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Mutant IDH 1 Expression in Non-Neoplastic And Neoplastic Non-Melanoma Epithelial Skin Lesions; IHC Expression And Clinicopathological Relations

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Abstract:
Background: *Cellular metabolism is known to be altered in cancer cells which switch to glycolysis and lactate fermentation for ATP production (Warburg Effect) providing a growth advantage for cancer cells. The "Warburg Effect" is mediated by dysregulation of metabolic enzymes. Isocitrate Dehydrogenase 1 (IDH1) is one of the metabolic enzymes; it catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate, with subsequent NADPH/NADH release. Recently, IDH1 mutation was discovered to be involved in multiple human cancers, such as adult gliomas, acute myeloid leukemias, prostate cancer, and colorectal cancer. This study aim to evaluate IHC expression IDH1 mutation in non-neoplastic and neoplastic non- melanoma epithelial skin lesions and correlate its expression with clinicopathological features of patients.*
Materials and methods: *Fifty patients were included in the study, twenty patients had non-neoplastic skin lesions (10 cases seborrheiac keratosis & 10 cases actinic keratosis) and thirty patients had neoplastic skin lesions (15 cases SCC & 15 cases BCC). Additionally, 5 control skin biopsies were taken from mastectomy specimens. Adequate tissue in paraffin blocks for IHC using IDH1-R132Hspecific antibody were assessed for IDH1 mutations.*
Results: *Positive mutant IDH1 IHC staining showed a cytoplasmic/nuclear staining. Increased IDH1 mutant expression was noticed in neoplastic non-melanoma compared to non-neoplastic skin lesions. IDH1 mutation was detected in 60% non-neoplastic skin cases (50 % of seborrheic keratosis and 70% of actinic keratosis) and in 73% neoplastic non-melanoma skin cases (100% of BCC and 47% of SCC). Positive staining was detected predominantly in basal cell layer in seborrheic keratosis, and scattered among various layers of epidermis in actinic keratosis. Additionally, positive staining was detected in dermal inflammatory cells and sebaceous glands. Among neoplastic skin lesions, 22/30 cases (73 %) were positive for mutant IDH1 with all 15 cases of BCC (100%) and 7 /15 cases of SCC (47 %). No statistically significant relation between mutant IDH1 and age , gender, number of sites and location of lesions was found. A statistically significant difference between expression of mutant IDH1in BCC and SCC with more intensity of staining among BCC than SCC cases was detected.*
In BCC patients, the intensity of IDH1 mutant expression (nuclear predominant over cytoplasmic staining) was marked among well differentiated BCC, and lessen among moderately and poorly differentiated BCC. Also, intensity of IDH1 mutant expression in BCC infiltrate was seen till the level of papillary dermis more than the level of reticular dermis. Regarding histopathological variants of BCC, no difference in intensity of staining was observed. In SCC patients, there was no statistically significant relation between mutant IDH1among different depths of infiltration. Although more expression of mutant IDH1 was noticed among well differentiated SCC than poorly differentiated cases, this difference was not statistically significant.
Conclusions: *IDH1 mutant expression is predominantly detected in basal cells, inflammatory cells and sebaceous glands. IDH1 mutant expression is more in skin lesions with basal cell proliferation (non neoplastic and neoplastic). IDH1 mutant expression among non-melanoma skin cancer (BCC,SCC) is more marked in BCC than SCC. In both BCC & SCC, IDH1 mutant expression decrease with loss of differentiation.*
Keywords: *IDH, IHC, skin cancer*

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I. Introduction

Isocitrate Dehydrogenase 1 (IDH1) is a member of family isocitrate dehydrogenases (IDH1-3). IDH are tricarboxylic acid cycle enzymes that play roles essential in cellular metabolism, including conversion of glucose and other nutrients into the molecule ATP within cells, to provide the energy needed for survival of cells. They catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate, with subsequent NADPH/NADH release. The $NADP^+$-dependent isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) function

at a crossroads of cellular metabolism in lipid synthesis, cellular defense against oxidative stress, oxidative respiration, and oxygen-sensing signal transduction **(1-3).**

In 2008, it was discovered that IDH1 mutations are involved in multiple human cancers. The gene is mutated in adult gliomas**(4,5),** particularly in tumors appearing in young adult patients, in acute myeloid leukemias and in other cancers**(6-8).** All mutations map to arginine residues in the catalytic pockets of IDH1 (R132) or IDH2 (R140 and R172) and confer on the enzymes a new activity. The resulting mutated protein undergoes a conformational change with subsequent loss of normal enzymatic function and the abnormal production of 2-hydroxyglutarate (2-HG) which inhibits enzymatic function of many alpha-ketoglutarate dependent dioxygenases, including histone and DNA demethylases, causing widespread changes in histone and DNA methylation and potentially promoting tumorigenesis **(9).** Effector pathways of mutant IDH remain incompletely understood and may differ between tumor types, reflecting clinical differences between these disorders**(10).**More than 100 types of tumors can occur on the skin; many o them have familial and/or inherited components, either in isolation or as part of a syndrome with other features. Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), which are known collectively as non-melanoma skin cancer, are two of the most common malignancies in the United States and are often caused by sun exposure, although several hereditary syndromes and genes are also associated with an increased risk of developing these cancers (11,12). Melanoma is less common than non-melanoma skin cancer, but 5% to 10% of all melanomas arise in multiple-case families and may be inherited in an autosomal dominant fashion(13). Many genes and hereditary syndromes are implicated in the development of cutaneous malignancy. Basal cell nevus syndrome, caused by pathogenic variants in PTCH1 and PTCH2 is associated with an increased risk of BCC, whereas syndromes such as xerodermapigmentosum (XP), oculocutaneousalbinism,epidermolysisbullosa, and Fanconi anemia are associated with an increased risk of SCC (14,15). CDKN2A is the major tumor suppressor gene associated with melanoma; pathogenic variants in CDKN2A account for 35% to 40% of all familial melanomas. Pathogenic variants in many other genes have also been found to be associated with melanoma includin, including CDK4, CDK6, BAP1, and BRCA2, (16). However, mutant IDH1expression and role in skin cancers is still not well understood.

II. Patients And Method

Patients

Fifty patients were included in the study, twenty patients had precancerous skin lesions (10cases seborrheiac keratosis &10 cases actinic keratosis) and thirty patients had cancerous skin lesions (15 cases SCC & 15 cases BCC). Additionally , 5 control skin biopsy taken from mastectomy specimen . Patients included in the study that referred from Dermatology outpatient clinic , Suez CanalUniversity Hospital, in a time period from January-2015 to September 2016,Ismailia, Egypt. Among the studied patients 22 cases were females and 28cases were males and their ages ranged from (20–50 years) and gave their written informed consent before their participation in the study.None of them received any medical treatment.The studied groups were subjected to history, complete physical examination.

Method

One section from each block was submitted, cut into 5 µm, mounted on glass slide, and stained with H&E stain. Another one serial from each block was prepared, cut into 5um thickness, mounted on positively charged glass-slides and immunostained with anti-IDH1 monoclonal antibody(Dianova, Hamburg, Germany, clone H09). The Mouse/Rabbit Immunodetector HRB/DAB Detection Kit (Bio,Sb, Santa Barbara, CA, USA) was used for the visualization of the antibody reaction.Sections were cut at 5um from paraffin embedded tissue blocks and mounted on positively charged slides and left to be dried in oven for 20 minutes at 56 degree c.Sections were then de-waxed in xylene, and rehyderatedthroughtaseries of graded alcohols. Antigen retrieval was done by using the microwave method, the slides were placed in coplin jars containing the antigen retrieval buffer (citrate buffer) ph 6.0, then the microwave was set at power 80% for 3 minutes and then at power 20% for 10 minutes then left for 20 minutes to cool and washed with phosphate buffer saline (PBS).Endogenous peroxidase activity was blocked with peroxidase blocking solution (ready to use) for 10 minutes then washed with PBS for 2 minutes.Slides are then incubated with the primary antibody after dilution with the primary antibody diluent at optimum concentration 1:200 for 2 hours at room temperature within the humidity chamber and are then washed with PBS solution 3 times for 2 minutes each. Two drops of Poly HRP enzyme conjugate are added to completely cover each slide and are incubated for 15 minutes, and are then rinsed with PBS solution 3 times for 2 minutes each. Color was developed using DAB substrate-chromogen solution that was prepared by adding DAB chromogen solution to DAB Buffer solution in a 1:1 ratio with the volume determined by the number of slides to be stained, and the incubated for 5-10 minutes at room temperature, and slides are then rinsed with tap water to remove excess substrate-chromogen solution (safe waste disposal).Mayer's haematoxylin was applied as a counter stain. The slides are then dehydrated in series of ethanol concentrations,

and mounted with DPX.Human endometrial and lymphoma tissue were used as positive control and normal brain tissue was used as negative control. Cytoplasmic intensity alone and/or along with nuclear staining were subjectively evaluated and was considered positive. The staining intensity was defined as follows:0, no staining; +1, weak; +2 moderate; +3, strong.

Statistical analysis;

For statistical analysis Fisher's Test was used to compare between other numerical data. P value was calculated using Minitab program and $P<0.05$ was considered significant while $P<0.01$ was highly significant.

III. Results

Patients clinical and demographic charateristics

Fifty patients were included in the study, twenty patients had non-neoplastic skin lesions (10 cases seborrheiac keratosis & 10 cases actinic keratosis) and thirty patients had neoplastic skin lesions (15 cases SCC & 15 cases BCC). Additionally , 5 control skin biopsy taken from mastectomy specimen . None of them received any medical treatment.Among the studied patients 22 cases were females and 28 cases were males and their ages ranged from (20–50 years),other clinic-pathological variables are summarized in (Table 1)

Table (1): Showing the clinical parameters of the studied patients

Variable	Number (%)
Age (Mean ±SD 32.5±13.31 years)	**50**
- **20-35yrs**	**12**
- **35-50yrs**	**38**
Gender	
- **Male**	**28**
- **female**	**22**
Non-neoplasticlesions	**20**
- **Seborrheiac Keratosis**	**10**
- **Actinic Keratosis**	**10**
Neoplastic Lesions	**30**
- **BCC**	**15**
- **SCC**	**15**
Duration of Disease	
- **Less than 6months**	**16**
- **6-12 months**	**24**
- **More than 1yr**	**10**
Number of sites of lesion	
- **Single One localized lesion**	**26**
- **Multiplesites of lesions**	**24**
Location of lesion	
- **Upper Extremities**	**10**
- **Face**	**19**
- **Chest**	**4**
- **Lower Extremities**	**10**
- **Others**	**7**
Total	**50**

BCC= basal cell carcinoma, SCC= Squamous cell carcinoma

As regard to cancerous patients (30 cases), 15 cases with BCC included 10 were well differentiated with solid /nested pattern and 3 cases were moderately differentiated with adenoid and pigmented pattern and 2 cases were poorly differentiated with squamoid pattern. 15 cases with SCC included 9 cases were well differentiated, 3 cases were moderately differentiated and 3 cases were poorly differentiated . All cancerous patients were in stage I (23 cases showed infiltration depth till papillary dermis, 4 cases showed infiltration till reticular dermis, and 3 cases (all were SCC) showed infiltration till subcutaneous tissue without nodal or distant metastasis, (Table 2)

Table (2); Histo-pathological features of neoplastic non- melanoma skin lesions

Variable	**Number (%)**
BCC Differentiation	15
- **Well differentiated**	10
- **Moderate differentiated**	3
- **Poor differentiated**	2
BCC Histological variant	15
- **Solid /nested**	10
- **Adenoid/pigmented**	3
- **Squamoid**	2

BCC depth of infiltration	
- **Papillary dermis**	13
- **Reticular dermis**	2
- **Subcutaneous tissue**	0
SCC Differentiation	15
- **Well differentiated**	9
- **Moderate differentiated**	3
- **Poor differentiated**	3
SCC depth of infiltration	
- **Papillary dermis**	10
- **Reticular dermis**	2
- **Subcutaneous tissue**	3
Total cancerous cases	30

Mutant IDH1 Immunohistochemical staining results;

Positive mutant IDH1 IHC staining showed a diffuse brownish cytoplasmic/nuclear staining

In the present study, human endometrial tissue served as positive control for IDH1 immunostaining andnormal human gastric tissue was considered as negative control. All 5normal control skin biopsy taken from mastectomy specimen cases (%) were negative for mutant IDH1. Among precancerous skin lesions cases (12/20 cases, 60%) were positive for mutant IDH1 with cases (5/10 cases, 50 %) of seborrheic keratosis and 7/10 cases (70%) of actinic keratosis were positive for mutant IDH1(**Figure 1&2**). Positive staining were detected as cytoplasmic/nuclear staining mainly in basal cells in seborrheic keratosis , and various layers of epidermis(not mainly basal cell layer) in actinic keratosis scattered cells in granular layer of epidermis and in dermal inflammatory cells and sebaceous glands.

Among non-melanoma cancerous skin lesions cases (22/30 cases, 73 %) were positive for mutant IDH1 with all 15 cases of BCC(100%) and 7/15 cases of SCC (47 %) (**Figure 3&4**), with more intensity of staining among BCC than SCC cases.

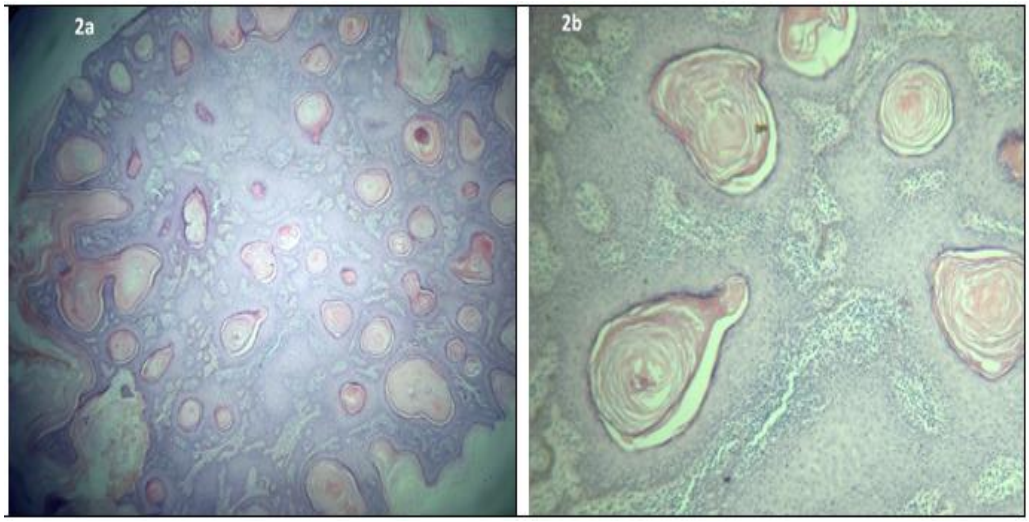


Figure 1: seborrheaic keratosis

a&bseborrheaic keratosis H&E x200 &400; hyperplastic epidermis with numerous keratin horn cysts (black astrix) , no atypia , scattered mitosis.

C&DIDH1 mutant IHC in seborrheaic keratosis: positive brownish cytoplasmic and nuclear staining mainly in basal cell layer of epidermis(black arrow).

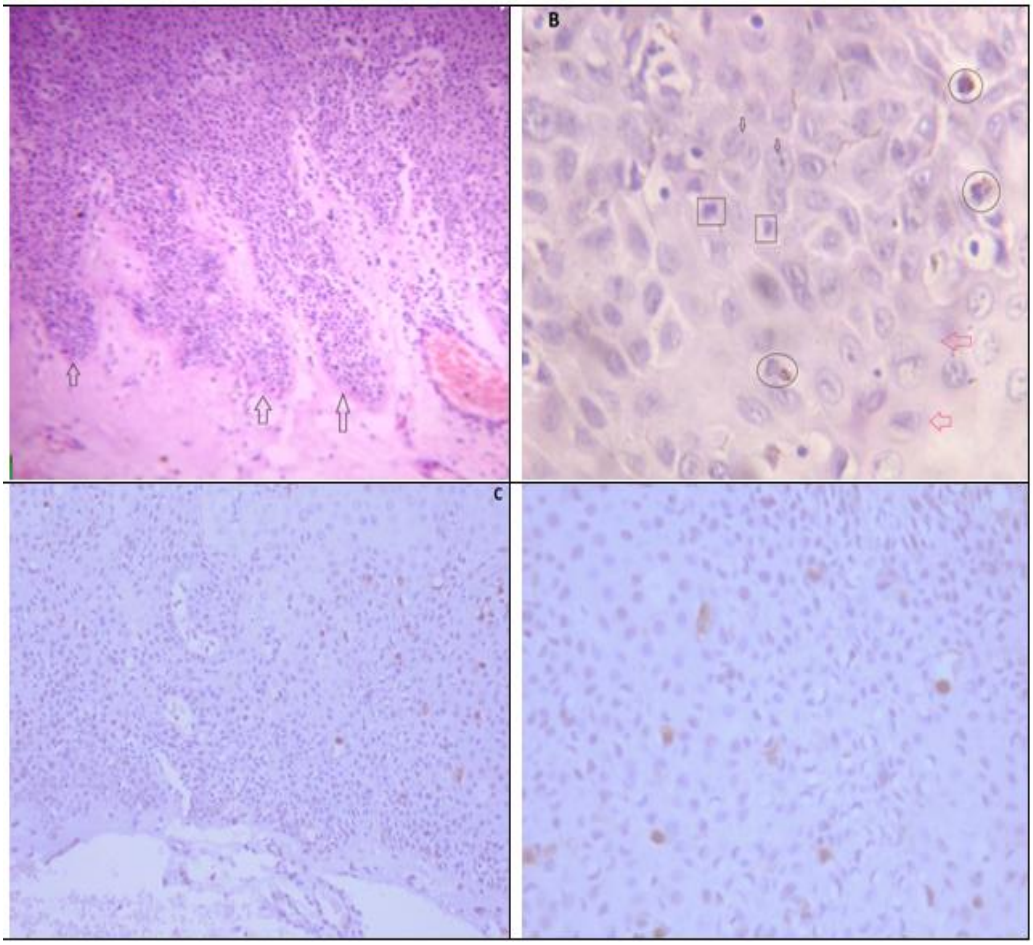
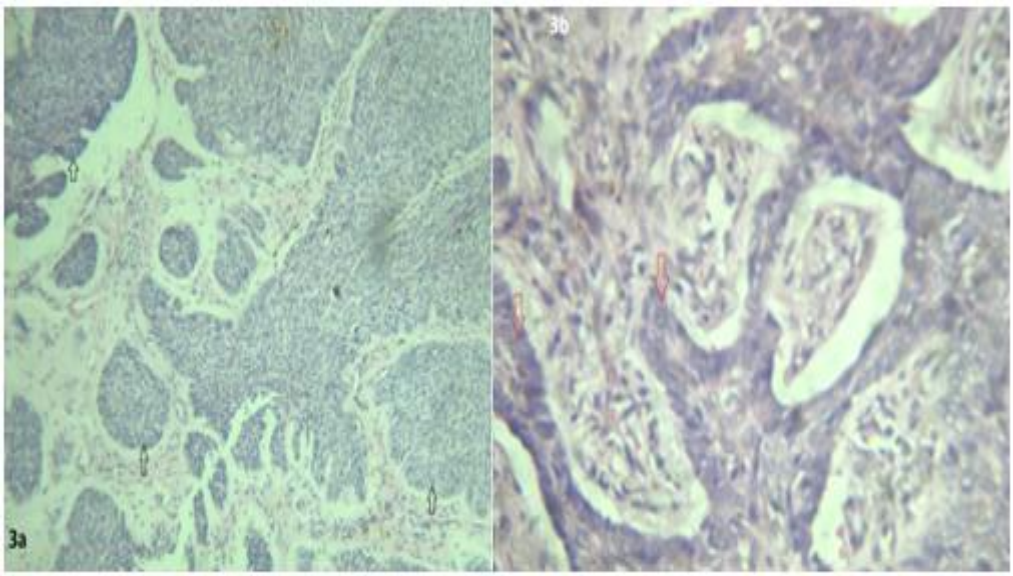


Figure 2: Actinic Keratosis

A&b H&E x200,400: The epidermis show mild hyperkeratosis with dysplasia of the basal keratinocytes and formation of small buds extending into the papillary dermis. There is prominent solar elastosis in the superficial dermis.

C&D:C&DIDH1 mutant IHC in seborrheaic keratosis: positive brownish cytoplasmic and nuclear staining scattered in various layers of epidermis not only basal cell layer.



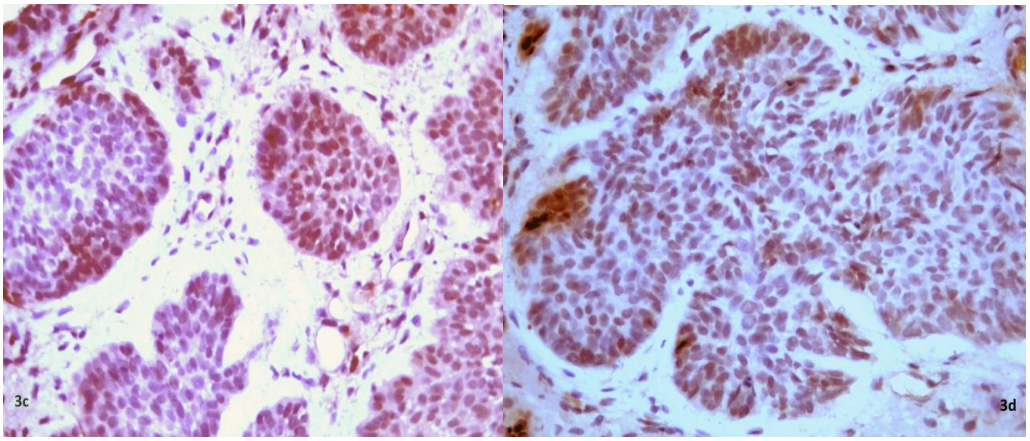


Figure 3 : BCC Cases ;

3a- BCC, H&Ex200 + 3b- BCC, H&Ex 400; nests of basaloid cells invading the underlying dermis with peripheral palisading (black arrow), stromal retraction around invading nestes , moderate inflammatory cells infiltrate in stroma.
3c- BCC& IDH1 mutant IHC stainx200, 3d x400: cytoplasmic /nuclear brownish staining in most of tumor cells and in stromal inflammatory cells

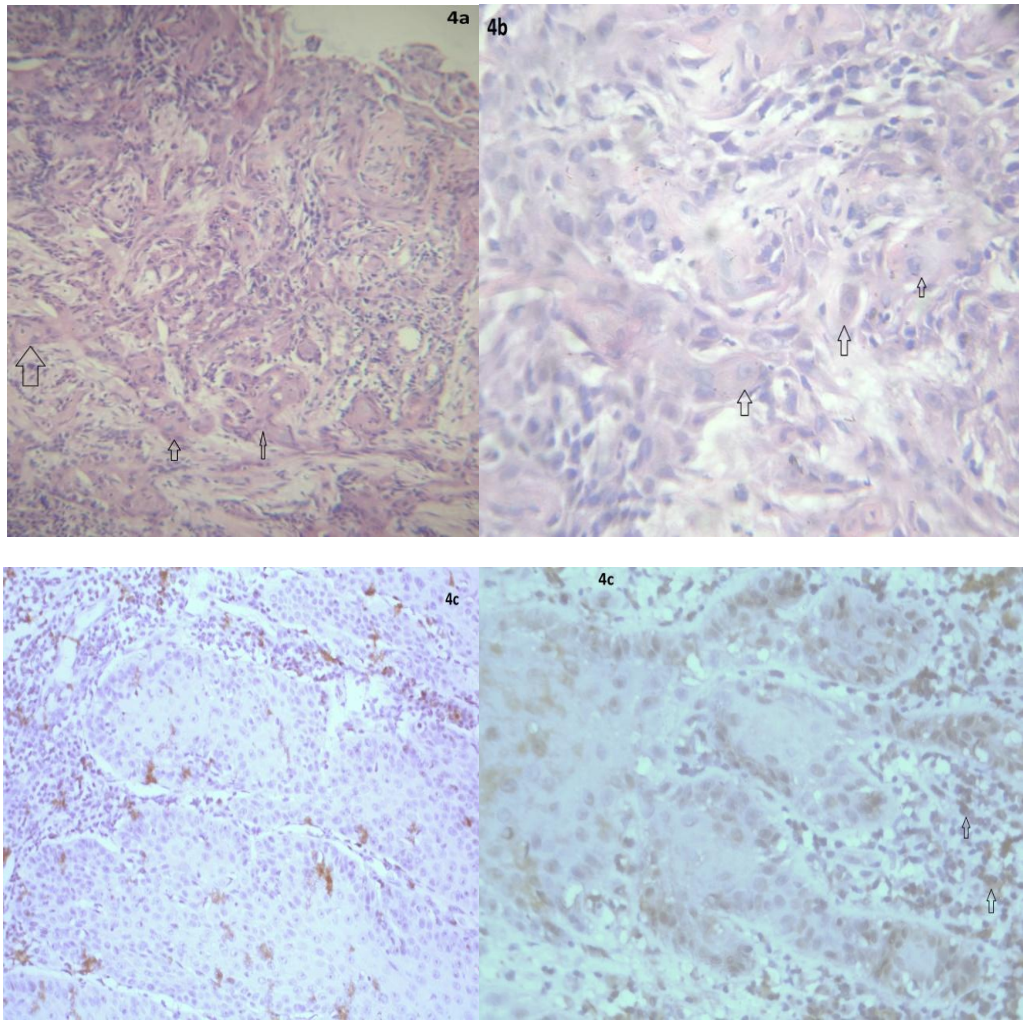


Figure 4 SCC Cases

4a-SCC H&Ex200+ 4b H&Ex400: solid nests of malignant squamoid cells infiltrating dermis showing intercellular bridging, individual keratinization (black arrow), mild nuclear pleomorphism, extensive

inflammatory cells infiltrate in surrounding stroma. 4c- IDH1 mutant IHC stain in SCC x200,4d IDH1 mutant IHC stain x 400: cytoplasmic / nuclear brownish staining of tumoral cells and extensive staining in non-neoplastic surrounding stromal inflammatory cells.

As regards of the relation between mutant IDH1 and variable clinicopathological features of studied patients , no statistically significant relation between mutant IDH1 and age, gender, number of sites and location of lesions (**Table3**).

Table (3) ; Relation between IDH1 mutant protein IHC staining and variable clinicopthological features of studied patients (p value)

Variable	IDH1 mutant Staining		P value
	+ve	-ve	
Age			
- 20-35yrs	**8**	**4**	1.0000
- 35-50yrs	**24**	**14**	
Gender			
- Male	**16**	**12**	0.0761
- female	**18**	**4**	
Number of sites of lesion			
- Single One lesion	**19**	**7**	1.0000
- Multiple sites of lesions	**17**	**7**	
Duration of Disease			
- Less than 6months	**8**	**8**	0.2106
- More than 6 months	**24**	**10**	
Location of lesions			
- Extremities (upper &lower)	**9**	**11**	0.2514
- Others (chest & face&…)	**19**	**11**	
Precancerous			
- Sebborrheic keratosis	**5**	**5**	
- Actinic keratosis	**7**	**3**	
Cancerous			0.3662
- BCC	**15**	**0**	
- SCC	**7**	**8**	

In spite more positive staining of mutant IDH1among cancerous skin cases than precancerous skin cases, there was no statistically significant relation between mutant IDH1among precancerous and cancerous skin lesions (p= 0.3662). A statistically significant difference between expression mutant IDH1in BCC and SCC (p= 0.0022) was detected. In BCC patients, the intensity of IDH1 mutant expression (nuclear predominant over cytoplasmic staining) in BCC were marked among well differentiated BCC , and lessen among moderately and poorly differentiated BCC. Also, more intensity of IDH1 mutant expression in BCC infiltrate till papillary dermis than BCC infiltrate till reticular dermis. As regard to histopathological variants of BCC no difference in intensity of staining. In SCC patients, there were no statistically significant relation between mutant IDH1among different depth of infiltration. Although more expression of mutant IDH1 among well differentiated SCC than poorly differentiated cases , this difference has no statistically significant relation (**Table 4).**

Table (4) ; Relation between IDH1 mutant protein IHC staining and variable histopthological features of cancerous studied patients (p value)

Variable	IDH1 mutant Staining		P value
	+ve	-ve	
Cancerous			
- BCC	**15**	**0**	*0.0022
- SCC	**7**	**8**	
SCC Differentiation			
- Well differentiated	**5**	**4**	0.6084
- Moderate differentiated	**1**	**2**	
- Poor differentiated	**1**	**2**	
SCC depth of infiltration			
- Papillary dermis	**6**	**4**	
- Reticular dermis	1	**1**	0.2821
- Subcutaneous tissue	**0**	**3**	

IV. Discussion

In cancer cells cellular metabolism is known to be altered. Cancer cells switch to glycolysis and lactate fermentation for ATP production (the "Warburg Effect). This metabolic switch provides a growth advantage for cancer cells. The "Warburg Effect" is mediated by dysregulation of metabolic enzymes. Isocitrate Dehydrogenase 1 (IDH1) is one of the metabolic enzyme catalyze the oxidative decarboxylation of isocitrate to

α-ketoglutarate, with subsequent NADPH/NADH release. Recently, IDH1 mutation was discovered to be involved in multiple human cancers, in 20% of adult gliomas, 10% of acute myeloid leukemias , prostate cancer, and colorectal cancer (**17-19**). Our study detected IDH1 mutation using IHC in 60% non-neoplastic skin cases (with 50 % of seborrheic keratosis 70% of actinic keratosis) and in 73% non-melanoma cancerous skin cases (with in all basal cell carcinoma and 47% of squamous cell carcinoma cases).

Although IDH1 is localized in the cytoplasm and in peroxisomes, we observed in both non neoplastic and neoplastic cells nuclear as well as cytoplasmic anti-IDH1-R132H immunostaining, as has been previously documented.it is unclear whether IDH1 protein is truly localized in the nucleus in vivo or whether the soluble protein penetrates the nucleus during tissue processing (an effect referred to as "antigen diffusion") (**20**). Positive staining were detected predominantly in basal cell layer in seborrheickeratosis , and scattered among various layers of epidermis(not only basal cell layer) in actinic keratosis. Additionally, positive staining were detected in dermal inflammatory cells and sebaceous glands.

Previous studies also noticed that non-neoplastic systemic organs showed positivity in the cytoplasm alone: the myocardium, peribronchial glands, interlobular ducts of the salivary gland, gastric fundic gland, columnar epithelia of the large bowel, hepatocytes, centroacinar cells, the intercalated ducts of the pancreas, renal proximal and distal tubules, adrenocortex, ovarian granulosa cells, and the choroid plexus epithelial (**21,22**). It was concluded that the immunopositivity detected in non-neoplastic systemic organs was due to cross-reactivity, because immunohistochemistry with an anti-mitochondrial antibody revealed that the mIDH1R132H staining pattern was identical to that of the mitochondria.These cells are rich in mitochondria containing IDH2, IDH3A, IDH3B, and IDH3G, and there is a possibility that mIDH1R132H cross-reacts with other subtypes of IDH. The mIDH1R132H developer targeted the region of the IDH1 amino acid sequence from codon 125 to 137 (CKPIIIGHHAYGD), which contains the R132H mutation. This sequence is similar to that of the region from codon 165 to 177 (TKPITIGRHAHGD) containing the substrate binding and enzymatic reaction sites of IDH2. We then carried out an immunohistochemical examination of non-neoplastic systemic organs with an anti-mitochondrial antibody. As a result, the localization of mIDH1R132H positivity in the renal proximal tubules, adrenocortical cells, choroid plexus epithelia, etc., was found to be identical to that of the mitochondria. Based on these considerations, we concluded that mIDH1R132H positivity in the cytoplasm of non-neoplastic systemic organs represents cross-reactivity. "True" mIDH1R132H positivity in gliomas was seen in both the cytoplasm and nuclei. Therefore, mIDH1R132H positivity should only be considered to be validated when a cell shows both cytoplasmic and nuclear staining (**23,24**).We observed that an increased in IDHI mutant protein in neoplastic skin lesions than non-neoplastic skin lesions**,** that may pointed to the suspected role of IDH1 mutation in tumoriogenesis.

Park and colleagues (**25**) have suggested the role of IDH1 and IDH2 as protectors against various insults. They have shown that IDH1 or IDH2 mutation increase lipid peroxidation, oxidative damage o DNA, intracellular peroxide generation, and decreased survival after oxidant exposure. More expression of IDH1 mutant protein by IHC in BCC than SCC may referred to the different biological behavior between the two commenest non-melanoma skin cancers and therefor, need different modalities in treatment.

References

[1]. Gabriel JL, Zervos PR, Plaut GW.Activity of purified NAD-specific isocitrate dehydrogenase at modulator and substrate concentrations approximating conditions in mitochondria.Metabolism.1986;35(7):661–667.

[2]. Jennings GT, Sechi S, Stevenson PM, Tuckey RC, Parmelee D, McAlister-Henn L. Cytosolic NADP(+)-dependent isocitrate dehydrogenase. Isolation of rat cDNA and study of tissue-specific and developmental expression of mRNA.J Biol Chem. 1994;269(37):23128–23134.

[3]. Nekrutenko A, Hillis DM, Patton JC, Bradley RD, Baker RJ. Cytosolic isocitrate dehydrogenase in humans, mice, and voles and phylogenetic analysis of the enzyme family.MolBiolEvol.1998;15(12):1674 –1684.

[4]. Sanson M, Marie Y, Paris S, et al. Isocitrate dehydrogenase 1 codon 132 mutation is an important prognostic biomarker in gliomas. J ClinOncol.2009;27(25):4150–4154.

[5]. Bleeker FE, Lamba S, Leenstra S, et al. IDH1 mutations at residue p.R132 (IDH1(R132)) occur frequently in high-grade gliomas but not in other solid tumors. Hum Mutat. 2009;30(1):7–11.

[6]. Kang MR, Kim MS, Oh JE, et al. Mutational analysis of IDH1 codon 132 in glioblastomas and other common cancers. Int J Cancer. 2009;125(2):353–355.

[7]. Park SW, Chung NG, Han JY, et al. Absence of IDH2 codon 172 mutation in common human cancers. Int J Cancer. 2009;125(10):2485–2486.

[8]. Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med. 2009;361(11):1058–1066.

[9]. Gross S, Cairns RA, Minden MD, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. J Exp Med. 2010;207(2):339–344.

[10]. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature.2009;462(7274):739–744.

[11]. Hartong DT, Dange M, McGee TL, Berson EL, Dryja TP, Colman RF. Insights from retinitis pigmentosa into the roles of isocitrate dehydrogenases in the Krebs cycle. Nat Genet. 2008;40(10):1230–1234.

[12]. Jo SH, Lee SH, Chun HS, et al. Cellular defense against UVB-induced phototoxicity by cytosolic NADP(+)-dependent isocitrate dehydrogenase. BiochemBiophys Res Commun.2002;292(2):542–549.

[13]. Liu X, Ling ZQ (Oct 2015). "Role of isocitrate dehydrogenase 1/2 (IDH 1/2) gene mutations in human tumors". Histology and Histopathology. **30** (10): 1155–60.
[14]. Vissers LE, Fano V, Martinelli D, Campos-Xavier B, Barbuti D, Cho TJ, et al. (Nov 2011). "Whole-exome sequencing detects somatic mutations of IDH1 in metaphysealchondromatosis with D-2-hydroxyglutaric aciduria (MC-HGA)". American Journal of Medical Genetics Part A. **155A** (11): 2609–16.
[15]. Shih AH, Abdel-Wahab O, Patel JP, Levine RL (Sep 2012). "The role of mutations in epigenetic regulators in myeloid malignancies". Nature Reviews. Cancer. **12** (9): 599–612.
[16]. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. (Feb 2009)."IDH1 and IDH2 mutations in gliomas". The New England Journal of Medicine. **360** (8): 765–73.
[17]. Guo C, Pirozzi CJ, Lopez GY, Yan H (Dec 2011). "Isocitrate dehydrogenase mutations in gliomas: mechanisms, biomarkers and therapeutic target". Current Opinion in Neurology. **24** (6): 648–52.
[18]. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al. (Sep 2009)."Recurring mutations found by sequencing an acute myeloid leukemia genome". The New England Journal of Medicine. **361** (11): 1058–66.
[19]. Turkalp Z, Karamchandani J, Das S (Oct 2014). "IDH mutation in glioma: new insights and promises for the future". JAMA Neurology. **71** (10): 1319–25.
[20]. Kim HJ, Fei X, Cho SC, Choi BY, Ahn HC, Lee K, Seo SY, Keum YS (Dec 2015). "Discovery of α-mangostin as a novel competitive inhibitor against mutant isocitrate dehydrogenase-1". Bioorganic & Medicinal Chemistry Letters. **25** (23): 5625–31.
[21]. Capper D, Zentgraf H, Balss J et al (2009) Monoclonal antibody specific for IDH1 R132H mutation. ActaNeuropathol 118:599–601.
[22]. Capper D, Weissert S, Balss J et al (2010) Characterization of R132H mutation-specific IDH1 antibody binding in brain tumors. Brain Pathol 2010:245–254.
[23]. Xu X, Zhao J, Xu Z, Peng B, Huang Q, Arnold E, Ding J (Aug 2004). "Structures of human cytosolic NADP-dependent isocitrate dehydrogenase reveal a novel self-regulatory mechanism of activity". The Journal of Biological Chemistry. **279** (32): 33946–57.

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