Morphological and morphometric study of cultured fibroblast from treated and untreated abnormal scar

Soad S. Ali, *MSc, PhD,* ***Nahid H. Hajrah,*** *MSc, PhD,* ***Nasra N. Ayuob,*** *MSc, MD,* ***Sabah S. Moshref,*** *FRCSs,* ***Osama A. Abuzinadah,*** *MSc, PhD.*

ABSTRACT

الأهداف: دراسة شكل وتركيب الخلية الليفيه المزروعه من الندب في المختبر بعد أخذها من الجلد المُصاب ومقارنتها بتلك الخلايا المأخوذه من عينات الجلد السليمة.

الطريقة: أُجريت هذه الدراسة في مركز أبحاث الملك فهد فى جامعة الملك عبد العزيز، جده، المملكه العربية السعودية خلال الفترة من ديسمبر 2008م إلى مارس 2010م، حيث جُمعت 52 عينة مُصابة بالجدرة (keloid) والندب الضخامية (hypertrophic scar). زُرعت الخلايا الليفيه المأخوذة من المناطق السليمة وكذلك تلك المأخوذة من الندب المصابة في المختبر ومن ثم دُرست هذه الخلايا من ناحية الشكل والتركيب وتم عمل مقارنة فيما بينهما.

النتائج: لقد كان هناك زيادة واضحة في عرض وطول الخلايا الليفيه المأخوذة من الندب الضخامية وندب الجدرة ونقص كبير في المؤشر القطبي الثنائي للخلية الليفيه، هذا بالإضافة إلى زيادة كبيرة جداً في المساحة الكلية لكل من الخلية والنواة وزيادة عالية جداً في نسبة النواة إلى السيتوبلازم (N:C ratio) وذلك بالمقارنة مع خلايا المناطق السليمة. وباستخدام طرق العلاج المختلفة حدث نقص كبير في المساحة الكلية لكل من الخلية والنواة المأخوذة من الندب المُصابة وذلك بالمقارنة مع العينات التي لم يتم علاجها. أما بالنسبه للتغيرات التركيبيه للخلية الليفيه المصابه فقد تبين وجود كمية كبيره من السيتوبلازم والزوائد والفجوات السيتوبلازميه وبعض الحبيبات المتكتله حول النواه وكذلك زياده فى حجم النواه مما يؤكد أن هناك زياده فى نشاط الخلية الليفيه.

خاتمة: أثبتت الدراسة التي أُجريت على شكل وتركيب الخلايا الليفيه المأخوذة من المناطق المُصابة بأن هذه الخلايا نشيطة بشكل واضح وذلك بالمقارنة مع خلايا المناطق السليمة، وأن طرق العلاج المختلفة التي أجريت على هذه الخلايا لم تمنع هذا النشاط الزائد بل قللت منه.

Objectives: To investigate the morphology of cultured fibroblasts derived from abnormal scars and compare it to those of human normal skin.

Methods: This study was carried out in the Surgical Clinic, Faculty of Medicine, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia between December 2008 and March 2010. Fifty-two samples of hypertrophic and keloid scar were collected. An in vitro study was conducted in which fibroblasts from normal foreskin; abnormal scars were cultured, studied morphologically and morphometrically.

Results: There was a highly significant increase in the length and breadth of fibroblasts from the hypertrophic and keloid scars, and highly significant decrease in the bipolarity index compared to control. There was a significant increase in the mean cell area, mean nuclear area and nuclear/cytoplasmic ratio of fibroblast of hypertrophic and keloid scars compared to control. There was a significant decrease in the mean cell area and mean nuclear area of the fibroblast of the treated keloid scar (with all used modalities) compared to untreated ones. Morphologically, abnormal scar fibroblasts has abundant spreading cytoplasm with numerous processes and large nuclei. The cytoplasm, of some cells, contained clumped granules in the peri-nuclear region, numerous vacuoles, and dense vesicles.

Conclusion: Morphological and morphometric study showed that hyperactive cultured fibroblasts was a characteristic feature of abnormal scars and the studied modalities of treatment reduced, but not completely nullify this activity.

Saudi Med J 2010; Vol. 31 (8): 874-881

From the Department of Anatomy (Ali, Ayoub), Faculty of Medicine, Department of Biology (Hajran, Abuzinadah), Faculty of Science, and the Department of Plastic Surgery (Moshref), Faculty of Medicine, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia.

Received 16th May 2010. Accepted 5th July 2010.

Address correspondence and reprint request to: Dr. Nasra N. Ayuob, Department of Anatomy, Faculty of Medicine, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arbia. Tel. +966 530112205. Fax. +966 (2) 6401000. E-mail: nasra_ayuob@yahoo.com

Wound healing is an interactive process that involves soluble mediators, extracellular matrix components, resident cells, keratinocytes, fibroblasts, endothelial cells, leukocyte subtypes,[1,2] and other recruited immune cells, macrophage, mast cells, plasma cells, and so forth. All the previously mentioned cells were reported to be involved in wound healing.[3] They were found to participate differentially in the classically defined 3 phases of wound healing: inflammation, proliferative, and tissue remodeling phases.[4,5] Abnormal scars are primarily a cosmetic concern, but their presence may have a significant negative impact on the affected individual. Consequently, patients often seek treatment to reduce the visibility and discomfort of a scar. Scars are a result of the natural healing process that occurs when the skin repairs itself after wounds, trauma, burns, surgical incision, or disease in predisposed individuals.[6,7] Sometimes, so much scar tissue is formed during deep wound healing resulting in a raised scar, if a scar remains within the boundaries of the original wounds; it is a hypertrophic scar. If it extends beyond the boundaries into the normal surrounding tissue, it is a keloid scar.[8,9] Both keloid and hypertrophic scars are characterized by abundant deposition of collagen and glycoprotein.[10] The objective of this work was to study the morphological and morphometric characteristics of the cultured fibroblasts from hypertrophic and keloid scars (treated and non-treated) and compare it from the normal skin using both light and electron microscopy, in order to find precise parameters to differentiate between abnormal scars that may help identifying better therapeutic, control, and treatment.

Methods. This study was carried out in the Surgical Clinic, Faculty of Medicine, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia between December 2008 and March 2010. Six samples were obtained after circumcision operation from the Maternity Hospital Surgical Clinic, Jeddah, and it was used as a standard of normal structure of human skin. Skin from chest region was also obtained during breast reduction operation. Fifty-two samples of (hypertrophic scar and keloid) were collected based on the clinical diagnosis from the surgical clinic in King Abdul-Aziz University Hospital (KAUH), and Military Armed Forces Hospital (MAFH) in Jeddah during 2008-2009. The age of the patients ranged from 10-50 years. Samples include: 8 of hypertrophic scars samples from different skin regions and keloid from ear lobe (n=16), sternum region (n=10), arms (n=3), thigh (n=4), foot (n=2), and shoulder (n=9). Classification of scars into hypertrophic scar or keloid (either treated or none treated) was based on initial clinical diagnosis (hospital reports). The treated samples according to the treatment regimen documented and reviewed by the researchers.[11]

Tissue culture studies (in vitro). Samples from normal human foreskin and abnormal scars tissue were transported in sterile media (Dulbecco's modified eagle's medium [DMEM] or minimum essential medium [MEM]) with antibiotic Penicillin/Streptomycin to tissue culture unit in King Abdul Aziz Medical Research Center (KFMRC). Collected samples were cut into small pieces for culture preparation. Primary culture was carried according to routine technique used in the unit.[12,13] The following were studied in cultured cells.

Ethical approval. The Biomedical Ethics Research Committee of Medical College, King Abdul-Aziz University approved this study as fulfilling the ethical requirements. Written consent was obtained from patients before operative excisions of abnormal scars in the plastic surgery clinics.

Light microscopic and morphometric studies. Passage II or III cells were stained with Comassi stain[14] and examined by inverted microscope and photographed. Morphometric studies of photographed cells include estimation of bipolarity index (length/width) using an ocular micrometer. Nuclear-cytoplasm ratio (N/C) was measured using software image-pro plus version 6.0 (**Figure 1a**).

Scanning electron microscopy of cultured fibroblasts. Normal and abnormal scar fibroblasts cultured on glass slides were fixed in 2.5% glutaraldehyde in 0.1M cacodylate for 30 minutes, followed by 2% osmium tetroxide in buffer solution. After fixation, the cells were processed for scanning microscopy. Samples were coated with 20 nm gold in a Joel Auto fine sputter coater (Coater JFC-1600, Japan) before examination in a Jeol JSM-6360LV scanning electron microscope (Jeol, London, UK).

Transmission electron microscopy of cultured fibroblasts. Normal and abnormal scar fibroblasts were fixed in 2.5% glutaraldehyde in 0.1 M, cacodylate buffers for 30 minutes (3 times). The fibroblasts were post-fixed in 1% osmium tetroxide and dehydrated in an ascending series of ethyl alcohol. Specimens were then embedded in epon-araldite. Ultrathin sections were cut and double stained with uranyl acetate and lead citrate before viewing in a Jeol JEM-1011 with Gatan digital camera at 80KV. All processing steps were carried in electron microscope (EM) unit, Faculty of Science, King Abdul-Aziz, Jeddah, Saudi Arabia.

Statistical analysis of the data was performed using the Statistical Package for Social Science (SPSS 12 for Windows) program Version 16. The data are expressed as means ± standard error (SE). Comparison of variables between groups was performed using the one-way

analysis of variance (ANOVA) and Student's t-test as appropriate. The least significance difference test (LSD) was employed to compare means for pairs of groups. *P*-values of less than 0.05 are considered statistically highly significant.

Results. ***A) Morphometric studies. Bipolarity index (L/W).*** There was a highly significant increase in fibroblast length and breadth of the hypertrophic and keloid scar compared to the normal skin, while there was highly significant decrease in the bipolarity index (L/W) of the fibroblast of the hypertrophic and keloid scar compared to the normal skin **Table 1**.

Nuclear/cytoplasmic ratio. There was an extremely significant increase in the mean cell area, mean nuclear area and the N/C ratio of the fibroblast of the hypertrophic and keloid scar compared to the normal skin. Also there was an extremely significant increase in both the mean cell area, mean nuclear area of the fibroblast of keloid compared to the hypertrophic scar **Table 2**. There was an extremely significant decrease in the mean cell area and mean nuclear area of the fibroblast of the treated keloid scar (by all studied modalities) compared to the untreated ones, while the N/C ratio showed no significant changes. But there was still extremely significant increase in all these parameters of the fibroblast of the treated keloid scar compared to normal skin **Table 3**.

B) Cell morphology. 1) Light microscopic study. In primary 24 hours (passage 0), monolayer cellular formation cannot be demonstrated. Only it could be recognized 10-15 days later. However, in 24 hours subculture (passage II or III) the cells tend to form monolayer. The cells appeared spindle shaped with few

Table 1 - Showed the mean cell length, breadth and bipolarity index (L/W) of normal, hypertrophic scars and keloid fibroblasts.

Parameters	Width (µm)	Length (µm)	Length/ Width
Normal Skin (n=120)			
Mean ± SD	31.19 ± 9.03	154.11 ± 37.41	5.46 ± 2.44
Range	11.47 - 57.03	73.06 - 233.47	1.89 - 13.27
HSc (n=120)			
Mean ± SD	51.21 ± 19.26	173.76 ± 45.98	3.86 ± 1.87
Range	16.24 - 122.17	88.85 - 281.10	1.34 - 10.14
Significance	P_1<0.001†	P_1<0.001†	P_1<0.001†
Keloid (n=120)	61.79 ± 26.18	186.07 ± 54.81	3.55 ± 1.86
Mean ± SD	13.96 - 185.85	30.68 - 371.70	0.51 - 13.04
Range	P_2<0.001†	P_2<0.001†	P_2<0.001†
Significance	P_3<0.001†	P_3<0.042*	P_3<0.241

P_1 - HSc versus normal, P_2 - Keloid verus normal, p3 - keloid versus HSc
†p<0.001 - highly significant, *p<0.05 significant. n - number of the measured fibroblasts.

Table 2 - Showed the mean cell area, mean nuclear area and N/C ratio of randomly selected normal human foreskin, hypertrophic and keloid fibroblasts.

Parameters	Mean cell area (µm)	Mean nuclear area (µm)	Mean (N/C) ratio
Normal skin			
Mean ± SD	370.7 ± 96.16	58.44 ± 12.72	1:6.2
Range	219.0 - 750.0	35.0 - 90.0	
Hypertrophic scar			
Mean ± SD	575.0 ± 141.6	62.40 ± 9.069	1:8.8
Range	421.0 - 950.0	40.00 - 79.00	
Significance	P_1<0.001†	P_1<0.046*	P_1<0.001†
Keloid skin			
Mean ± SD	1253 ± 379.4	131.4 ± 27.92	1:8.8
Range	476 - 273	(87 - 205)	
Significance	P_2<0.001†	P_2<0.001†	P_2<0.001†
	P_3<0.001†	P_3<0.001†	P_3>0.05

P_1 - hypertrophic scar versus Normal skin, P_2 - keloid skin versus normal skin, P_3 - keloid skin versus hypertrophic scar. Significant at p<0.05. Number of cells measured was 50 for each group chosen by random selection.
*significant, p<0.05, †extremly significant, p<0.001

Table 3 - Comparison between mean cell and nuclear area and (N/C) in keloid fibroblasts from treated and untreated lesions.

Parameters	Mean cell area (µm)	Mean nuclear area (µm)	Mean (N/ C) ratio
Normal skin			
Mean ± SD	370.7 ± 96.16	58.44 ± 12.72	1:6.2
Range	219.0 - 750.0	35.0 - 90.0	
Untreated keloid scar			
Mean ± SD	1253.0 ± 379.4	131.4 ± 27.92	1: 8.8
Range	476.0 - 2730	87.0 - 205.0	
Significance	‡P<0.001	‡P<0.001	‡P<0.001
Monotherapy (Zinc tape)			
Mean ± SD	1010.0 ± 298.7	101.6 ± 15.23	0.107 ± 0.028
Range	574.0 - 1701.0	60.00 - 130.0	0.060 - 0.171
Significance	‡P<0.001	‡P<0.001	‡P<0.001
	‡P_1<0.001	‡P_1<0.001	P_1>0.05
Ditherapy (zinc oxide tape and steroids injection)			
Mean ± SD	910.6 ± 241.1	117.3 ± 29.06	1:7.5
Range	569.0 - 1650	79.00 - 188.0	‡P<0.001
Significance	‡P<0.001	‡P<0.001	P_1<0.005†
	‡P_1<0.001	P_1<0.05*	
Ditherapy (zinc oxide tape and surgery)			
Mean ± SD	740.5 ± 270.3	92.42 ± 33.91	1: 7.8
Range	322.0 - 1650	49.00 - 188.0	
Significance	‡P<0.001	‡P<0.001	‡P<0.001
	‡P_1<0.001	‡P_1<0.001	P_1<0.05*
Tritherapy (zinc oxide tape, steroids injections and surgery)			
Mean ± SD	570.4 ± 175.4	67.56 ± 14.67	1: 81
Range	322.0 - 919.0	49.00 - 104.0	
Significance	‡P<0.001	‡P<0.001	‡P<0.001
	‡P_1<0.001	‡P_1<0.001	P_1<0.09

Number of cells measured was 50 for each group chosen by random selection. P normal skin versus others, P_1 untreated versus others.
*P<0.05 significant, †P<0.05 highly significant, ‡P<0.001 extremely significant.

cellular processes that usually have pointed ends. The cytoplasm stained homogenously blue with Comassi, occasionally some cells showed tiny vacuoles. The nuclei are rounded to oval in shape. They had one or 2 prominent nucleoli. The cytoplasmic volume is relatively scanty compared to nuclear size. Morphometric measurements confirm the above description (**Table 2**). Few cells appeared to have abundant cytoplasm and more cellular processes (**Figure 1b**).

Fibroblasts from hypertrophic scar (umbilical region) looked larger than fibroblasts cultured from normal foreskin. Spindle forms are infrequently seen. They appeared more spreading and of different shape. They had abundant cytoplasm relative to nuclear size, see also morphometric results (**Table 2**). Cellular extensions are also more numerous. The processes sometimes have foot like rounded ending (facet like) (**Figures 1c & 1d**).

Keloid scars removed from different body regions (ear, sternum, thigh, and arm) were studied. It was observed that although fibroblasts from all samples had the same morphological appearances that differentiate them from normal fibroblasts, there were minimal regional variations. Generally, keloid fibroblasts have abundant cytoplasm relative to nuclear size. The most characteristic feature was numerous cytoplasmic processes extending mostly from polar ends. Most processes end in facet shaped manner (filopodia-like). The nuclei are rounded to oval and have one or more nucleoli. Keloid fibroblasts showed different shapes; large satellite cells with numerous polar processes, pyramidal cells with pointed one end and broad opposite one and an oblong shaped cells (**Figures 2a & 2b**).

Most treated cells showed denser less cytoplasm and relatively smaller nuclei. Different shaped keloid fibroblasts; large satellite cells with numerous polar processes, pyramidal cells with pointed one end and broad opposite one and an oblong shaped cells were seen (**Figures 2c-2f**).

2) Scanning electron microscopy. Cultured fibroblasts looked relatively small in size with few numbers processes. The cytoplasm is scanty compared to the large vesicular nucleus. There is a little cytoplasmic material separating the nucleus from the surface membrane. The nuclei of the cells contain large multiple nucloeli. Cytoplasmic processes are short, sometimes exhibits terminal dilation. Some cells showed cytoplasmic whitish granular contents which may be located near the cytoplasmic membrane. Around the cell (peri-cellular region) few of these granules were seen associated with fine fibrillar threads (**Figures 3a - 3b**). Fibroblasts cultured for 24 hours

from hypertrophic scar lesions are of various shaped and sizes. They lost their spindle shape, the cytoplasm is abundant. The cells have numerous processes with abnormal shaped expanded ends that appeared facet-like (**Figures 3c-3d**). White rounded shape granules were observed within the cytoplasm and in association with cell membrane and processes. Similar granules were also seen in the vicinity (peri-cellular region) of enlarged fibroblast. The cultured fibroblast from untreated keloid scar had abundant cytoplasm compared to nuclear size. The cytoplasm contained clumped granules in the peri-nuclear region. The nuclei of the cells had abnormal shaped nucleoli (**Figure 4a**). Cultured fibroblasts from treated keloid scar showed abundant dense granular cytoplasm and broad short blunt cellular processes. The nuclei of the cells were large vesicular with prominent large nucleoli, but sometimes appeared shrunken. The contact between cells was increased (**Figures 4b-4d**).

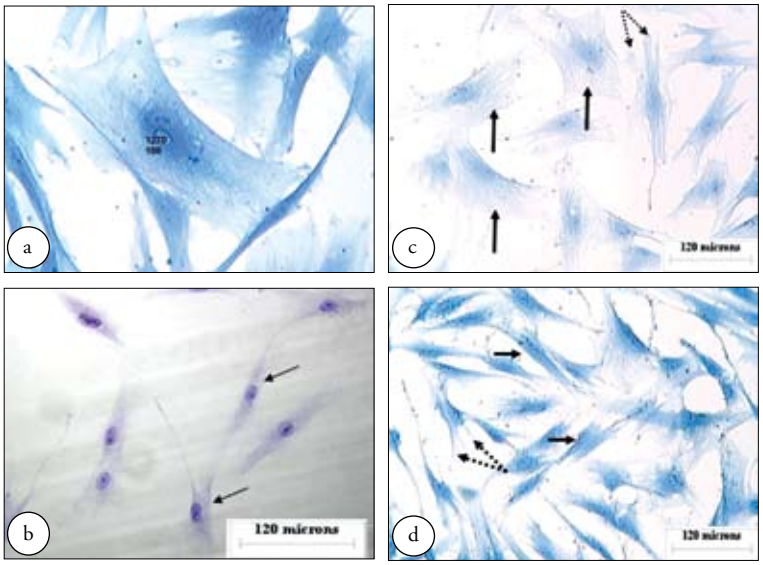

Figure 1 - **a**) Nuclear-cytoplasm ratio (N/C) was measured using software image-pro plus version 6.0. **b**) Human foreskin spindle shape fibroblasts with few cytoplasmic extensions and rounded to oval vesicular nuclei with one or 2 nucleoli (arrows). **c**) Cultured fibroblasts of postoperative hypertrophic scar from umbilical region of black female 47-year-old. Notice the large sized spreading fibroblasts with abundant cytoplasm (black arrows) relative to nucleus and extensive cellular extensions (dotted arrows). **d**) Cultured fibroblasts from posttraumatic hypertrophic scar of leg of a young girl (12-year-old) appear relatively spreading (black arrows), but less than the previous sample. The cells have relatively abundant cytoplasm compared to nuclear. Many cells have numerous cellular extensions (dotted arrows). All stained by Comassi stain.

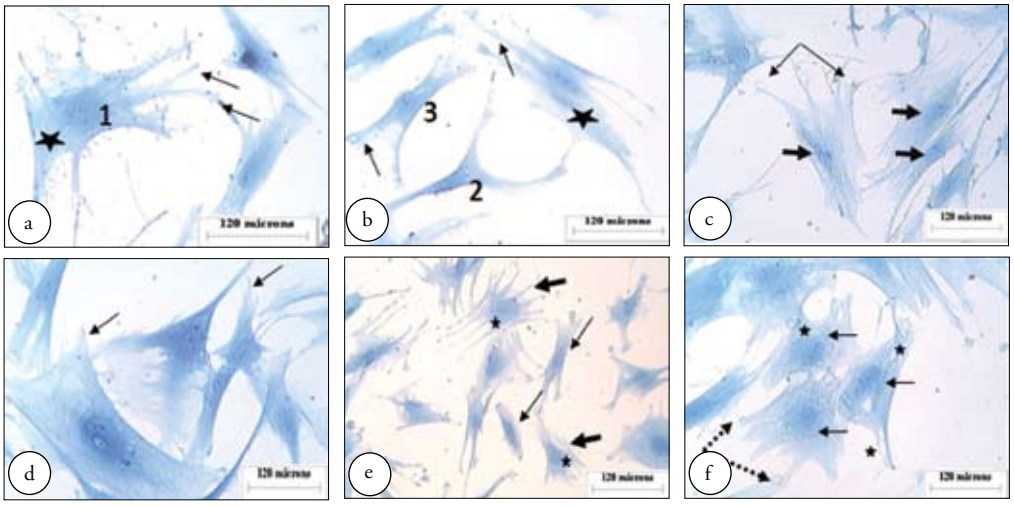

Figure 2 - **a-b**) Cultured keloid fibroblasts from ear lobule region of a female (17-year-old) showing different shapes of fibroblasts; satellite (1), pyramidal (2), and oblong (3). Notice the abundant cytoplasm (stars) and the abnormal numerous cell processes. Some end in blunt foot like shape (thin arrows). **c**) Cultured keloid fibroblasts from ear lobe region, (treated with steroid) of 51 years female showing abundant cytoplasm relative to nucleus (thick arrows). The cells are satellite in shape with numerous cellular processes (thin black arrows). **d**) Cultured keloid fibroblasts from 20-year-old male (sternal region) treated with zinc oxide with different shapes (some appear shrunken and others still have abundant cytoplasm relative to nuclear size). Cytoplasmic processes are numerous, but shorter and with blunted ends (facet-like) (arrows). **e**) Different shaped cultured keloid fibroblasts (thick arrows; from female 50-year-old) from sternal region treated by few zinc oxide + steroid showing abundant cytoplasm (stars), numerous processes. Notice the presence of some shrunken cells with small ill defined nuclei (thin arrows). f) Fibroblasts from recurrent keloid lesion treated with low zinc oxide + steroid from the thigh region of a 5-year-old boy showing abundant cytoplasm (stars) with vesicular content in peri-nuclear region (black arrows) relative to nuclear size. Notice the numerous broad ended processes (facet-like) (dotted arrows).

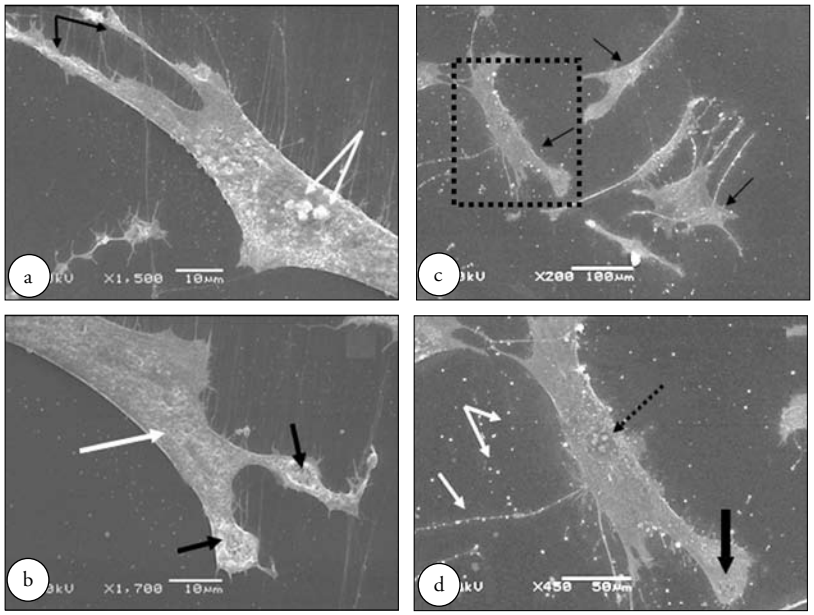

Figure 3 - Scanning electron micrograph of **a**) normal human fibroblasts from chest region showing scanty cytoplasm surrounding the large vesicular nucleus with multiple nucleoli (white arrows) and few cell processes (black arrows). **b**) Another pole of the same fibroblast showing white granular (white arrow) and short fibrillar content within the cytoplasm, with a bulb like dilated end foot (black arrows). **c**) Different shaped cultured fibroblasts from hypertrophic scar (arrows). Notice the abundant cytoplasm and numerous cell processes associated with white secretory granules. **d**) Magnified fibroblast from the previous figure to show the multiple nucleoli within the nucleus (dotted black arrow), the expanded foot like processes (thick black arrow) and the white secretory granules (tropocollagen) associated with cell processes and around the cell (white arrows).

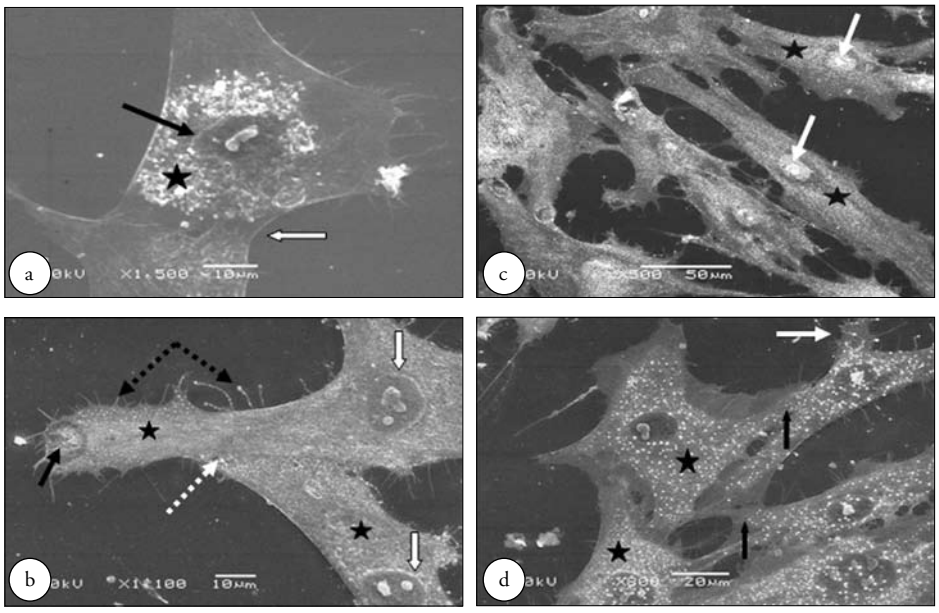

Figure 4 - Scanning electron micrograph of **a**) cultured untreated keloid fibroblast from sternum male (33-year-old) showing the region of cell contact (white arrow). The cytoplasm is abundant compared to nuclear size. Notice the abnormal shaped nucleolus (black arrow). The cytoplasm contained clumped granules in the peri-nuclear region (black star). b) Cultured keloid fibroblasts from female thigh treated with both zinc oxides + corticosteroid. Showing contact (dotted white arrow) between 2 cells with abundant dense cytoplasm (black stars), broad cellular process with facet-like ending (black arrow). Fine thread like extensions from the cell surface associated with white granules similar to those seen within the cytoplasm (black dotted arrows). The nuclei of the cells are large vesicular with prominent large nucleoli (white arrows). **c**) Cultured fibroblasts from female steroid treated keloid (50-year-old) in the ear lobule region shows more detailed structure of fibroblasts. Notice the dense cytoplasm (stars) and shrunken nuclei with few processes (white arrows). d) Cultured treated keloid fibroblasts from female ear lobe (37-year-old). The cytoplasm is dense and granular (stars) with short blunt processes (white arrows). Notice how cells contact each other (black arrows).

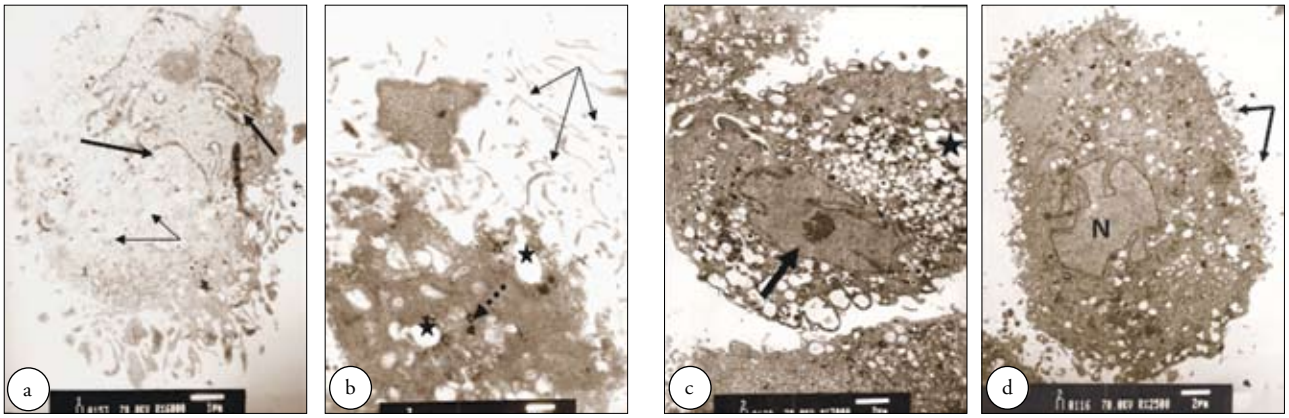

Figure 5 - Transmission electron micrograph of **a)** cultured human foreskin fibroblasts (after addition of Trypsin) showing highly folded nuclear membrane (thick black arrows) and prominent vacuoles (thin arrows) most probably dilated endoplasmic reticulum. b- Cultured fibroblast from hypertrophic scar showing numerous thin cytoplasmic extensions in the vicinity of the cell (black arrows). Note the electron dense bodies (black dotted arrows) and empty vacuoles (black stars), which may be rough endoplasmic reticulum (RER). **c)** Cultured keloid untreated fibroblast showing infolded nuclear membrane well defined nucleus (thick arrow). The cytoplasm was electron dense filled with numerous cytoplasmic vacuoles (black stars). **d)** Treated keloid cultured fibroblast showing nucleus (N) with irregular contour, cytoplasmic vacuoles, dense bodies. Few short cytoplasmic processes compared to control (arrows).

3) Transmission electron microscopy. The ultrastructure of normal fibroblast showed that its nuclei were folded with prominent nucleoli. Although cell organelles characterizes normal fibroblasts were not visualized, the cytoplasm showed empty vacuoles, which may be dilated endoplasmic reticulum. Dense granules were also observed at the periphery of the cells. Few short cell processes were observed extending from one pole of the cells (**Figure 5a**). Fibroblasts from hypertrophic scars showed also vacuoles and dense bodies, but less than keloid cells. In all samples the nuclei have folded nuclear membrane, a sign of active proliferating cells (**Figure 5b**). Fibroblasts from untreated keloid lesions showed dense cytoplasm with numerous vacuoles, dense bodies and few strands of rough endoplasmic reticulum (RER). Numerous fine processes were seen projecting from cell surface, some cells had numerous vacuoles, and some possessed dense vesicles (**Figure 5c**). Fibroblast from treated keloid showing nucleus with irregular contour, cytoplasmic vacuoles, dense bodies and few short cytoplasmic processes compared to control (**Figure 5d**).

Discussion. In this in vitro study, we used cell cultured fibroblasts; the key cell in fibrotic lesion. Morphometric and morphologic description were carried out to demonstrate differences between normal and abnormal scar forming cultured fibroblasts. The present morphological studies of cultured fibroblasts showed that both hypertrophic and keloid fibroblasts are more active in culture media compared to those cultured from human foreskin. Light and scanning electron microscopy showed that they have common characteristic features that demonstrate high rate of synthetic activity. Hypertrophic scar (HSc) and keloid fibroblasts had large nuclei with prominent nucleoli. Abundant cytoplasm and numerous processes were among the characteristic features. Some processes had facet like endings. Keloid cells were found to be larger and so more active compared to those of HSc. This was proven by morphometric studies carried out in the present study. Nuclear cytoplasmic ratio and bipolarity index ratio (cell length/cell width) were found to be larger in keloid followed by hypertrophic scar compared to control. This indicates active cells in these lesions.[15] It was reported that the above measured parameters reflect the degree of cellular elongation and activity.[16] It was reported that the growth potential of keloid fibroblasts was higher than that of normal dermal cells.[17] Nuclear cytoplasmic ratio was considered as an indication of proliferative activity of cells. In the present study, the mean N/C, was observed to be approximately 1:6 in human foreskin fibroblasts, 1:9 in HSc cells, and untreated keloid cells, and 1:8 in all treated cells. Hence, fibroblasts of abnormal scars examined herein appeared to have a tendency for spreading in width rather than in length indicating proliferating or regenerating state rather than malignant behavior. Malignant cells were reported to have N/C equaling to 1:1.[18] Treatment was also observed to decrease the spreading feature of keloid cells. Keloid fibroblasts derived from scars treated with drugs known to inhibit cell proliferations such as steroids and zinc oxide had less nuclear cytoplasmic ratio indicating less proliferative and synthetic activities. It was reported that intraregional steroid injection induced decreasing collagen contents and fibroblast density in keloid scars.[19] The researchers found that using either steroid alone or steroid with

zinc oxide reduced the size of keloid lesions, which was most probably due to the decreased of fibroblast synthetic activity.[11,20] The presence of inflammation in human foreskin may explain the occasional presence of large sized cells observed in cultured fibroblasts in tissue culture obtained from such specimens.[21] However, morphometric measurements (N/C and L/W) ratios of normal fibroblasts showed that they were still differ from those of keloid or hypertrophic derived fibroblasts.[22]

Using H-thymidine incorporation showed that both keloid and hypertrophic scar fibroblasts are much more active than normal dermal fibroblasts.[3]

Fibroblast besides its role as structural elements has the ability to serve as a resident sentinel cell.[23] For example, fibroblasts, when activated by substances released during tissue injury or derived from infectious microorganisms or by other environmental factors, produce chemokines that initiate the recruitment of bone marrow derived cells. The same authors emphasized the concept that fibroblasts are key sites of chemokines synthesis, which initiates a cascade of events involved in wound healing and clearance of invading microorganisms.

One of the limitations of the study was the marked diversity of the fibroblasts in different anatomical regions of the skin. This was noticed also by other researchers who said that fibroblasts, even from a single tissue, are not composed of a homogeneous population, but rather consist of subsets of cells much like lymphocytes.[24,25] This concept may to some extent explain the variation in cell behavior in proliferative activity and morphological diversity observed in culture media.[26] Moreover, regional diversity, of the fibroblasts, may reflect the specialized functions of the tissue of origin.[27] Another limitation was the expensiveness of the tissue culture materials used and the drop out of the cases during follow up.

References

1. Santoro MM, Gaudino G. Cellular and molecular facets of keratinocyte reepithelization during wound healing. Experimental. ***Cell Research*** 2005; 304: 274-286.
2. Wolfram D, Tzankov A, Pülzl P, Piza-Katzer H. Hypertrophic scars and.keloids--a review of their pathophysiology, risk factors, and therapeutic management. ***Dermatol Sur***g 2009; 35: 171-181.
3. Meenakshi J, Jayaraman V, Ramakrishnan KM, Babu M. Keloids and hypertrophic scars: a review. ***Indian J Plast Surg*** 2005; 38: 175-179.
4. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. ***Physiol Rev*** 2003; 83: 835-870.
5. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. ***Trends Cell Biol*** 2005; 15: 599-607.
6. Alster TS, Tanzi EL. Hypertrophic scars and keloids: etiology and management. ***Am J Clin Dermatol*** 2003; 4: 235-243.
7. Köse O, Waseem A. Keloids and hypertrophic scars: are they two different sides of the same coin? ***Dermatol Sur***g 2008; 34: 336-346.
8. English RS, Shenefelt PD. Keloids and hypertrophic scars. ***Dermatol Surg*** 1999; 25: 631-638.
9. Niessen FB, Spauwen PH, Schalkwijk J, Kon M. On the .nature of hypertrophic scars and keloids: A review. ***Plast Recon Surg*** 1999; 104: 1435-1458.
10. Mercandetti M, Cohen AJ. Wound Healing: Healing and Repair (Updated: 2009 October). Available from URL: http://emedicine.medscape.com
11. Moshref S. Topical zink oxide adhesive tape for keloid management. ***Egyp J Sur*** 2006; 25: 183-235.
12. Mather JP and Roberts PE. Introduction to Cell and Tissue Culture. New York (NY) and London (UK): Plenum Press; 1998.
13. Khorshid FA. Comparative study of keloid formation in humans and laboratory animals. ***Med Sci Monit*** 2005; 11: 212-219.
14. Mutalik S.Treatment of keloids and hypertrophic scars. ***Indian J Dermatol Venereol Leprol*** 2005; 71: 3-8.
15. Lepekhin E, Gron B, Berezin V, Bock E, Dabelsteen E. Differences in motility pattern between human buccal fibroblasts and periodontal and skin fibroblasts. ***Eur J Oral Sci*** 2002; 110: 13-20.
16. Soll DR, Voss EV, Arnum-finney B, Wessels D. Dynamic Morphology System: A method for quantitating changes in shaps, pseudopod formation, and motion in normal and mutant amoebae of Dictyostelium discoideum. J***ournal of Cellular Biochemistry*** 1988; 37: 177-192.
17. Luo S, Benathan M, Raffoul W, Panizzon RG, Egloff DV. Abnormal balance between proliferation and apoptotic cell death in fibroblasts derived from keloid lesions. ***Plast Reconstr Surg*** 2001; 10: 87-96.
18. Kumar V, Abbas AK, Fausto N, Mitchell RN. Basic Pathology 8th ed. USA: Elsevier; 2007.
19. Kaur S, Amanjeet, Thami GP, Mohan H. Intra-lesional steroid induced histological changes in the skin. ***Indian J Dermatol Venereol Leprol*** 2003; 69: 232-234.
20. Al-Attar A, Mess S, Thomassen JM, Kauffman CL, Davison SP. Keloid pathogenesis and treatment. ***Plast Reconstr Surg*** 2006; 117: 286-300.
21. Alster TS, Tanzi EL. Hypertrophic scars and keloids: etiology and management. ***Am J Clin Dermatol*** 2003; 4: 235-243.
22. Kumar V, Abbas AK, Fausto N. Tissue Renewal and Repair Regeneration, Healing, and Fibrosis. Pathologic Basis of Disease 7th ed. USA: Robbins and Cortran, Elsevier; 2005. p. 87-118
23. Marneros AG, Krieg T. Keloids--clinical diagnosis, pathogenesis, and treatment options. ***J Dtsch Dermatol Ges*** 2004; 2: 905-913.
24. Awonusonu F, Srinivasan S, Strange J, Al-Jumaily W, Bruce MC. Developmental shift in the relative percentages of lung fibroblast subsets: role of apoptosis postseptation. ***Am J Physiol Lung Cell Mol Physiol*** 1999; 277: L848-L859.
25. Hetzel M, Bachem M, Anders D, Trischler G, Faehling M. Different effects of growth factors on proliferation and matrix production of normal and fibrotic human lung fibroblasts. ***Lung*** 2005; 183: 225-237.
26. Kulasekaran P, Scavone CA, Rogers DS, Arenberg DA, Thannickal VJ, Horowitz JC. Endothelin-1 and transforming growth factor-beta1 independently induce fibroblast resistance to apoptosis via AKT activation. ***Am J Respir Cell Mol Biol*** 2009; 41: 484-493.
27. Alexander GM, Thomas K. Keloids-clinical diagnosis, pathogenesis, and treatment options. ***J Dtsch Dermatol Ges*** 2004; 2: 905-913.