IMMUNOHISTOCHEMICAL STUDY OF POST-BURN KELOID IN EGYPTIAN PATIENTS

THESIS

Submitted for the Partial Fulfillment

of the M.Sc. Degree in Pathology

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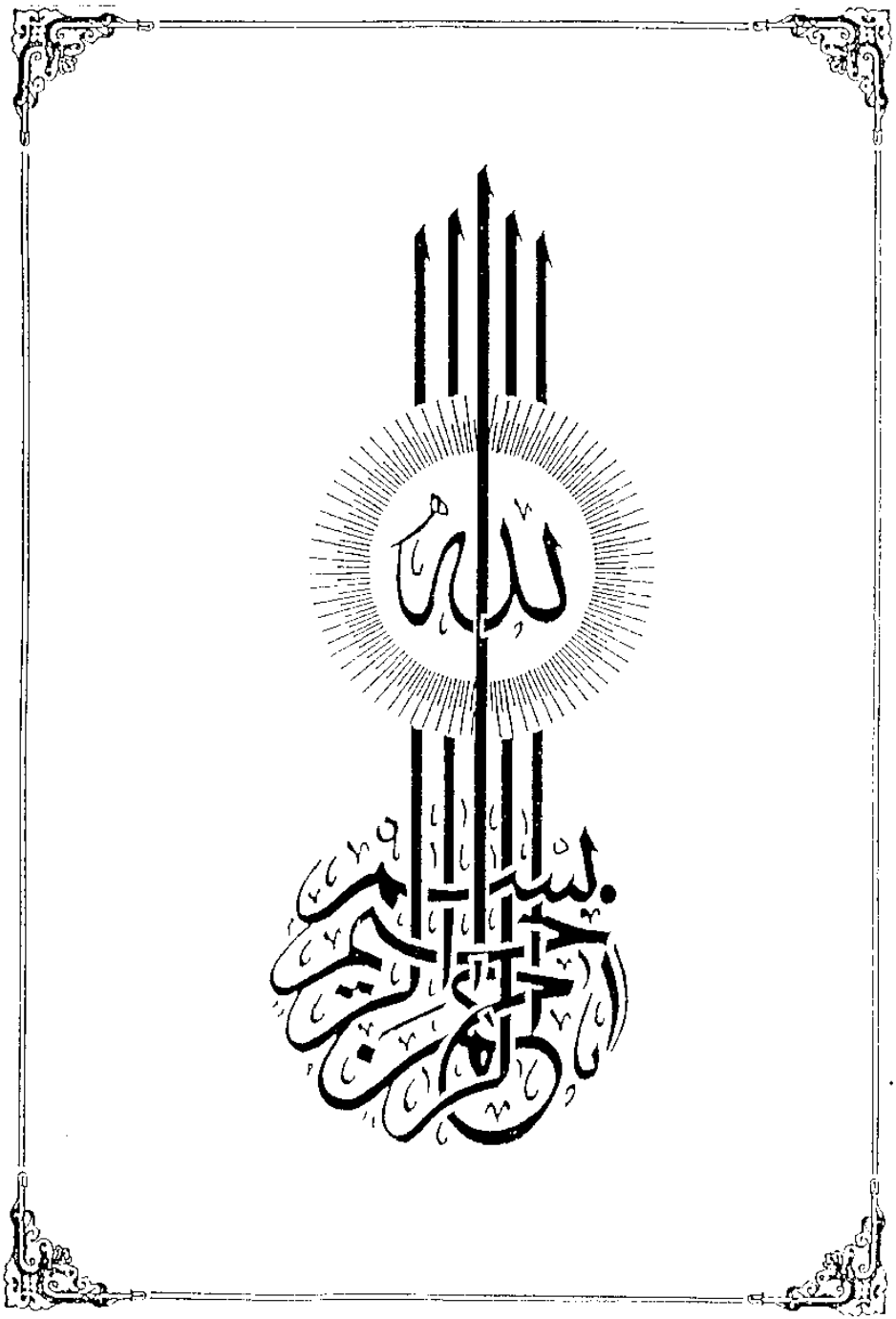
1996

بسم ا لله الرحمن الرحيم



ACKNOWLEDGMENT

I would like to express my deepest and cordial appreciation to ***Prof. Dr. Laila El Shabrawy***, Professor of Pathology , Ain-Shams University , for her constant support and encouragement and whose guidance has shown me my way in this work.

I wish also, to acknowledge my heartful and deepest gratitude to ***Dr. Wafaa Abdel Aal***, Asst. Professor of Pathology , NRC , for her faithful supervision , guidance and encouragement. She supplied me with a lot of her own experience. The credit of bringing this work to light goes to her.

I am greatly indebted to ***Dr. Tarek El Sharkawy***, Lecturer of Pathology , Ain-Shams University , who gave me a lot of his pointfull ideas, assistance and encouragement.

I am greatly indebted to ***Dr. Mona Mouris***, Asst. Professor of Pathology, NRC , who gave me a lot of her experience, and new ideas.

Much thanks and appreciation is directed to ***Dr. Ashraf Haider and his lab team*** in Shistosomiasis research (Grant No. 09- 02-77) who supplied me with all the equipments and ideas I used to accomplish this work.

TABLE OF CONTENTS

TABLE OF FIGURES AND CHARTS

INTRODUCTION

Chapter one

INTRODUCTION

Aberrations of growth may occur even in what may begin initially as normal wound healing. The accumulation of excessive amounts of collagen may give rise to a raised *tumorous scar* known as a keloid in which the mechanism of formation is still not known (Cotran et al., 1994).

Keloid is a common condition which occurs as a complication of wound healing after surgical operations or after burn and represents a difficult therapeutic problem.

Keloid formation appears to be an individual predisposition , and for reasons unknown this aberrations is somewhat more common in *blacks* (Kumar et al., 1992). The tendency to develop keloids appears to diminish with age. Any surgical incision in a known *'keloid former'* is more likely to develop into a keloid than a similar incision in a random patient and recurrence following simple excision of a keloid is highly probable.

Certain areas of the body have a particular tendency to produce keloids ; the presternal area is probably the most prone of all and here oddly enough the

shape of the keloid often shows a sex difference- in the male it tends to be irregular in outline , in the female the pull of the breasts commonly gives a butterfly outline. The deltoid area is another notorious site(McGregor , 1989).

Every young scar is a new growth of connective tissue and has some features of a neoplasm. However , just as atypical proliferation of epithelium in a healing wound or other tissue defect eventually subsides , so is the stage of proliferation of fibrocytes and blood vessels in a scar followed by a stage of maturation and return to relatively normal tissue homeostasis. Keloid tissue , on the other hand , remains immature for prolonged periods and has other peculiar characteristics (Mehregan , 1986).

AIM OF THE WORK:

In this study , we are going to evaluate the role of the tumor suppressor genes in keloid formation and recurrence , also the prolefrative activity of keloid will be investigated by using immunohistochemical techniques.

Patients and methods :

Biopsies from 20 patients with post-burn and post-operative keloid from outpatient clinic of Ain Shams and Kasr El Einy university hospitals from different age and sex group will be taken and subjected to immunohistochemical analysis.

The following monoclonal antibodies will be used to determine the expression of p53 protein and the proliferation marker PCNA (Proliferating Cell Nuclear Antigen).

1 - *Monoclonal antibody anti p53* , Dako USA.

2 - *Monoclonal antibody , anti PCNA* , Dako USA.

(Anti Proliferating Cell Nuclear Antigen)

Methods :

Histochemical techniques using H&E and special stains for the collagen (Van Gieson and Masson Trichrom as special stains for the keloid itself).

Immunohistochemical techniques using Peroxidase-antiperoxidase (ABC Universal detection kit).

ANATOMY AND EMBRYOLOGY OF SKIN

Chapter two

ANATOMY AND EMBRYOLOGY OF SKIN

The skin is a vitally important organ, has a complicated structure, and serves many functions (Montagna and Parakkal., 1974 ; Pinkus, 1976). 'Normal skin', however, is an abstraction. Topography and differences of age, sex, and genetic constitution introduce so many variations that few general statements can be made.

Embryology of the skin

1 - The epidermis

a - Keratinocytes :

- At 5 to 6 weeks old, the epidermis in a few areas still consists of only a single layer of ectodermal cells (Breathnach, 1971). However, in most areas , there are already two layers, the basal cell layer, or stratum germinativum, and above it, the periderm layer.
- When the fetus reaches the age of 10 weeks, another row of cells, the stratum intermedium, forms between the two layers through upward movement of cells of the basal cell layer.
- At 19 weeks, there are two to three layers of intermediate cells, and the cells of the periderm begin to flatten.

- At 23 weeks , keratinization has taken place in the upper cell layer of the stratum intermedium, and small keratohyaline granules are apparent in the subjacent cells. The cells of the periderm have largely been shed, leaving only fragments of degenerated periderm cells above the keratinized cells of the newly formed stratum corneum (Holbrook and Odland , 1975).

b - Melanocytes :

The appearance of melanocytes in the epidermis takes place in a craniocaudal direction, in accordance with the development of the neural crest, from which the melanocytes are derived. Melanocytes can be identified in the epidermis of the head region during the latter part of the third fetal month; in the more caudal body regions, the earliest formed melanin can be observed only in the latter part of the fourth month. Because melanocytes are functionally immature during their migration through the fetal dermis, they cannot be identified by histochemical methods until they have reached the epidermis (Becker and Zimmermann , 1955).

c - Langerhans cells :

Langerhan cells, which are bone-marrow-derived cells, begin to appear in the epidermis by 7 weeks of gestation. At this stage, they are fewer, smaller, and less dendritic than at a later fetal stage.

d - Merkel cells :

Merkel cells arise, between weeks 8 and 12 of gestational age, from precursor stages of epithelial cells of early fetal epidermis that still express simple epithelial cytokeratines. Some Merkel cells detach from the epidermis and

migrate temporarily into the upper dermis, where some of them associate with small nerves (Moll et al., 1986).

The epidermal appendages

The embryonal stratum germinativum, or basal cell layer, differentiates not only into basal cells, which give rise to keratinizing epidermis, but also into hair germs, also called primary epithelial germs, which give rise to the hair, sebaceous glands, and apocrine glands, and into eccrine gland germs , which give rise to the eccrine glands (Chart 1) (Lever et al., 1990).

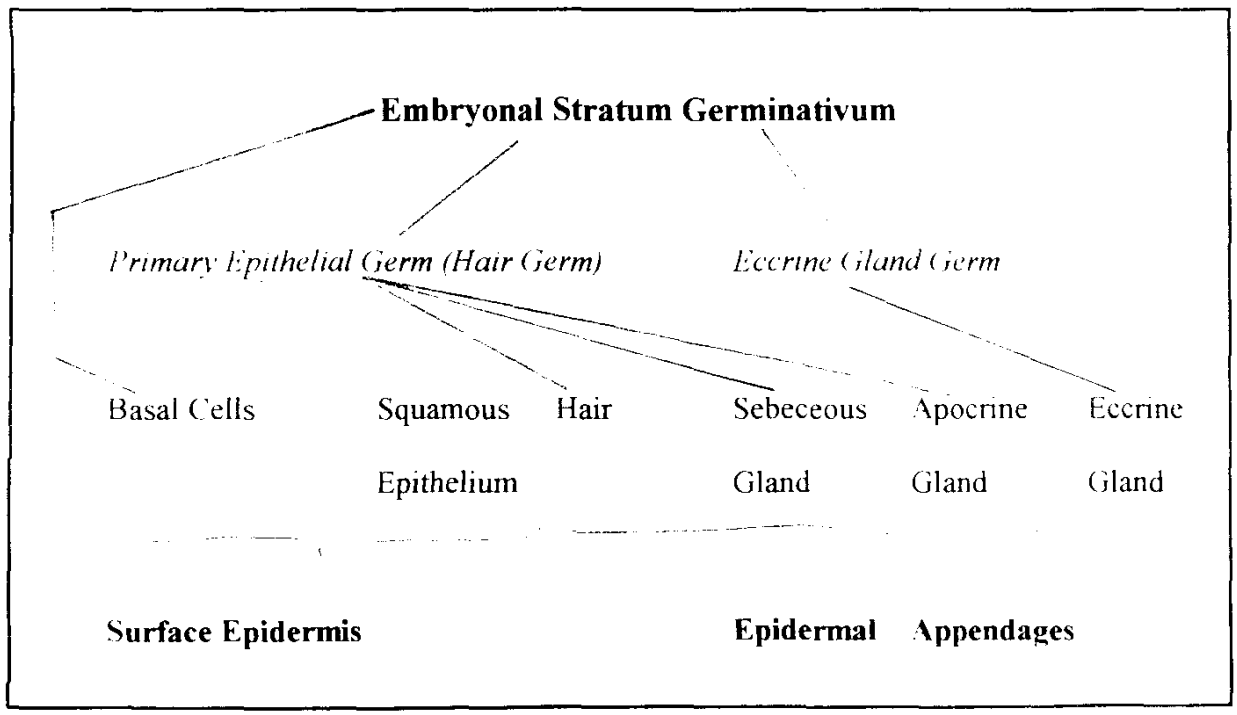


Chart 1. Embryology of the epidermis

a - Hair : The general development of hair begins in the fourth fetal month in the face and scalp and gradually extends in a cephalocaudal direction. Hair germs, or primary epithelial germs, in their earliest stage of development

consist of an area of crowding of deeply basophilic cells in the basal cell layer of the epidermis. Subsequently the areas of crowding develop into buds that protrude into the dermis. Beneath each bud lies a group of mesenchymal cells from which the dermal hair papilla is later formed. As the primary epithelial germ grows deeper into the dermis under induction by the underlying mesenchymal cells, it forms first the hair peg and then, as the hair matrix cells and the dermal hair papilla develop, the bulbous hair peg (Pinkus , 1958).

As The bulbous peg stage is reached, differentiation occurs in the lower and upper portions of the hair follicle and in the overlying epidermis. Differentiation in the lower portion of the follicle leads to the formation of the hair cone and subsequently to the formation of the hair, the cuticle, and the two inner root sheaths (Hashimoto , 1970).

b - Sebaceous glands : The development of sebaceous glands from the middle bulge of hair follicles begins on the scalp and face in the fourth month of fetal life.

c - Apocrine glands : Apocrine glands develop only in certain areas from the upper bulge of hair follicles that are in the early bulbous peg stage and show a hair cone. The formation of apocrine glands begins late in the fourth month and continues until late in embryonic life, as long as new hair follicles develop.

d - Eccrine glands : Eccrine glands are present in mammals only on the soles. Accordingly, the eccrine glands develop in humans earlier on the palms and soles than elsewhere. On the palms and soles, eccrine gland germs are first seen early in the fourth gestational month (Hashimoto et al., 1965). In the early part