Egypt, J. Plast. Reconstr. Surg., Vol. 35, No. 2, July: 245-251, 2011

The Prognostic Values of the Immunohistochemical Expression of Ezrin and Larger Safety Margins in Basal Cell Carcinoma of the Face

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ABSTRACT

The aim of this study was to assess the expression pattern of Ezrin in normal skin and basal cell carcinoma and to correlate the results with clinicopathological data in addition to wider safety margins. We excise the basal cell carcinoma with wider safety margins (5mm) and we have used immunohistochemical technique to examine the expression of Ezrin in paraffin-embedded tissues of basal cell carcinoma (n=45) and normal control skin (n=5).

In all patients there were safety margins of 0.5mm in all directions of the tumor, peripheral and deep margins, and histopathological study of the excised lesions revealed clear surgical margins and deep layer from the tumor cells and follow-up for 3 years revealed no recurrence in any of our patient. The best cosmetic appearance was obtained in cases reconstructed by rhomboid flaps and only one case reconstructed by forehead head flap needed revision. In normal skin, Ezrin was dominantly development, especially in regulating the growth and metastatic capacity of cancer cells. However, little is known about the expression of Ezrin in normal epidermis and various skin tumors.

In this study, we examined the immunohistochemical expression of Ezrin in basal cell carcinoma and normal skin. Our aim was to determine whether there is a correlation between the degree of Ezrin expression and tumor aggressiveness in addition to wider safety margins and their role in recurrence rate.

INTRODUCTION

Basal Cell Carcinomas (BCCs) are the most common forms of skin cancer **[1]**. They rarely metastasize but are often locally aggressive, with a small percentage showing multiple recurrences. They also have a possible, although as yet unproven, adnexal origin and often show differentiation toward adnexal structures **[2,3]**. Usually, basal cell carcinoma (BCC) appears as a slowly growing tumor arising on sun-exposed areas of the body; the face being the most common site **[4]**. It is well-known that recurrence rates are higher for larger and previously recurrent tumors, as well as for more aggressive histologic subtypes **[5-9]**.

In this study, we investigated the value of immunohistochemical expression of Ezrin in basal cell carcinoma. Ezrin has been demonstrated to have a role in Physical and functional connecting the actin cytoskeleton to the cell membrane. Ezrin is a member of the Ezrin-radixin-moesin (ERM) family, which share homology with the amino-terminal membrane-binding domain of erythrocyte band 4.1 and possesses membrane-cytoskeleton linking functions **[10]**. Ezrin plays a positive role in maintaining cell shape and cell polarity, as well as participating in membrane-trafficking pathways, cell migration, cell signaling, growth regulation, and differentiation **[11]**. Because of its unique functions, Ezrin is also actively involved in the biology of tumor.

PATIENTS AND METHODS

The current study was carried out at Minia University Hospital, Egypt, during the period from January 2003 to February 2007. It included 45 patients that were presented by Basal Cell Carcinoma (BCC). The median age of the patients, was 64 year (ranged between 56 and 72 years). Table (1) demonstrates all clinicopathological data of the patients included in the study (including age, gender as well as the site of the lesion).

All patients were subjected to thorough history taking, routine clinical examination in addition to essential laboratory investigations and tissue biopsy (punch biopsy) analysis. Five normal skin biopsies have been retrieved from the archives and have been included in the study. All patients have given written informed consents for the surgical interference as well as for enrollment in the study.

Surgical procedure:

The different presentations of the patients were: BCC of the cheek (10), nasolabial fold (7), nose (5), upper lip (5), forehead (5), lower eye lid (3), outer canthus (3), inner canthus (2), and scalp (5) cases (Table 2).

There were variations in surgical techniques according to the site, size and availability of the tissue around the tumor, but all the surgical procedures were performed as follows: Clinically clear margins were determined around the tumor by sterile marker, a 5mm safety margins around the tumor were marked (Fig. 1). Local anesthesia was obtained with infiltration of lignocaine 0.1%: with adrenaline one in 100,000, which was injected beneath and around the area marked for excision and subsequent reconstruction, and the excised specimen was marked with a suture to provide orientation. The excised specimen was submitted for histologic confirmation of the diagnosis and examination for clearness of the surgical margins. Reconstruction was performed with different types of local faciocutanous flaps (rhomboid, rotational, Island and advancement flap), the wound were closed in layers using 4/0 Vicryl and 5/0 proline. 5-7 days postoperatively the sutures were removed and follow-up of the patient in the hospital clinic was carried out at one to three months for the first year, and every six to 12 months for the next 2 years.

Pathological specimens:

All surgically removed tumor biopsies from the 45 patients included in the study, have been subjected to histopathological examination. Each specimen was immediately fixed in 10% buffered Formalin, processed in different grades of alcohols and finally processed into paraffin wax blocks. Paraffin serial sections of 4μm thick were cut from each specimen and stained for routine Haematoxilin & Eosin (H&E) and Ezrin immunostaining. All tumors were histologically graded.

Immunohistochemical staining technique:

Streptavidin-biotin peroxidase complex procedure was applied for staining. Four μm-thick sections from archival paraffin-embedded tissues were cut, placed on poly-L-lysine coated slides, deparaffinized with xylene, and rehydrated through graded alcohol. Endogenous peroxidase activity was blocked by incubation with 0.3% Hydrogen peroxide/Methanol for 30 minutes. Antigen retrieval was achieved by microwave treatment, where the slides were placed in sodium citrate buffer (0.01M, pH 6.0) for 10 minutes. The sections were incubated with mouse IgG monoclonal antibody directed against Ezrin (NeoMarkers, Lab Vision, and Fremont, CA, USA). The antibody was diluted at its optimum concentration (1:200 dilutions) in PBS containing 5% (w/v) bovine serum albumin and the sections were incubated for 1 hour at room temperature. Thereafter, the slides were thoroughly rinsed with PBS and a biotinylated goat anti-mouse secondary antibody was applied to sections for 30 minutes at room temperature. Visualization of the reaction was performed with an avidin-biotin complex immunoperoxidase system using 3, 3'diaminobenzidine as a chromogen for 10 minutes. Then, the slides were rinsed in running tap water and lightly counterstained with Mayer's haematoxylin. Finally, sections were dehydrated in ascending grades of alcohol, cleared in xylene and mounted with DPX.

Positive and negative control:

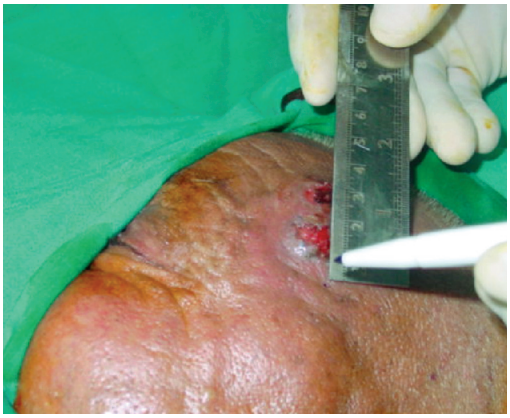
Each staining batch included both positive and negative control sections. Negative control sections were treated with phosphate-buffered saline (PBS) instead of primary antibody. Also in tumor samples, examining adjacent normal skin served as an internal control. Metastatic lung adenocarcinoma was used as a positive control.

Assessment of immunohistochemical staining:

Assessment of immuno-staining was done by Dr. Abdleghany MI, without previous knowledge of the clinical data of the cases. The results were expressed using a semiquantitative scale as following: 0/Negative = complete absence of staining by tumor cells; Positive 1+ = 1-30% of the cells were stained; Positive 2+ = 31-75% of the cells were positively stained; Positive 3+ = 76-100% of the cells expressed immunostaining **[12]**. To determine the expression pattern of the immunostaining of Ezrin, at least 10 high-power fields were randomly selected for microscopic examination. Cytoplasmic and/or membranous staining localization were also evaluated.

Statistical analysis:

The nonparametric Mann-Whitney U-test and the Kruskal-Wallis rank test were used to examine the relationship between the pattern of Ezrin expression and the clinico-pathologic data. Statistical analyses were performed using the SPSS Version 10 for Windows (SPSS Inc., Chicago, IL) program package.



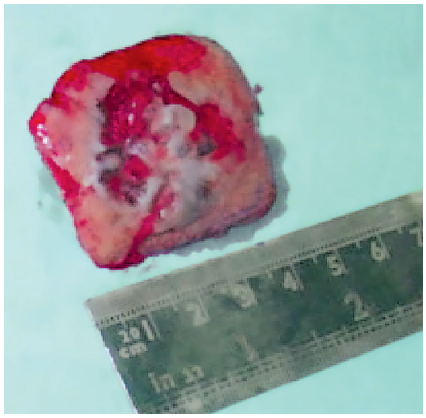


Fig. (1): Intraoperative marking with 5mm safety margin and the specimen after complete excision with safety margin.

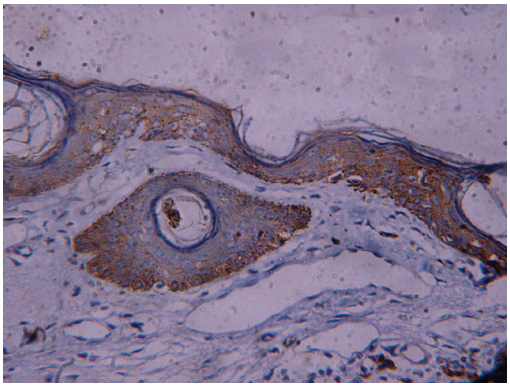


Fig. (2): Immunohistochemical expression of Ezrin in normal skin shows weak positive (1+) membranous pattern of staining and the intensity of staining decreases from the basal layer towards the upper layers (IHC, Original magnification X100).

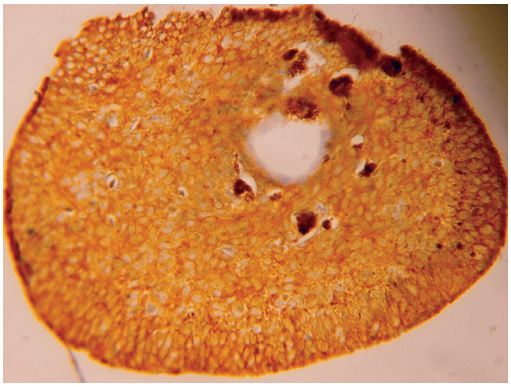
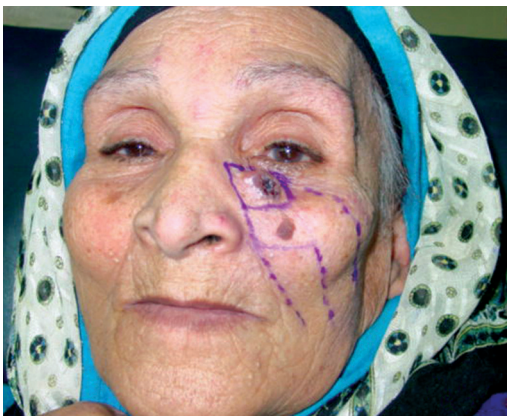


Fig. (3): Heterogenous strong (2+ -3+) cytoplasmic immunoexpression of Ezrin is exhibited by the tumor sheets of basal cell carcinoma (IHC, Original magnification X200).



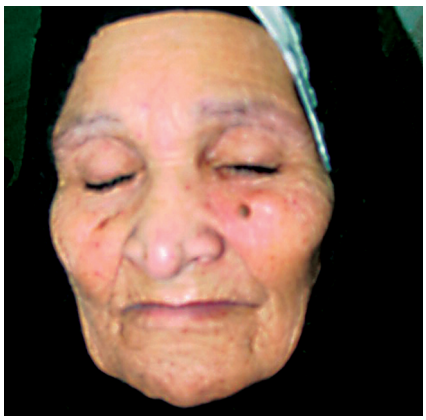
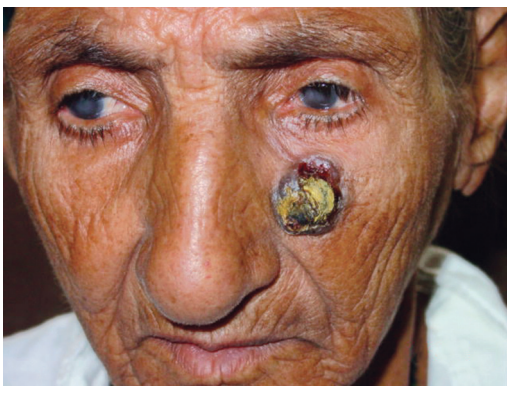


Fig. (4): Pigmented BCC left cheek and 6 months after excision and reconstruction by rhomboid flap.



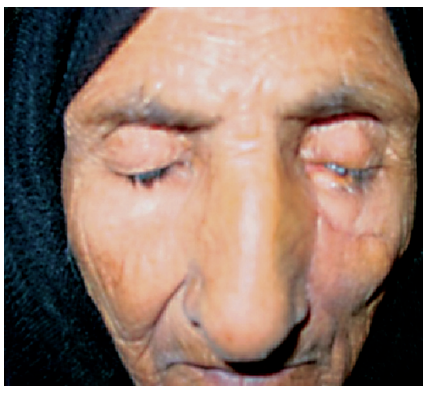
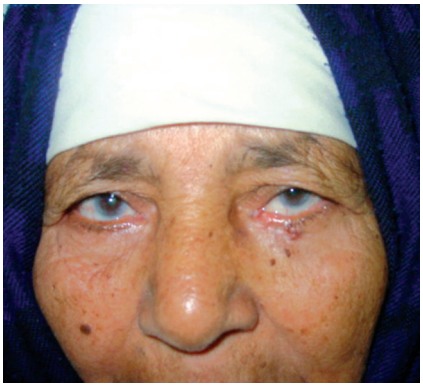


Fig. (5): Nodular BCC left cheek and 9 months after excision and reconstruction by rhomboid flap.



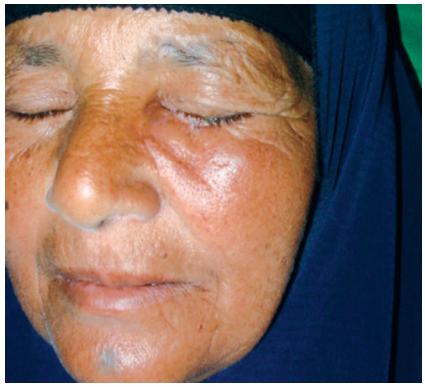
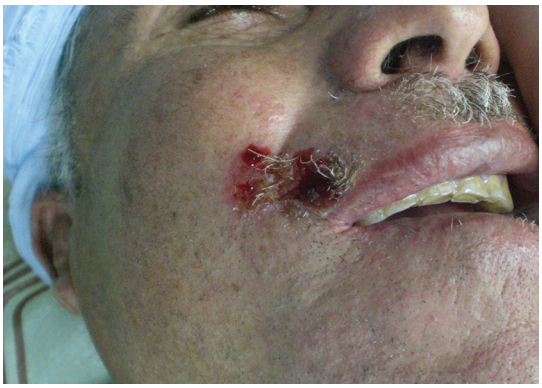


Fig. (6): Nodular BCC left lower eye lid and 3 months after excision and reconstruction by rhomboid flap.



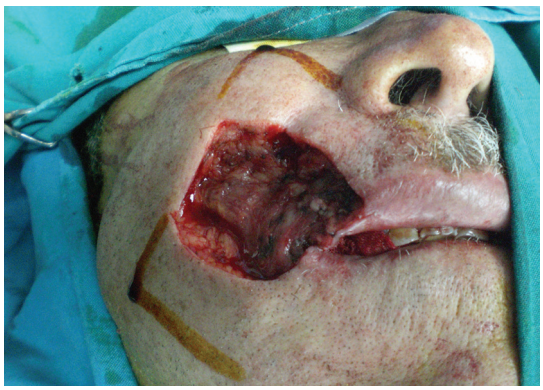
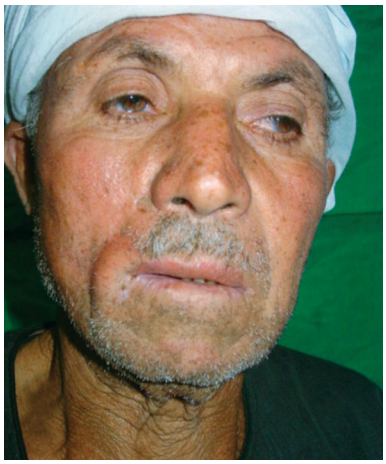


Fig. (7A): Recurrent BCC upper lip intraoperative and after excision and marking of the flaps design.



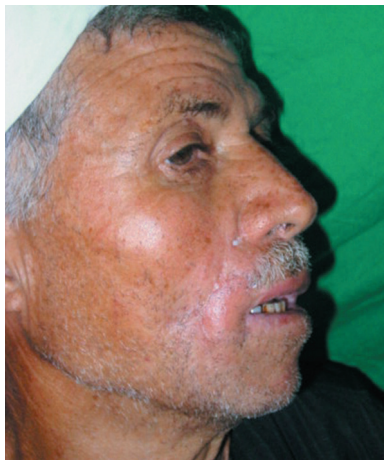
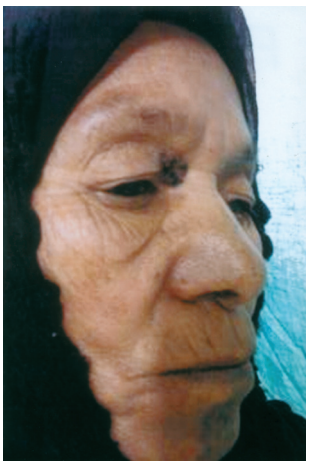


Fig. (7B): Reconstruction by double rhomboid flap late postoperative (one year PO).



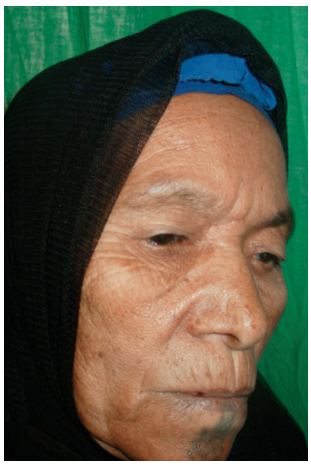
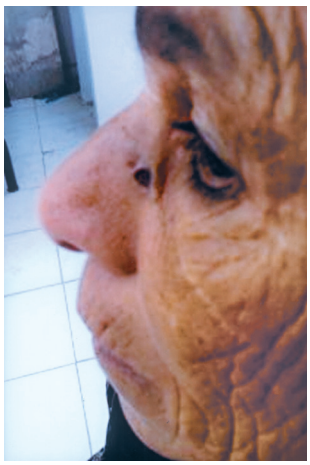


Fig. (8): Pigmented BCC right medial canthus and 6 months after excision and reconstruction by glabellar flap.



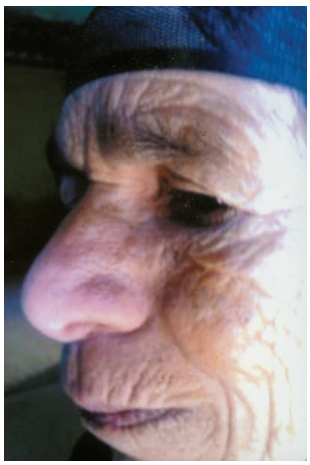


Fig. (9): Ulcerative BCC left side of the nose and 2 years after excision and reconstruction by nasolibial (island) flap.


Fig. (10): Ulcerative BCC right cheek and 6 months after excision and reconstruction by island flap.

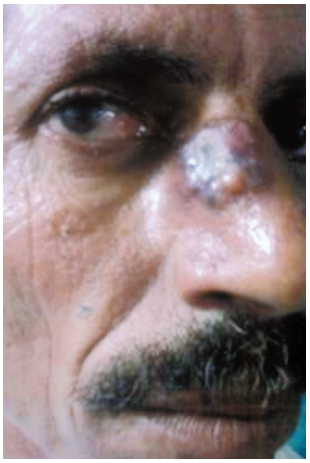
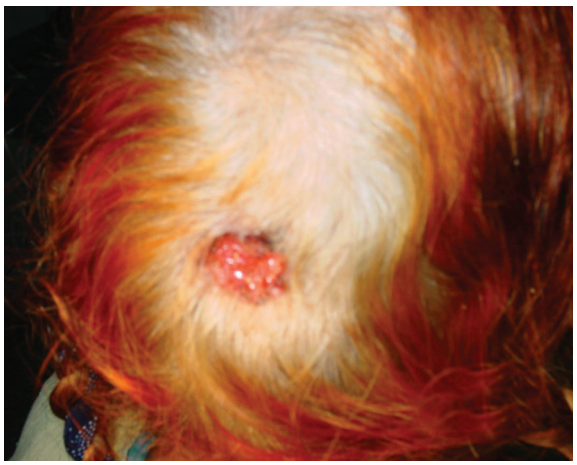
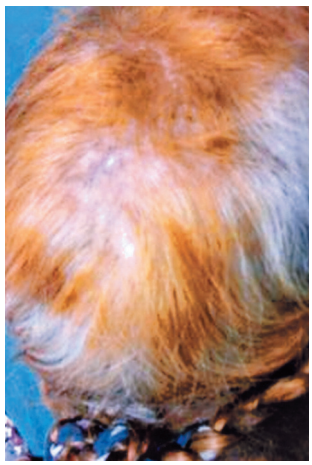



Fig. (11): Nodular BCC right side of the nose and 2 months after excision and reconstruction by forehead flap.

Fig. (12): Ulcerative BCC of the scalp and one year after excision and reconstruction by rotational flap.

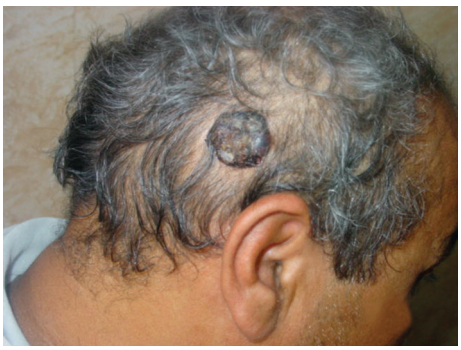
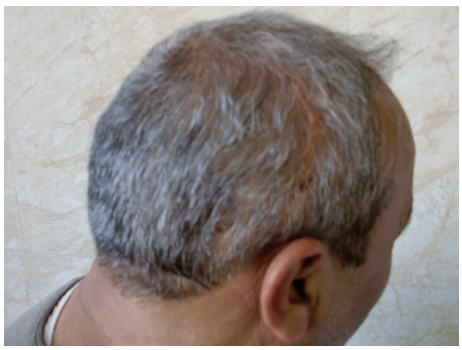



Fig. (13): Nodular BCC of the scalp and 2 years after excision and reconstruction by rotational flap.

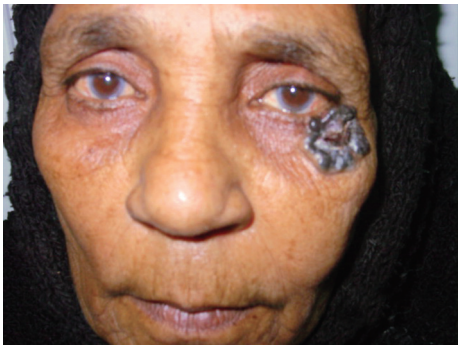
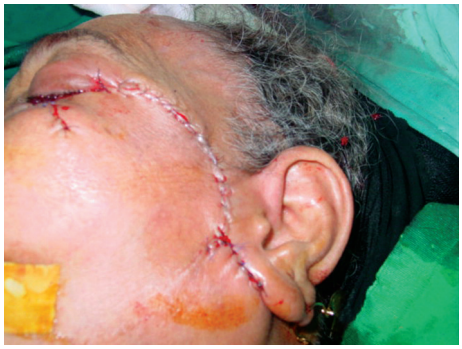



Fig. (14): Pigmented BCC right cheek and lower eye lid and intraoperative picture after excision and reconstruction by rotational flap.

Table (1): Demonstrates the site and gender distribution of the cases.

Site	Gender		Number
	M	F	
Cheek	7	3	10
Nasolabial fold	3	4	7
Nose	3	2	5
Upper lip	4	1	5
Forehead	5	0	5
Lower eye lid	2	1	3
Outer canthus	1	2	3
Inner canthus	0	2	2
Scalp	2	3	5

Table (2): Demonstrates the surgical procedures.

Procedure	Number	Percentage
Rhomboid	30	67
Rotational	11	25
Advancement	2	4
Island	2	4

RESULTS

I- *Immunohistochemical expression of Ezrin:*

Normal skin:

All (100%) normal skin specimens showed weak positive (1+) membranous pattern of staining for Ezrin (Fig. 2). It has been noticed that the intensity of staining decreased from the basal layer towards the upper layers.

Basal cell carcinoma:

The immuno-expression of Ezrin was markedly upregulated in all specimens of BCC. Most tumor sheets, especially solid nests showed heterogeneous, very strong cytoplasmic staining (Fig. 3).

II- *Surgical outcome:*

Through 4 years a 45 patients with Basal Cell Carcinoma (BCC) of the face treated by surgical excision with safety margins and reconstruction of the defect by local flap. The size of the lesion with the safety margins varies from 2-4cm in diameter. In all patients there were safety margins of 0.5mm in all directions of the tumor, peripheral and deep margins, and histopathological study of the excised lesions revealed clear surgical margins and deep layer from the tumor cells and follow-up for 3 years revealed no recurrence in any of our patient. The best cosmetic appearance was obtained in cases reconstructed by rhomboid flaps (Figs. 4-7) and only one case reconstructed by forehead head flap needed revision.

DISCUSSION

Most basal-cell carcinomas are treated worldwide by surgical excision **[13]**. The recurrence rate for basal cell carcinoma after complete conventional surgical excision is low in the order of 1% **[14]**. The standard surgical margin for primary nonmelanoma skin cancers is 4mm **[15,16]**. In a study done by Kimai Asadi et al., they found that narrow-margin elliptical excision with margins up to 3mm is only 80% effective in clearing these tumors **[17]**. In our study we used wider safety margin (5mm) in 45 patients with different forms of basal cell carcinoma of the face and scalp, the wide safety margin does not affect the cosmetic appearance after excision of the Basal Cell Carcinoma but had reduced the recurrence rate and tumor infiltration to the resection margins to 0%

Also we used different reconstructive procedures according to the tissue availability (rhomboid and rotational flaps had the best cosmetic appearance while the results of island and advancement flaps was satisfactory).

In normal skin, Ezrin is localized to the membrane of basal cells, and the intensity of staining decreases from the basal layer towards the upper layers. As the basal layer and parabasal layer cells form the proliferative pool of a normal skin, it is reasonable that Ezrin is expressed in the cell membrane, as in other normal tissues. In our study, the expression of Ezrin was markedly up regulated in BCC compared with the adjacent or overlying normal skin and normal controls. Loss of membranous localization of Ezrin and its cytoplasmic accumulation were evident in basal cell carcinoma, suggesting that these two changes are fundamental in keratinocyte transformation. Also, when correlated with clinicopathological data, the expression of Ezrin was increased with higher grades and within areas of squamous differentiation reflecting the importance of such expression in predicting the behavior of these tumors. Park et al. (2010) mentioned that, according to their studies of Ezrin in squamous cell carcinoma and other epithelial lesions including basal cell carcinoma, it was not possible to judge whether the changes described in their results are a primary event in carcinogenesis or a secondary phenomenon **[12]**. However, even if secondary, dysregulation of the Ezrin and in particular the cytoplasmic up-regulation observed may facilitate the malignant process. They concluded that, the quantitative and qualitative changes in Ezrin expression would be of well importance in the pathogenesis of those keratinocyte tumors. However, to elucidate such role for Ezrin, further

experimental studies are strongly recommended. Overall, the current study have shown that the change of cellular localization of Ezrin in addition to its expression level in basal cell carcinoma lesions suggest that the localization of Ezrin by immunohistochemical staining may be used as a useful tool in predicting the prognosis of such cases, especially its expression pattern was found to correlate with the tumor differentiation and grade. It will be helpful to evaluate the invasiveness of these tumors by measuring the expression of this marker.

REFERENCES

1- Quinn A.G., Sikkink S. and Rees J.L.: Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. Cancer Res., 54: 4756-9, 1994.

2- Kocsard E.: Solar keratoses and their relationship to non-melanoma skin cancers. Australas. J. Dermatol., 38 (Suppl. 1): S30, 1997.

3- Wade T.R. and Ackerman A.B.: The many faces of basal-cell carcinoma. J. Dermatol. Surg. Oncol., 4: 23-8, 1978.

4- Richmond-Sinclair N.M., Pandeya N., Ware R.S., et al.: Incidence of basal cell carcinoma multiplicity and detailed anatomic distribution: Longitudinal study of an Australian population. J. Invest. Dermatol., 2008; Published online July 31. DOI: 10.1038/jid.234, 2008.

5- Kuijpers D.I., Thissen M.R. and Neumann M.H.: Basal cell carcinoma: Treatment options and prognosis, a scientiic approach to a common malignancy. Am. J. Clin. Dermatol., 3: 247-59, 2002.

6- Netscher D.T. and Spira M.: Basal cell carcinoma: An overview of tumor biology and treatment. Plast. Reconstr. Surg., 113: 74-94, 2004.

7- Silverman M.K., Lopf A.W., Bart R.S., et al.: Recurrence rates of treated basal cell carcinomas. Part 3: Surgical excision. J. Dermatol. Surg. Oncol., 18: 471-6, 1992.

8- Rowe D.E., Carroll R.J. and Day C.L.J.: Long-term recurrence rates in previously untreated (primary) basal cell carcinoma: Implications for patient follow-up. J. Dermatol. Surg. Oncol., 15: 315-28, 1989.

9- Rowe D.E., Carroll R.J. and Day C.L.Jr: Mohs surgery is the treatment of choice for recurrent (previously treated) basal cell carcinoma. J. Dermatol. Surg. Oncol., 15: 424-31, 1989.

10- Crepaldi T., Gautreau A., Comoglio P.M., et al.: Ezrin is an effector of hepatocyte growth factor-mediated migration and morphogenesis in epithelial cells. J. Cell Biol., 138: 423-434, 1997.

11- Dear T.N., Ramshaw I.A. and Kefford R.F.: Differntial expression of a novel gene, WDNM1, in nonmetastatic rat mammary adenocarcinoma cells. Cancer Res., 48: 5203-5209, 1998.

12- Park H-R., Min S.K., Min K., et al.: Differential expression of Ezrin in epithelial skin tumors: Cytoplasmic Ezrin immunoreactivity in squamous cell carcinoma. International Journal of Dermatology, 49: 48-52, 2010.

13- Goldberg L.H.: Basal cell carcinoma. Lancet, 347: 663-67, 1996.

14- Griffiths R.W., Suvarna S.K. and Stone J.: Do basal cell carcinomas recur after complete conventional surgical excision? Br. J. Plast. Surg., 58: 795-805, 2005.

15- Wolf D.J. and Zitelli J.A.: Surgical margins for basal cell carcinoma. Arch. Dermatol., 123: 340-4, 1987.

16- Thomas D.J., King A.R. and Peat B.G.: Excision margins for nonmelanotic skin cancer. Plast. Reconstr. Surg., 112: 57-63, 2003.

17- Kimyai-Asadi A., Murad Alam, Leonard H., et al.: Eficacy of narrow-margin excision of well-demarcated primary facial basal cell carcinomas. J. Am. Acad. Dermatol., 53: 464-8, 2005.