to identify mean contrast between two gatherings with respect to parametric information and the Mann-Whitney test (z) was utilized to think about two non-parametric information. Articulation of involucrin when treatment was thought about utilizing Stuart-Maxwell test. Correlations between various degrees of articulation and various degrees of reaction were finished utilizing Kruskal Wallis test for numerical information and Fisher's careful test for absolute information. The relating P-values were acquired. A P-esteem < 0.05 was considered factually huge, while a P-esteem > 0.05 was considered statically non-noteworthy. The factual

investigation was led utilizing Statistical Package for Social Sciences (SPSS), form 25 (IBM, Armonk, New York, United states)**.**

This study include twenty patients with multiple warts and ten apparent healthy individuals as control group. There were no significant difference between both groups as regard gender and age (*P*=0.602 and 0.914 respectively) Table (1).

3. Results

Table (1) Comparison between patient and control group regarding sociodemographic characteristics.

Variable		Patients group (no.=20)		Control group (no.=10)		Test	P
		No.	%	No.	%		
Gender	**Female**	12	60.0	5	50.0	X^2=0.271	0.602
	Male	8	40.0	5	50.0		
		Mean ±SD	**Range**	**Mean ±SD**	**Range**		
Age (years)		28±8	19-45	28±7	19-40	Z= -.0132	0.914

Regarding clinical response in the 1st injected wart, 35% of warts showed partial response, 40.0% showed complete response while only 25.0% showed no response. In the 2nd injected wart 55% of warts showed partial response, 20.0% showed complete response while only 25.0% showed no response. In non-injected warts 66.7% showed partial response and 33% showed no response Fig (1).

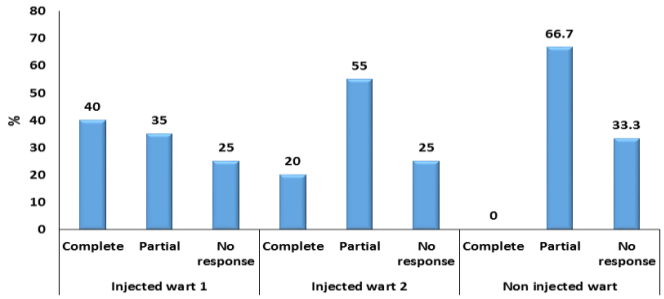

Fig (1) Clinical response to vitamin D_3 in injected and non-injected warts

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There was a significant association between response grades in the 1st injected wart and smoking (*P*= 0.01). Sixty percent of those with no response were smokers, 71.4% of those with partial response were smokers compared to absent of smoking in those with complete response**.**

Also in 1st injected wart, the mean number of sessions was significantly higher in those with partial and no response (4.0) compared to those with complete response (2) (*P* < 0.001) Table (2).

Table (2) Comparison between degrees of clinical response in the 1st injected wart and different clinical variables.

Variable		Clinical response			Test	P value
		Complete (n = 8)	Partial (n = 7)	No response (n = 5)		
Age (years)	Mean ±SD	25 ±5	31	28	KW = 1.995	0.376
Gender	Male n (%)	4 (50.0)	5 (71.4)	3 (60.0)	FET= 0.836	0.844
	Female n (%)	4 (50.0)	2 (28.6)	2 (40.0)		
Smoking	Yes n (%)	0 (0.0)	5 (71.4)	3 (60.0)	FET = 9.333	0.01×
Duration (months)	Mean ±SD	6 ±2	7 ±6	10	Z =3.567	0.168
Number of sessions	Mean ±SD	2 ±0.71 a	4 ±0 b	4 ±0 b	Z =17.709	<0.001×
Site	Hand	6 (75.0)	2 (28.6)	3 (60.0)	FET = 3.180	0.274
	Leg	0 (0.0)	3 (42.8)	1 (20.0)	FET = 2.574	0.312
	Foot	2 (25.0)	2 (28.6)	1 (20.0)	FET = 0.359	1.0

KW: Kruskal Wallis test. FET: Fisher's exact test.

Table (3) Involucrin expression in both groups before and after vit d3 injection

	patient group		Control group		Statistical test	P value
	No (20)	%	No (10)	%		
Involucrin before						
Mild	9	45.0	0	00.0	FET=10.49	0.005××
Moderate	8	40.0	3	30.0		
Strong	3	15.0	7	70.0		
Involucrin after						
Mild	5	25.0	0	00.0	FET=3.04	0.22
Moderate	6	30.0	3	30.0		
Strong	9	45.0	7	70.0		

Chi-square test was used

There was a significant difference between patient and control groups regarding to involucrin expression Table (3) and Fig (2).

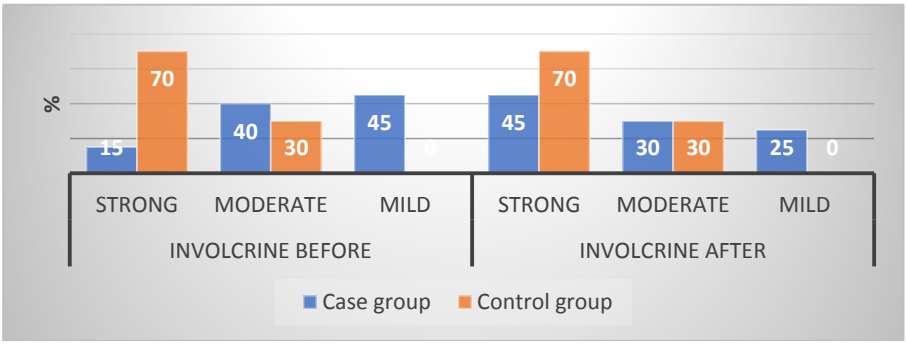

Fig (2) Involucrin expression in both groups before and after vit.D_3 injection.

There was a significant increase in involucrin degree of expression after injection of vitamin D3 .Before injection, 45.0% of patients showed mild expression, 40.0% showed moderate expression while 15.0% showed strong expression. After injection, 30.0% of patients showed moderate expression and 45.0% showed strong expression Table (4).

Table (4) Expression of involucrin before and after injection of vitamin D_3 in patients group.

	Before		After		P value
Involucrin degree					
Mild	9	45.0	5	25.0	0.11
Moderate	8	40.0	6	30.0	
Strong	3	15.0	9	45.0	
Injected warts					
Complete	7	35.0	1	5.0	0.047
Partial	8	40.0	14	70.0	
No	5	25.0	5	25.0	

4. Discussion

The investigation included 20 patients with various verruca vulgaris and 10 obvious solid people as control gathering. Patients looked for treatment as a result of repeat after various medicines, dread from spread and its appearance.

As in regards to the reaction in infused moles, the outcomes with nutrient D3 were 40% finished reaction, 35% fractional reaction, 25% no reaction in the first infused mole and 20%complete reaction, 55%partial reponse and 25% no reaction in the second infused mole. These outcomes were coordinated with Abou-Taleb et al.,2019 study ,who proclaimed 43.5%of cases demonstrated total leeway, 43,5% indicated halfway reaction and 13% indicated no response[12].

Be that as it may, these consequences of nutrient D3 didn't go with Raghukumar et al. [5] study which revealed 90% complete leeway, 6.66% incomplete reaction and no reaction in 3.33%. This can be clarified by the higher number of patients included , higher number of plantar warts(48%), higher portion of intralesional nutrient D3

injected(up to 0.5ml per sore), more moles infused per meeting (greatest 5 moles), shorter span periods between session(3 weeks) and more meetings (4 meetings) ,higher convergence of nutrient D3(600,000 IU,15mg/ml)

Concerning mole reaction, this outcomes with nutrient D3 was 66.7 %. This was unique in relation to what was accounted for by Aktaş et al., [8] who accomplished 80% removed mole reaction, and this might be clarified by the consideration of plantar moles just in that review. Another conceivable clarification is the higher level of female patients in Aktaş et al. [8] study (65% versus 40% in our examination). Females are known to have unrivaled both intrinsic and humoral resistant reactions which attach pathogen freedom [10].

These consequences of nutrient D3 could be disclosed by its capability to direct epidermal cell expansion and separation and balance cytokine creation. Also, Toll-like receptor initiation of human macrophages upregulates the declaration of VDR and nutrient D1 - hydroxylase qualities, prompting articulation and emission of antimicrobial peptides. In this way, intralesional nutrient D was endeavored to be utilized as an invulnerable energizer in the event of hard-headed moles [8].

The perception of freedom of untreated moles in the current examination demonstrates the advancement of a far reaching cell interceded safe reaction against HPV. It has been demonstrated that fruitful intralesional antigen immunotherapy is related which further enact cytotoxic T cells and normal executioner cells to annihilate HPV-contaminated cells [11].

As to reactions, (90%) percent of this examination announced torment at time of infusion which was coordinated with Raghukumar et al., [5] and El-Taweel et al ., [13]. What's more, (10%) percent of the patients experienced vasovagal assault as abrupt beginning of hypotension and troublesome breathing that going on for a couple of minutes. This might be because of dread as mental factor is the primary driver of vasovagal assault.

Another significant finding was the less fix rate in smoker patients in nutrient D3 rewarded gathering. This might be disclosed by that presentation to tobacco smoke has been accounted for to repress the proliferative reactions of T lymphocytes. Smoke-incited impedance of cell-intervened resistance was described by diminished creation of IFN-g as a result of diminished actuation of a few interpretation factors which act in show to upregulate the creation of this cytokine by T cells [14. Notwithstanding that, It was discovered that cigarette smoking meddles with authoritative of nutrient D to its receptors thus hinder its activity by repressing VDR translocation from the core to the cytoplasm [15]. Likewise, smoking was found to weaken nutrient D work. The activities of the nutrient D hormone 1,25-dihydroxy nutrient D3 (1,25(OH)2D3) are intervened by the atomic nutrient D receptor (VDR), which when enacted influences interpretation of at any rate 913 qualities and effects forms running from calcium digestion to articulation of key antimicrobial peptides [16].

Concerning quantities of meetings in this investigation , it was watched highier number of meetings in those with fractional and no reaction (4.0) contrasted and complete reaction (2) in both infused moles. That didn't go with kavya et al., [9] who found that greater treatment meetings would accomplish better reaction. Different examinations show more meetings until complete freedom with highier number of patients, highier portion of intralesional nutrient D3 infusion, highier centralization of nutrient D3, highier number of plantar mole not verruca vulgaris as this investigation and highier number of infused mole per meeting that expansion the chance of reaction in next meetings.

In this investigation involucrin articulation was essentially highier in cases contrasted with control which was coordinated with Barcelos et al., [17] who indicated that plane mole (PW) showed early involucrin articulation at more profound epidermal layers.

In ordinary epidermis involucrin articulation is seen in the upper spinous layer. In the current examination, involucrin articulation was seen in basal and spinous layer which coordinated with Callegaro and Sotto., [18] who watched involucrin articulation in MB-bearing cells and in spinous and basal layers of MC sores. Additionally this didn't go with Chen et al., [19] who demonstrated involucrin articulation in psoriatic skin was in all spinous keratinocytes yet not the basal nor the cornified cells were recolored with involucrin. These perceptions show the gifted articulation of involucrin(an epidermal terminal marker) which proposes a previous terminal separation .

Interestingly Barcelos et al., [17] which show Involucrin articulation happened from the low epidermal spinous layer to the granular layer in 42.85% and central basal articulation in 33.33% in plane mole (PW) Here, There was a critical increment in involucrin level of articulation after infusion of nutrient D3 .As Before infusion, 45.0% of patients indicated gentle articulation, 40.0% demonstrated moderate articulation while 15.0% demonstrated solid articulation. After infusion, 30.0% of patients demonstrated moderate articulation and 45.0% indicated solid articulation.

That was coordinated with Chen et al., [19] who appeared over-communicated involucrin in psoriatic skin which charachterized by quickened epidermal turnover.

5. Conclusion

There are histopathological changes after injection of vitamin D3 in verruca vulgaris including marked decreasing in epidermal thickness and decreasing of inflammatory cells in the dermis. In verruca vulgaris, Involucrin expression is higher after injection of vitamin D3.

References

[1] S.k.Loo, W.Y.Tang,Warts (non-genital) .BMJ Clin.Evid, Vol.17, PP.10,2014.

[2]E. Gyöngyösi, A. Szalmás, A .Ferenczi , Effects of human papillomavirus (HPV) type 16 oncoproteins on the expression of involucrin in human keratinocytes, Virol. J,Vol . 9, PP.36,2014.

[3] E.R.Li, D.M.Owens, P.Djian,.Expression of involucrin in normal, hyperproliferative and

neopla,Vol.9(6), PP.431–438.

[4] W.Z .Mostafa ,R.A.Hegazy, "Vitamin D and the skin Focus on a complex relationship: A review. J. Adv. Res,Vol. 6(6), PP.793–804,2015.

[5] S. Raghukumar, B.C.Ravikumar, K.N.Vinay, Intralesional vitamin D3 injection in the treatment of recalcitrant warts: a novel proposition . J. Cutan. Med. Surg, Vol. 21(4), PP.320–324, 2014.

[6] G.Fathy, M.A. Sharara, A.H.Khafagy, Intralesional vitamin D3 versus Candida antigen immunotherapy in the treatment of multiple recalcitrant plantar warts: A comparative case–control study . Dermatol. Ther, Vol.32(5), PP. e12997 ,2019.

[7] k. Al-Ghamdi, A.Kumar, N.MoussaThe role of vitamin D in melanogenesis with an emphasis on vitiligo .Indian J. Dermatology, Venereol. Leprol, Vol.79(6), PP.750, 2013.

[8] H.Aktaş, C. Ergin, B.Demir, Intralesional vitamin D injection may be an effective treatment option for warts. J. Cutan. Med. Surg, Vol.20(2), PP. 118–122,2016.

[9] M.Kavya ,B.M.Shashikumar ,M.R Harish,Safety and efficacy of intralesional vitamin D3 in cutaneous warts: An open uncontrolled trial. J. Cutan. Aesthet. Surg., Vol .10(2), PP. 90-94, 2016.

[10] A. Alikhan, J.R. Griffin,C.C.Newman,Use of Candida antigen injections for the treatment of verruca vulgaris: A two-year mayo clinic experience. J. Dermatolog. Treat, Vol.27(4), PP.355–358, 2016.

[11] A. Nofal, E .Salah, E. Nofal, Intralesional antigen immunotherapy for the treatment of warts: current concepts and future prospects. Am. J. Clin. Dermatol, Vol.14(4), PP.253–260, 2013.

[12] D.A.E.Abou-Taleb , H.A.Abou-Taleb , O.El-Badawy, ,Intralesional vitamin D3 versus intralesional purified protein derivative in treatment of multiple warts: A comparative clinical and immunological study. Dermatol. Ther,Vol.32(5), PP.e13034, 2019.

[13] A.E.El-Taweel , R.M. Salem, A.H. Allam ,Cigarette smoking reduces the efficacy of intralesional vitamin D in the treatment of warts. DermatolTher, Vol. 32(2), PP.e12816 , 2019.

[14] R.Anderson,Cigarette smoking and mechanisms of susceptibility to infections of the respiratory tract and other organ systems. J. Infect, Vol.67(3), PP.169–184,2013.

[15] S.T.Uh, S.M.koo , Y.K.Kim Inhibition of vitamin d receptor translocation by cigarette smoking extracts. Tuberc. Respir. Dis, Vol.73(5),PP.258–265, 2014.

[16] J.W.Pike,M.B.Meyer,The vitamin D receptor: new paradigms for the regulation of gene expression by 1, 25-dihydroxyvitamin D3 .Rheum. Dis. Clin, Vol.38(1),PP.13–27,2012.

[17] A.C.Barcelos ,M.N.Sotto,Comparative analysis of the expression of cytokeratins (1, 10, 14, 16, 4), involucrin, filaggrin and e- cadherin in plane warts and epidermodysplasia verruciformis plane wart- type lesions. J. Cutan. Pathol, Vol.36(6), PP.647–654,2009.

[18] C.F.Callegaro,M.N.Sotto,Molluscum contagiosum: immunomorphological aspects of keratinocytes markers of differentiation and adhesion. J. Cutan. Pathol, Vol. 36(12), PP.1279–1285,2009.

[19] J.Q.Chen , X.Y.Man , W. li,Regulation of involucrin in psoriatic epidermal keratinocytes: the roles of ERK1/2 and GSK-3β .Cell Biochem. Biophys, Vol.66(3), PP.523–528, 2013.