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# EXPRESSION OF STEM CELL MARKER, NESTIN, IN THE BASAL EPITHELIAL LAYER OF SKIN EPIDERMIS BY MEANS OF IMMUNOHISTOCHEMICAL TECHNIQUE.

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**Abstract:**

*The epidermis builds up from a lonely layer of ancestor cells. The basal layer cells directly made touch with the basement membrane, which involves reproducing cells. The keratinocyte cells of the basal layer gaining a system of intermediate filaments. In the epidermis of skin, stem cells characterised from differentiated keratinocytes according to their levitating expression of nestin. The present study investigated the expression of nestin, a recognized indicator of epidermal stem cells in ordinary human skin. Skin keratinocytes have confirmed useful, because of their fitting and ability to be cultured. The make use of cultured keratinocytes licenese a much greater surface to be covered and needs a tiny area of unaltered skin from which the skin cells were harvested for culture.*

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## INTRODUCTION:

The skin is the most important line of defence to guard the body from dryness, injury, and contamination. On the way to convene these needs, the skin have evolved complicated differentiation procedure that results in a rough, water-impermeable external covering that is persistently renewable. Human skin consists of dermal and epidermal parts. The epithelium of epidermis is a stratified squamous, based on a basement membrane [1].

The epidermis builds up from a solitary layer of progenitor cells (ectodermal in origin) throughout a firmly regulated successions of events taking place during embryonic maturity [2-4]. So, The epidermis is consider as a stratified squamous epithelium with absolute barrier meaning [5]. This complete process needs a particular balance among differentiation and proliferation to guarantee the incurably differentiated cells that are sloughed off are substituted by offspring of stem cells, which segregate, differentiate and travel outer towards the exterior of the skin [6].

The cells directly make contact with the basement membrane, called basal layer, which includes proliferating cells. The keratinocyte cells of the basal layer acquire a system of intermediate filaments, although they are fairly undifferentiated. Since the group of basal cells extends because of division, a few cells separate from the basement membrane and start to move toward the surface of the skin. There is no apparent morphologically discrete region of the basal layer where progenitor cells might be situated. It has been acknowledged from the 1970s that the epidermis is structured into columns of growing cells [7].

It was originally hypothesized that the whole basal layer consisted of progenitor cells, then afterwards that the Langerhans cells were progenitor cells. Many studies proposed that progenitor (stem cells) might cover 3–7% of cells in basal layer [7].

As the description of every stem cell population stays a difficult mission, a new approach to reach this aim consists in developing new markers discriminating differentiated cells from progenitors. Attractive results have been obtained in numerous tissues: neural, hepatic and most of hematopoietic tissues, by means of a lot of monoclonal antibodies [10-14].

In the epidermis of skin, stem cells possibly will be discriminated from differentiated keratinocytes according to their elevated expression of nestin [15-17].

Nestin is the sixth class of an intermediate filament expressed in the plasma membrane and cytoplasm of neural stem cells (12, 18]. Its expression has in addition been found in melanomas. [19].

Nestin is expressed in the hair follicle of the mice. Nestin-expressing cells be capable of differentiate in vitro into keratinocytes, melanocytes ,smooth muscle cells ,neurones and glial cells. Nestin-expressing cells are positive for a comparatively undifferentiated stem cell, signifying that they are pluripotent cells [20-25].

In the present study, we essayed the opportunity that nestin could be a indicator of skin stem cells, and titled a numeral of significant issues concerning this cell denizens.

## PATIENTS AND METHODS:

**Subjects and skin specimens:**

A totality of 100 patients with epidermal cyst were taken on and informed permission was obtained from the Department of Dermatology, Baghdad Hospital, Baghdad; Iraq from June 2015 to January 2016. For the entire patients. Sex, age residence and occupation were recorded. The group consisted of 68males and 32 females, with an age range of 10–39 years as shown in table(1). Patients didn't treated with any systemic drugs and didn't apply any topical drugs to the area of biopsy for more than a few weeks. Skin specimens was obtained throughout surgical excision of an epidermal cyst from the extremities, which were fixed in 10% formalin and embedded in paraffin. Immunohistochemical staining were performed on successive sections of the skin samples.

**Immunohistochemical procedure:**

Samples were immersed throughout the night in a recently prepared fixative containing 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.3). After that, samples were dehydrated in graded successions of ethanol to xylene and embedded in paraffin wax. Immunohistochemical staining was carried out by a peroxidase-labeled streptavidin -biotin technique. In brief, paraffin sections (4 mm) were slice and placed on positive charged -glass slides. Subsequently, sections stained for nestin were laded in autoclave in 10 mM citrate buffer (pH 6.0) for 5 min.

Sections were treated with 3% H2O2 in methanol for 15 min to satisfy activity of endogenous peroxidase. Following washing in PBS, incubation of sections were finished during the night at 50 C with a 1:500 dilution of anti-nestin antibody [abcam, USA.

**Table 1: Samples according to age groups.**

| Age group | Male | | Female | | Total No. |
|---|---|---|---|---|---|
| | No | % | No | % | |
| G1 **(10-19)** | **22** | 32.4 | **11** | 34.5 | **33** |
| G2 **(20-29)** | **24** | 35.2 | **9** | 28 | **33** |
| G3 **(30-39)** | **22** | 32.4 | **12** | 37.5 | **34** |
| Total N0. | **68** | 100% | **32** | 100% | **100** |

**Scoring procedure:**

The positive cell indicates the cell which includes nestin protein and appears brown in color due to treatment with DAB solution. but, the negative cell means it doesn't have nestin protein and seems blue in color due to counterstaining with hematoxillin solution. The nestin protein is confined in the plasma membrane and cytoplasm, therefore the cytoplasm looks brown and nucleus looks blue (Pai SA& Patil PU, 2008)(27).

Nestin expression was estimated under light microscopy at X100, X200, X400 and X600.Immunohistochemical technique was specified percentage scores, based on the quantity of signals. Positive cells were counted in ten dissimilar fields of 100 cells for every sample and the average of positive cells of the ten fields was determined conveying cases to one of the three subsequent score categories: Score(1)=1-25% , Score(2)=26-50%, Score(3)>50% (28).

**RESULTS:**

This study investigated the expression of nestin, a recognized marker of epidermal stem cells in ordinary human skin. In Skin specimens, immunostaining showed that the basal layer was positive for nestin as seen in figures (2,3 ,4&5). These results propose that nestin is expressed in dynamically proliferating keratinocytes in the basal layer of epidermis. Nestin was positive in the epidermis of the entire samples of skin .The immunohistochemical stain verified cytoplasmic staining. No considerable discrepancies in the potency of nestin expression according to sex, age as shown in figure (1) and table (2&3). Accordingly, these results show that nestin expressing cells in the skin are present in keratinocyte
located in the basal layer of epithelium.

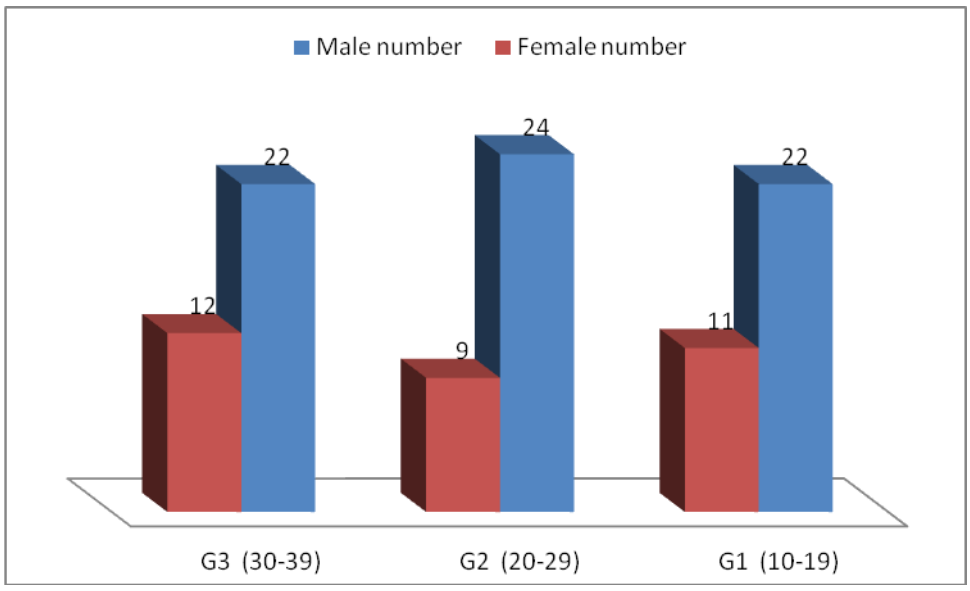


**Fig 1: Samples according to age groups.**

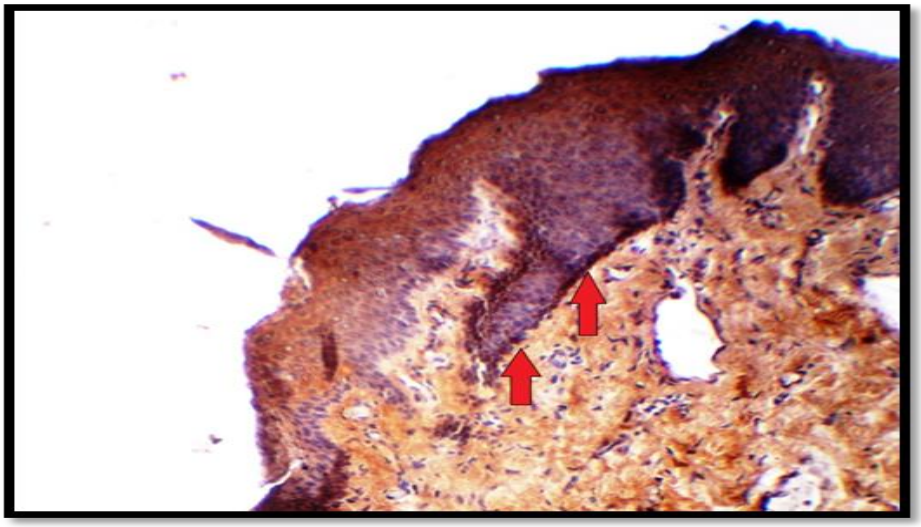

**Fig 2: Nestin expression in basal epithelial layer of skin epidermis X100.**

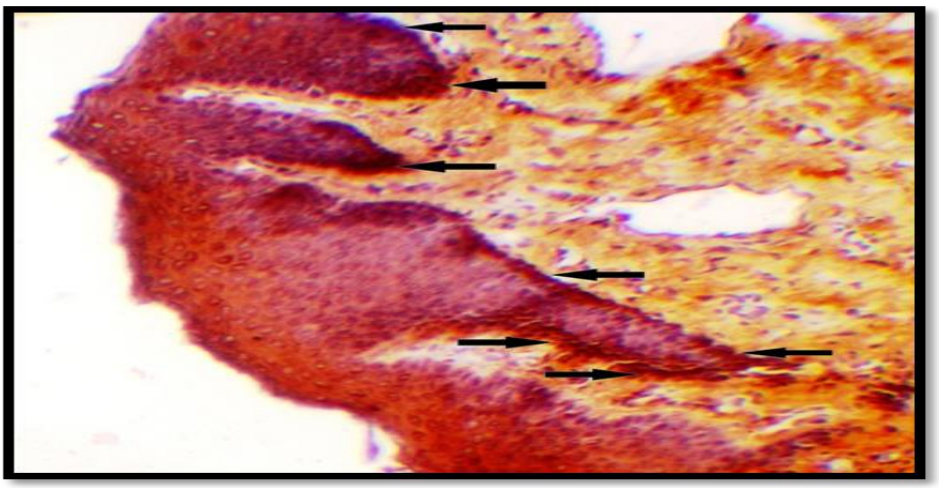

**Fig 3: Nestin expression in basal epithelial layer of skin epidermis X200.**

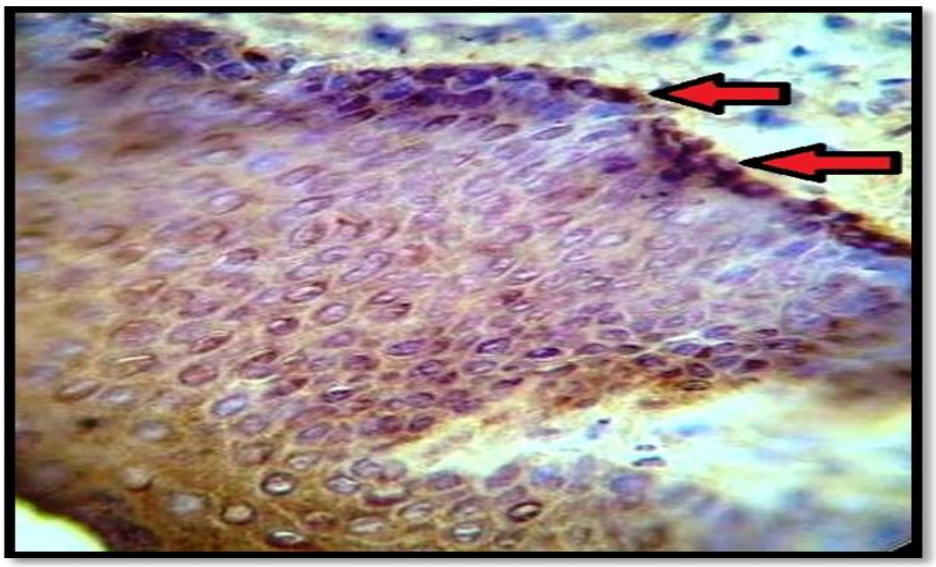

**Fig 4: Nestin expression in basal epithelial layer of skin epidermis X400.**

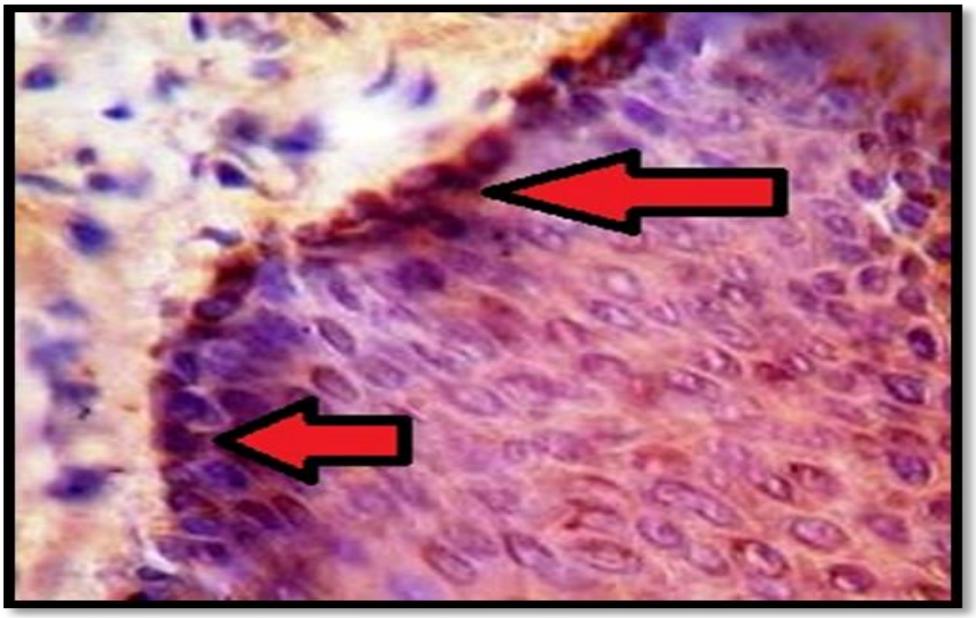

**Figure (5): Nestin expression in basal epithelial layer of skin epidermis X600.**

**Table 2: Stem cell score according to gender.**

| Stem Cell Score | Group | | Total number |
|---|---|---|---|
| | **Male** | **Female** | |
| <25 | 23 | 11 | 34 |
| 25-50 | 21 | 12 | 33 |
| >50 | 25 | 9 | 34 |
| Total number | 68 | 32 | 100 |

**Table3:Stem cell score according to age group.**

| Stem Cell Score | Age group | | |
|---|---|---|---|
| | **10-19** | **20-29** | **30-39** |
| <25 | 11 | 9 | 8 |
| 25-50 | 12 | 14 | 17 |
| >50 | 10 | 10 | 9 |
| Total number | 33 | 33 | 34 |

## DISCUSSION:

The turnover period of the epidermis is (311 hour) in ordinary skin. Cell division take places in the stem cells in the basal layer of epidermis. Stem cells are characterized by a sluggish growth rate, a extended cell cycle and the capacity to differentiate into cells of different families [29-31 ] .

The stem cells proliferation are firmly regulated to preserve homeostasis in the epidermis; therefore, it is vital to understand the circumstances and mechanisms that organize their proliferation and differentiation [32-34 ] .

Even though, several markers for epidermal stem cells have been projected[36 ] , the marker side view of the epidermal stem cell remains to be completely defined. Our results reported that nestin-expressing basal epithelial stem cells are fairly undifferentiated and pluripotent [20-23 ] .

The study, showed that skin nestin -expressing cells are situated in the basal layers of epidermis. Even if co-expression of nestin has been accounted in the hippocampal neurogenic cells of post-ischaemic tissue of monkeys [37 ] , the
co expression of nestin in the epidermal cells of human have not been accounted. It is fascinating to note that nestin expression was observed in proliferating human epidermal cells. Our results suggest that nestin- expressing cells (keratinocytes) may possibly have an essential role in the pathogenesis of skin diseases such as psoriases, eczema and other chronic proliferating skin diseases.
Labelling technique used to exemplify the nestin expressing cell residents, provided confirmation that the existence of basal stem cells in skin epithelial layer was expected from early inspections. Certainly, in partial thickness injuries, the regeneration of epidermis consequences from the migration of basal stem cells [38-40] .

Though, in the majority of studies, the recognition of skin stem cells is a hard task and rests on indirect proofs. The most consistent feature of stem cells is their slow-cycling character[7] .

Nestin, first recognized in the cytoplasm of neural stem cells in addition to migrating and proliferating cells through embryogenesis and in different adult tissues go through regeneration, for example the central nervous system, liver, pancreas, and digestive tract .

Nestin expression has been establish in glioblastoma multiforme, primordial ectodermal tumors of the CNS and melanoma [19 ]. Nestin expression appears to associate with the elevated proliferative and migrational action of these tumors.

## CONCLUSION:

This study was taken on to estimate nestin as a biochemical marker for stem cells of skin in order to address a number of long-lasting questions regarding these stem cells in the field of dermatology. In conclusion, skin nestin expression supplies an additional means to allow additional characterization of stem cells in skin under ordinary and pathological circumstances in situ and in vitro. Taken this facts strongly propose that nestin is a prospective biochemical marker for skin stem cells.

Fundamental research into biology of stem cell is partially oriented toward the ultimate opportunity of harvesting stem cells from a patient, modifying or growing them, and reimplanting them to take care of disease. Skin keratinocytes have proved helpful for this by now, because of their convenience and capability to be cultured. The make use of cultured keratinocytes permits a much greater surface to be covered and needs a minor area of unaltered skin from which to harvest the skin cells for culture.

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