C-KIT EXPRESSION IN MALIGNANT EPIDERMAL CUTANEOUS TUMORS

SDUAHER

By

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IN
PATHOLOGY

Under the guidance of

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OCTOBER 2015

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Date: 25/5/15

Place: Kolar

Signature of the Candidate

Dr. SHARITHA M N

Dedicated with

REVERENCE

to

My FAMILY

LIST OF ABBREVIATIONS

Sl No	Abbreviation	Expansion
1.	BAX	BCL-2 ASSOCIATED X PROTEIN
2.	bcl-2	B CELL LYMPHOMA 2
3.	BCC	BASAL CELL CARCINOMA
4.	CDKN2A	CYCLIN DEPENDENT KINASE INHIBITOR 2A
5.	CD	CLUSTER OF DIFFERENTIATION
6.	CEA	CARCINOEMBRYONIC ANTIGEN
7.	CK	CYTOKERATIN
8.	DAB	DIAMINO BENZIDINE
9.	DNA	DE OXY RIBONUCLEIC ACID
10.	DPX	DISTERNE, PLASTICISER, XYLENE
11.	EMA	EPITHELIAL MEMBRANE ANTIGEN
12.	Gli 1	GLIOBLASTOMA 1
13.	GIST	GASTROINTESTINAL STROMAL TUMOR
14.	H&E	HEMATOXYLIN & EOSIN
15.	HLA DR7	HUMAN LEUKOCYTE ANTIGEN
16.	HPV	HUMAN PAILLOMA VIRUS
17.	HRP	HORSE RADISH PEROXIDASE
18.	IHC	IMMUNOHISTOCHEMISTRY
19.	MDM2	MOUSE DOUBLE MINUTE HOMOLOGUE 2
20.	MIT-F	MICROPHTHALMIA TRANSCRIPTION FACTOR
21.	MM	MALIGNANT MELANOMA
22.	M-CSF	MONOCYTE-COLONY STIMULATING FACTOR
23.	NMSC	NON MELANOMA SKIN
24.	PCR	POLYMERASE CHAIN REACTION
25.	PDGF	PLATELET DERIVED GROWTH
26.	PTCH	PROTEIN PATCHED HOMOLOGUE 1
27.	PI3K	PHOSPHOTIDYLINOSITOL3 KINASE

28.	PTEN	PHOSPHATASE AND TENSIN HOMOLOGUE
29.	RAS	RAT SARCOMA
30.	SCC	SQUAMOUS CELL CARCINOMA
31.	SCF	STEM CELL FACTOR

ABSTRACT

INTRODUCTION:

C-kit is a tyrosine kinase receptor and its role in cancer is important as it has been used as a target for drugs that inhibit their activity. C-Kit (CD117) is normally expressed in basal cells of epidermis and also in the melanocytes. Alterations in C-Kit expression is seen in variety of neoplasms like mastocytosis, germ cell tumors, endometrial carcinoma, ovarian tumors and adenocarcinoma of prostate.

OBJECTIVES OF THE STUDY:

1. To evaluate C-kit expression in malignant epidermal tumors.
2. To correlate C-kit expression with age, sex, tumor differentiation, histological type, size of the tumor and level of invasion.

MATERIALS AND METHODS:

Fifty three cases of formalin fixed, paraffin embedded tissue blocks of SCC, BCC and MM were retrieved from the archives of Department of Pathology, SDUMC, Kolar. Medical records were reviewed to get clinical details like age, sex, clinical presentation and size of the tumor. Immunohistochemistry was done using C-kit monoclonal antibody. H & E slides were reviewed to record the histological type and Clarke's level of invasion. IHC stained slides were evaluated for C-kit expression.

RESULTS:

Fifty three cases were included in the study, out of which 58% were SCC and 21% were BCC and MM each. Male to female ratio in SCC, BCC and MM were 2.03:1, 0.57:1 and 1.7:1 respectively. Predominant age group affected in SCC was 61-70yrs, 51-60yrs in BCC and MM. Most common affected site was leg and foot in SCC(38.7%), infra orbital in BCC(36.3%) and sloe and heel in MM(73%).Clinically, 61.2% of SCC presented as ulceroproliferative growth, 54.5% of BCC presented as ulcerative lesions and 54.5% of MM presented as ulceroproliferative growth.WDSCC constituted 72.4% of SCC and 64% of BCC were of nodular type. In MM, 64% of cases showed Clarke's level of invasion-IV. C-kit was detected in 41.85% of SCC, 72.68% of BCC and 91.29% of MM.

CONCLUSION:

C-kit expression was detected in 41.85% of SCC, 72.68% of BCC and 91.29% of MM. Weak and moderate C-kit expression was seen in SCC and BCC. 54.5% of MM showed strong C-kit expression and 9.09% showed weak and moderate expression. There was no correlation between the age and sex of the patient, size of the tumor, different grades of differentiation, histological type and C-kit expression in squamous cell carcinoma and basal cell carcinoma. There was no significant association between Clarke's level of invasion and C-kit expression in malignant melanoma

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C-KIT EXPRESSION IN MALIGNANT EPIDERMAL CUTANEOUS TUMORS

INTRODUCTION:

Cutaneous tumors are divided into tumors of epidermis, skin adnexa, melanocytes, neuro endocrine cells and the dermis. Malignant tumors of the epidermis include squamous cell carcinoma and basal cell carcinoma. Malignant tumors of the melanocytes include malignant melanoma.

Immunohistochemistry is an important auxiliary tool for diagnosis and differential diagnosis, and for predicting the outcome of many skin tumors. Tumor cells in basal cell carcinoma are positive for bcl-2, Ber-EP4, CK5 and CK17 and CD10. Expression p53 is related to aggressive variants of BCC.[1] Keratinocytes in squamous cell carcinoma are reactive for HMW cytokeratins than BCC. Tumor cells in SCC are positive for EMA and CK5/6 [2]. Melanoma cells are positive for S-100, HMB-45, Melan-A and Mit-f [1].

C-Kit (CD117) is an immunohistochemical marker which is normally expressed in basal cells of epidermis and also in the melanocytes. Cells with C-kit possess unique properties of cancer stem cells including self-renewal, differentiation, high tumorigenic potential and chemo resistance [1]. C-kit is a tyrosine kinase receptor and its role in cancer is important as it has been used as a target for drugs that inhibit their activity. C-kit expression in GIST has been well documented and target therapy with Imatinib mesylate is proven.[3]

Alterations in C-Kit expression are seen in variety of other neoplasms like mastocytosis, germ cell tumors, endometrial carcinoma, ovarian tumors and adenocarcinoma of prostate[3, 4, 5]. The expression of C-kit has been found in squamous cell carcinoma of head and neck and vulva[6,7].Various studies have been done to determine the C-kit expression in malignant melanomas and recently, in basal cell carcinoma[8,9]. However, limited studies have been done to see its expression in cutaneous squamous cell carcinoma.

C-kit is a potential target for site specific therapy in certain solid tumors especially like gastrointestinal stromal tumors. Though C-kit expression has been studied in other tumors in India, there are a very few studies demonstrating the expression of C-kit in cutaneous tumors. The identification of this marker in cutaneous malignances may possibly lead to the development of effective treatment protocols.

Although numerous studies have been to done to evaluate C-kit expression in cutaneous tumors in other countries, extensive review of literature revealed limited study done in India to evaluate the same.

OBJECTIVES OF THE STUDY:

1. To determine the expression of c-kit in various malignant epidermal cutaneous tumors

2. To correlate c-kit expression with age, sex, size of the tumor, tumor differentiation, histological type and level of invasion.

Squamous cell carcinoma:

SCC and BCC are together called as non melanoma skin cancers. Although BCC is the most common carcinoma worldwide, several studies have indicated SCC as the most common carcinoma in India[10] BCC and SCC originate either in the basal cells or squamous cells of the epidermis. . It is most commonly seen in the elderly individuals, and rare in childhood and adolescence.[11] Risk factors for the development of SCC include sun exposure and chronic inflammation. NMSC is an increasing problem for health care services worldwide which causes significant morbidity. The rising incidence rates of NMSC are probably caused by a combination of increased exposure to ultraviolet (UV) or sun light, increased outdoor activities, changes in clothing style, increased longevity, ozone depletion, genetics and in some cases, immune suppression. Most squamous cell carcinomas arise in areas of direct exposure to the sun such as forehead, face, neck and dorsum of hands. Ears, scalp and vermillion part of the lower lip may also be involved. After sun exposure, the next common cause is squamous cell carcinoma arising in the scars in burns and stasis ulcers, termed as marjolin's ulcer. It may also occur at sites of chronic ulceration, trauma, burns, frost bite, fistula tracts, tattoos, vaccination scars, pilonidal sinus and epidermal cyst.it is also seen in organ transplant recipients, where scalp is the most common site affected.[1] Metastasis in squamous cell carcinoma arising from sun damaged skin is lower compared to the metastasis rates in carcinomas arising at the site of chronic inflammation, scars or in organ transplant recipients.[2] Less important etiologic agents include radiation therapy, arsenic exposure, coal tar, smoking and hydrocarbons. Human papilloma virus may play a role in immunosuppressed individuals, genital lesions and digital squamous cell carcinoma.[1]

As with other cancers, SCC exhibits impaired genomic maintenance that facilitates acquisition of new mutations. The mechanism leading to genomic instability in keratinocytes likely results from

UVB induced inactivation of P53, since most SCC's harbor UVB mutations such as CC to TT and C to T transitions.[12]

Ultra violet radiation damages the DNA of epidermal cells and causes mutation of P53 gene, which is a tumor suppressor gene. Mutant P53 accumulates in the nucleus and the expansion of P53 correlates with sun exposure and sun damage. Heat shock protein 105, epidermal growth factor receptors and COX-2 expression is seen in minority of tumors.[1]

In addition to mutation in P53 gene, loss of expression of CDKN2A may result in tumor progression in squamous cell carcinomas. Also, dysregulated DNA mismatch repair may be seen in a few squamous cell carcinomas.[1]

Clinically, squamous cell carcinoma of the skin most commonly presents as a shallow ulcer surrounded by a wide, elevated, indurated border. Often the ulcer is covered by a crust that conceals a red, granular base. Occasionally, raised, fungoid, verrucous lesions without ulceration occur.[2]

The conditions which are considered morphological expressions of squamous cell carcinoma include solar keratosis and its analogues- arsenical and radiation keratoses, Bowen's disease and bowenoid papulosis, giant condyloma and verrucous carcinoma and proliferating trichilemmal cysts.[2]

PATHOLOGY OF SQUAMOUS CELL CARCINOMA:

Histopathologically, invasive SCC will frequently bear close resemblance to their precursor AK lesions, but can be distinguished from the latter via the presence of infiltrative cells passing through the basement membrane into the dermis. SCCIs can be subdivided into three broad histologic grades based on their associated degree of nuclear atypia and keratinization. The majority of SCCI's are well differentiated, with tumor cells containing only slightly enlarged, hyper chromatic nuclei with abundant amounts of cytoplasm. They produce large amounts of keratin, resulting in the formation of extracellular keratin pearls. Intercellular bridges are frequently visible. These tumors are generally associated with a very low-malignant potential, with the likelihood of metastasis being approximately 0.5%. In contrast, SCCI can also present as a poorly differentiated tumor with greatly enlarged, pleomorphic nuclei demonstrating a high degree of atypia and frequent mitoses. Keratin production in these cells are markedly reduced. It usually demonstrates a much more aggressive clinical behavior, with an increased rate of metastasis and recurrence. A third, moderately differentiated subtype exists which share features of both well-differentiated and poorly differentiated tumors.[13]

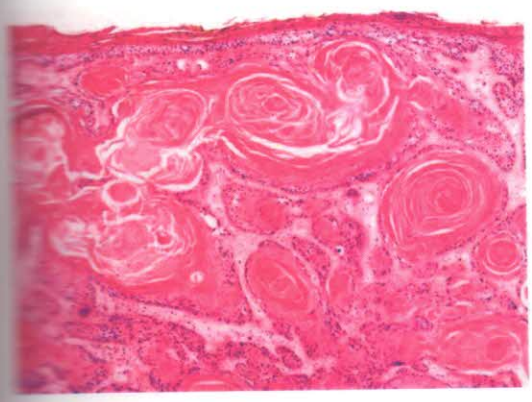


FIG 1: WELL DIFFERENTIATED SCC

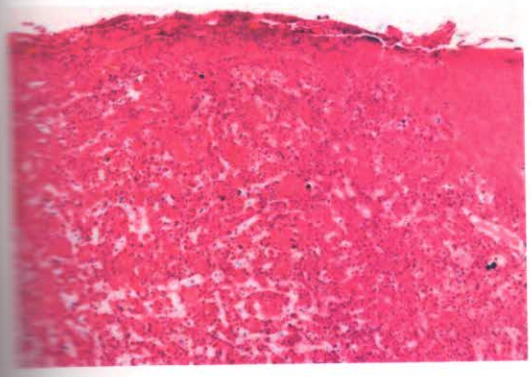


FIG 2: MODERATELY DIFFERENTIATED SCC

HISTOLOGICAL VARIANTS OF SQUAMOUS CELL CARCINOMA: [1,2,11]

Spindle-cell squamous cell carcinoma:

It is an uncommon variant of squamous cell carcinoma that arises in sun damaged and irradiated skin.it is also most commonly associated with SCC arising in organ transplant recipients. They may be entirely composed of spindle cells or may have a well differentiated squamous cell component. The spindle cells have vesicular nuclei, scanty eosinophilic cytoplasm and indistinct cell borders with variable pleomorphism and many mitotic figures. Some spindle cell SCC may co-express cytokeratin and vimentin suggesting a metaplastic change in squamous cell carcinoma.

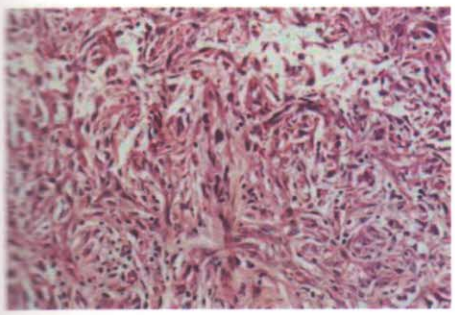


FIG 3: SPINDLE CELL SCC

ADENOID SQUAMOUS CELL CARCINOMA:

SCC may show tubular and alveolar formation as a result of dyskeratosis and acantholysis. These lesions have been termed as adenoid squamous cell carcinoma or acantholytic squamous cell carcinoma. Histologically, there are tubular and alveolar lumina lined with one or several layers of epithelium. In areas in which the lumina are lined with a single layer of epithelium, the epithelial cells resemble glandular cells, but in areas with several layers of epithelium, squamous and partially keratinized cells usually form the inner layers. The lumina are filled with desquamated acantholytic cells, many of which are partially or fully keratinized. These tumors represent

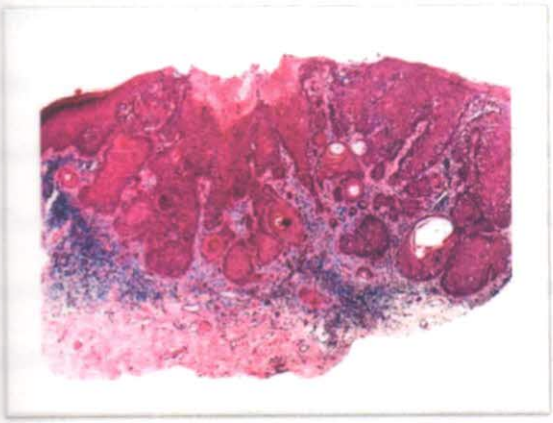


FIG 4:ACANTHOLYTIC SCC,SCANNER

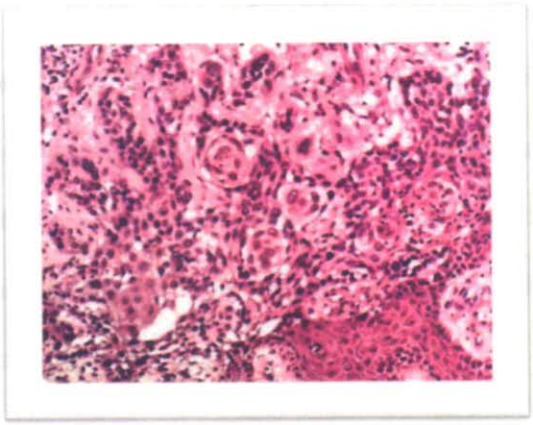


FIG 5: ACANTHOLYTIC SCC, LOW POWER

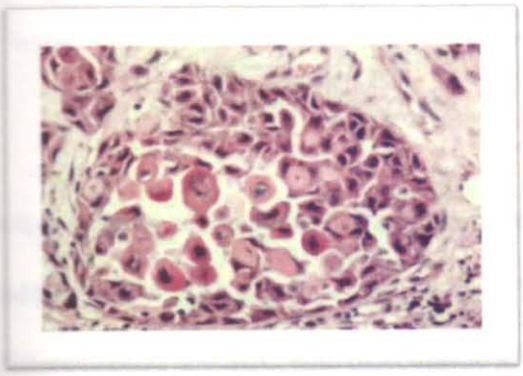


FIG 6: ADENOID SCC, HIGH POWER

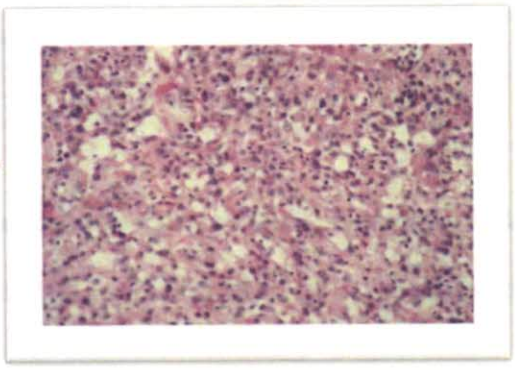


FIG7: PSEUDOVASCULAR ADENOID SCC

MUCIN PRODUCING SQUAMOUS CELL CARCINOMA:

This variant is associated with aggressive clinical course. This tumor has different designations like mucoepidermoid carcinoma and adenosquamous carcinoma. Varying numbers of mucin-producing cells are found within tumors that have the appearance of a squamous cell carcinoma. These cells generally appear large and pale and stain positively with the PAS method and with mucicarmine. Occasionally, true glandular lumina are present. Some of the lumina resemble distorted eccrine ducts and stain positively for carcinoembryonic antigen.

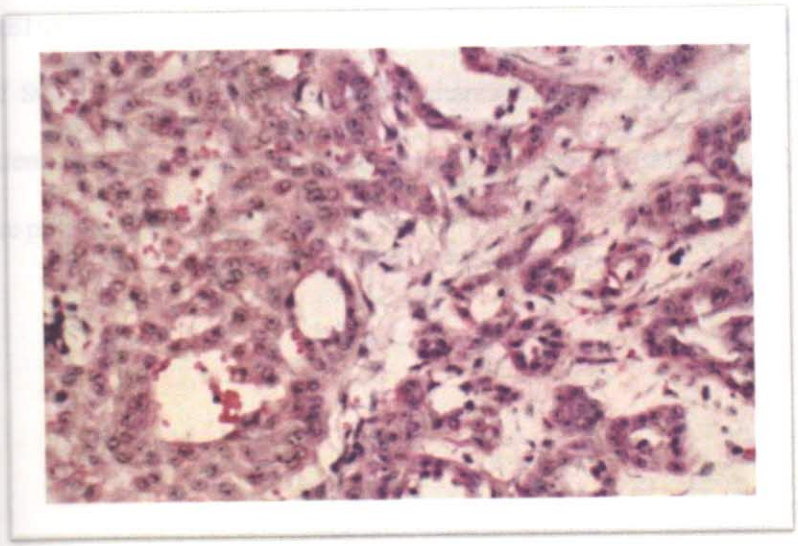


FIG 8: ADENOSQUAMOUS CARCINOMA

Other variants of squamous cell carcinoma include clear cell and signet ring SCC, pigmented SCC, inflammatory SCC, pseudo hyperplastic SCC, basaloid SCC, follicular SCC, SCC with rhabdoid differentiation and SCC with osteoclastic giant cells.[1]

IMMUNOHISTOCHEMISTRY IN SCC:

Variants of squamous cell carcinoma like adenoid SCC, mucin producing SCC and spindle cell cutaneous squamous cell carcinoma may be difficult to diagnose by H & E stain. The SCC is positive for high molecular weight cytokeratin and EMA.spindle cell cutaneous SCC should be differentiated from desmoplastic melanoma, leiomyosarcoma and atypical fibroxanthoma.in a study, a panel of IHC markers were used to differentiate between these tumors. S-100 was positive in melanoma, smooth muscle actin and desmin showed positivity in leiomyosarcoma and P63, AE1/AE3 was positive in squamous cell carcinoma. Adenosquamous carcinoma is a rare variant of SCC, arising from acrosyringia, characterized by formation of mucin secreting glands. In adenosquamous carcinoma, tumor cells are positive for EMA and CK and cells forming the glands are positive for CEA.[14, 15]

TABLE 1: HISTOLOGICAL DIFFERENCES BETWEEN LOW AND HIGH GRADE SCC: [1]

Low-Grade SCC
Well to moderately differentiated: intercellular bridges and keratin pearls
Tumor cells arranged in solid or sheet-like patterns
Association with solar damage and precursor actinic keratosis
Diameter less than 2 cm
Depth less than 2 mm
High-Grade SCC
Poorly differentiated: clear-cell, sarcomatoid, or single cell features
Presence of infiltrating individual tumor cells
Arising *de novo* or in site of prior injury (ulcer, burn scar, or osteomyleitis)
Perineural and/or perivascular invasion
Diameter greater than 2 cm
Depth greater than 2 mm

BASAL CELL CARCINOMA:

Basal cell carcinoma is the most common non melanoma cutaneous cancer worldwide. But, its incidence is only second to squamous cell carcinoma in India.[10] It shows a locally invasive behavior and low metastatic potential and easily treatable by surgery. Cells of BCC originate from pluripotent cells of inter follicular epidermis and outer sheath of hair follicle.[16] Basal cell carcinomas generally occur in adults, although they may be seen in children. Males are more commonly affected than females. BCC is more predominantly seen in areas of skin exposed to sun. Most frequently, they occur in head and neck region. Rarely, they may involve shoulders, back or chest.[1] Three rare forms of basal cell carcinoma is associated with early onset. They are linear, unilateral basal cell nevus, nevoid basal cell carcinoma syndrome and Bazex syndrome.[11] The most common predisposing factor for basal cell carcinoma is a light skin color in association with prolonged exposure to strong sunlight. The predisposing effect of sun exposure is particularly evident in patients with xeroderma pigmentosum, in whom both basal cell carcinoma and squamous cell carcinoma are common. Additional factors that predispose a person to develop basal cell carcinoma are increased exposure to X- rays and, less commonly, burn scars and other scars.[17] The risk factors for BCC recurrence are: large size of a tumor (over 2 cm); particular localization – central facial site (periocular, perioral, nasal); perineural and perivascular invasion; aggressive histology (morphoeic, micronodular); and prior recurrence of BCC.[17]

Morphological classification of BCC includes: nodular (with micronodular), infiltrative (with morphoeic), superficial and mixed subtype. The nodular subtype occurs most commonly on the head (mainly the nose and forehead), neck and upper back, while the micronodular subtype occurs most commonly around the eyes. The morpheaform localizes mainly on the nose, eye angles, forehead and cheeks. It is very rare on the trunk. The lesions of superficial BCC, usually multifocal,

are localized on the trunk. In some cases superficial BCC may appear on the head, within the parietal part of the scalp.[17]

According to Lever's, five clinical types of basal cell carcinoma occur: (a) noduloulcerative basal cell carcinoma, including rodent ulcer, by far the most common type; (b) pigmented basal cell carcinoma; (c) morphea-like or fibrosing basal cell carcinoma; (d) superficial basal cell carcinoma; and (e) fibroepithelioma.[2]

The nevoid basal cell carcinoma syndrome is an autosomal dominant disorder with low penetration. Small nodules appear between puberty and 35 years of age, and there may be hundreds or thousands of them. They are haphazardly distributed over the face and body. During adulthood, many of the basal cell carcinomas undergo ulceration. Occasionally, even death occurs as the result of invasion of an orbit and of the brain. There also may be metastases to the lung. Half of adult patients with the nevoid basal cell carcinoma syndrome show numerous palmar and plantar pits 1 to 3 mm in diameter. These pits usually develop during the second decade of life. Most patients show multiple skeletal and central nervous system anomalies among which are odontogenic keratocysts of the jaws, anomalies of the ribs, scoliosis, mental retardation, and calcification of the falx cerebri. In several reported cases, there were also cerebellar medulloblastomas or fibrosarcomas of a mandible or maxilla. In the jaw cysts, an ameloblastoma may arise.[2]

The Bazex syndrome is dominantly inherited and shows as its main features (a) follicular atrophoderma characterized by widened follicular openings like mainly on the extremities; and (b) multiple, small basal cell carcinomas on the face, and usually arising first in adolescence or early adulthood, but occasionally in late childhood. In addition, there may be localized anhidrosis or generalized hypohidrosis, and congenital hypotrichosis on the scalp and elsewhere.[11]

PATHOGENESIS OF BASAL CELL CARCINOMA:

The usual site of origin of basal cell carcinoma appears to be the surface epidermis. Occasionally, however, the tumor may originate from the outer root sheath of a hair follicle. There is increasing evidence to suggest genetic factors play an important role in susceptibility of few individuals to basal cell carcinoma. Patched homologue 1 gene mutations are seen in both nevoid BCC and sporadic cases of BCC.PTCH 1 gene mutations, receptors for sonic Hedgehog, leads to accumulation of transcription factor, Gli 1, which may play an important role in development of BCC.SOX 9, a downstream target of sonic hedgehog pathway is expressed in all basal cell carcinomas.10 BMI 1 gene which is up regulated by sonic hedgehog pathway is over expressed in BCC. Mutation in BAX and P53 gene is seen in a few sporadic cases. Some cases also show association with HLA DR7 and HLA DR4.[18]

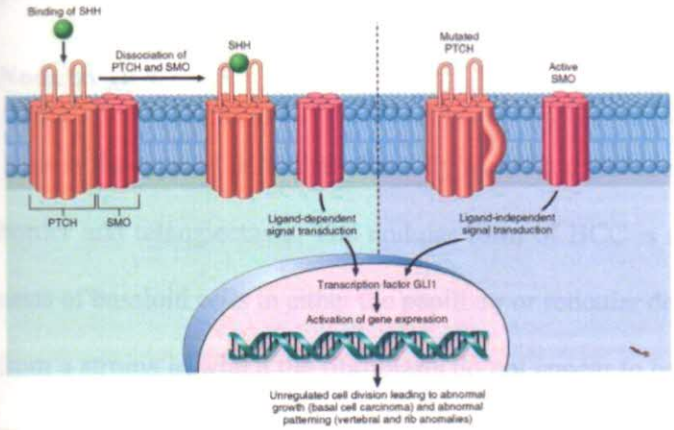


FIGURE 25-16 Normal and oncogenic hedgehog signaling. *Left*, Normally, PTCH and SMO form a receptor complex that binds sonic hedgehog (SHH). In the absence of SHH, PTCH blocks SMO activity. When SHH binds PTCH, SMO is released to trigger a signal transduction cascade that leads to activation of GLI1 and other transcription factors. *Right*, Mutations in *PTCH*, and less often in *SMO*, allow SMO to signal without ligand binding and underlie the nevoid basal cell carcinoma (Gorlin) syndrome.

FIG 9: PATHOGENESIS OF BASAL CELL CARCINOMA

HISTOPATHOLOGICAL VARIANTS OF BASAL CELL CARCINOMA:

Crowson classified BCC's as belonging to indolent-growth or aggressive-growth subsets. The indolent-growth variants include superficial and nodular BCC. The aggressive growth tumors are infiltrative BCC, metatypical BCC, and morpheiform or sclerosing BCC.[19]

Superficial BCC is characterized by a proliferation of atypical basaloid cells that form an axis parallel to the epidermal surface and demonstrate slit-like retraction of the palisaded basal cells from the subjacent stroma. The resulting cleft-like spaces often contain Alcian blue-positive mesenchymal mucoid material, a presumed product of the stromal cells. Some cases manifest melanin pigmentation of the epithelium and in the histiocytes in the subjacent stroma; of pigmented BCCs, most are held to reflect superficial tumors, although nodular BCCs constitute the most frequent form of pigmented BCC. A band like, often heavy, lymphoid infiltrate may be present. [19]

Nodular BCC:

This is the type of BCC that clinically shows a translucent pearly papule or nodule with a rolled border and telangiectasia. The nodular form of BCC is characterized by discrete large or small nests of basaloid cells in either the papillary or reticular dermis accompanied by slit-like retraction from a stroma in which the fibroblasts do not appear to be plump. The surrounding stroma shows myxoid change, is rarely fibrotic and may show calcification in discrete islands of tumor or in adjacent stroma. Mitoses and individual cell necrosis are uncommon.[19]

Micronodular BCC manifests a plaque-like indurated lesion with a poorly demarcated contour. Histologically, tumor nests with roughly the same shape and contour as nodular BCC are seen, but which are nonetheless smaller and widely dispersed in an often asymmetric distribution extending

deeper into the dermis and/or subcutis. Retraction spaces are not common and the surrounding stroma shows either a myxoid or collagenized morphology suggesting that these lesions may be an intermediate step between nodular and aggressive growth subtypes.[19]

Morpheaform or sclerosing BCC is characterized by columns of basaloid cells one to two cells thick enmeshed in a densely collagenized stroma containing proplastic fibroblasts. Individual cell necrosis and mitotic activity is brisk considering the relative tumor volume and the neoplasms themselves are poorly demarcated, showing widespread invasion of the reticular dermis and penetration into the subcutaneous tissue. Slit-like retraction from the stroma is less common than for the nodular and superficial variants. Pronounced stromal fibroplasia and fibrosis surrounds the tumor tongues.[19]

Infiltrative growth BCC comprises, at the light microscopic level, irregularly sized and shaped nests of tumor cells; the nests show sharp angulation of their peripheral contours, occasional foci of slit-like retraction, and frequent mitotic activity and individual cell necrosis of the neoplastic cells. The stroma is frequently fibrotic with plump proplastic stromal fibroblasts. The nests are variable in size and shape with jagged contours. Typically the elongated tumor cell strands of the infiltrative growth component are 5–8 cells in thickness. Like the morpheaform variant, these tumors are poorly circumscribed and may show invasion of subcutis and adjacent muscular and other structures. Perineural infiltration is a distinct risk in this variant as in the morpheaform BCC.[19]

IMMUNOHISTOCHEMISTRY IN BCC:

Basal cell carcinoma is positive for BerEP4, a keratin marker and negative for epithelial membrane antigen (EMA). EMA expression in BCC is restricted to squamoid areas.[20] In another study, they showed that CD10 may be a useful marker to differentiate basal cell carcinoma from squamous cell carcinoma. In this study, CD10 was positive in 48% of cases of BCC.[21]

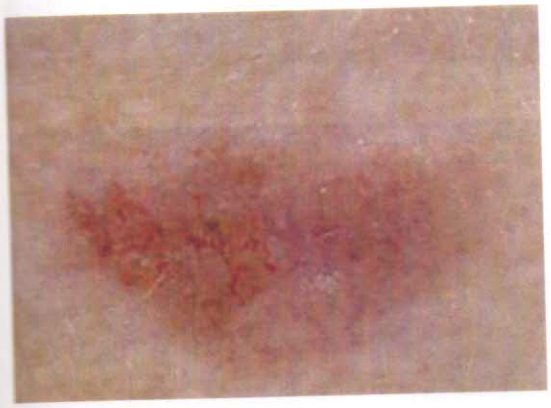


FIG 10:NODULAR BCC

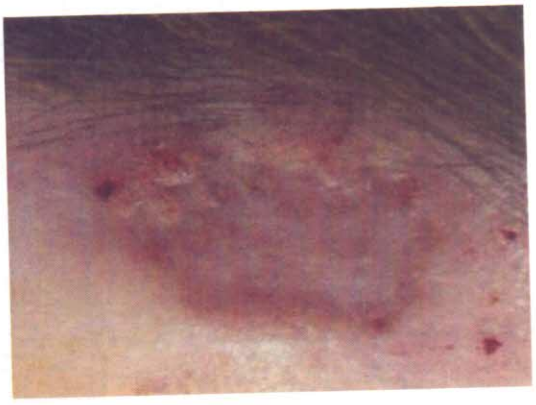


FIG 11: MORPHOEIC BCC

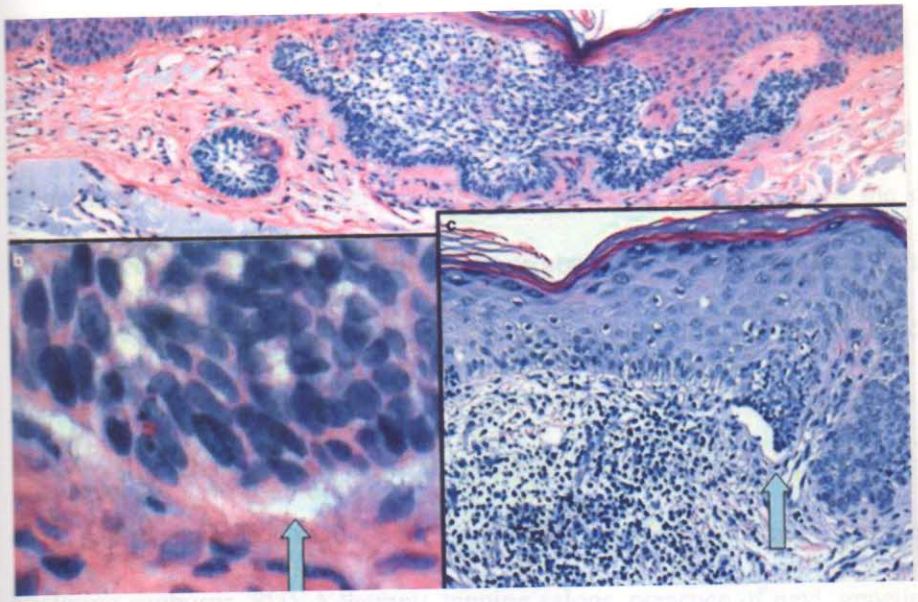


FIG 12: NODULAR BCC

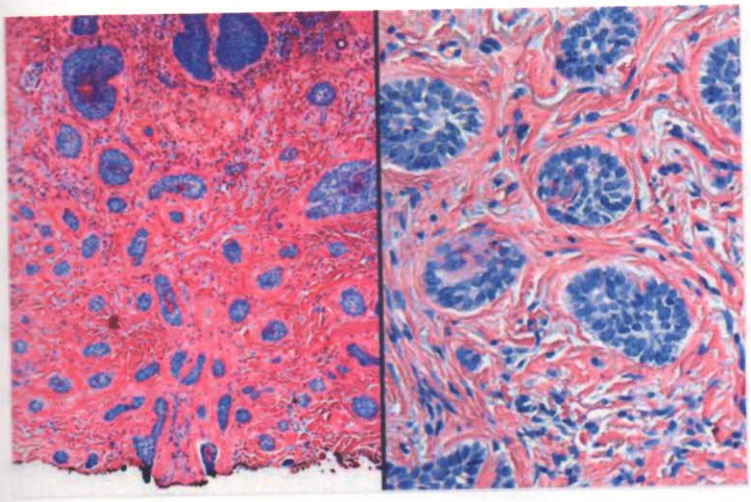


FIG 13: MICRONODULAR BCC

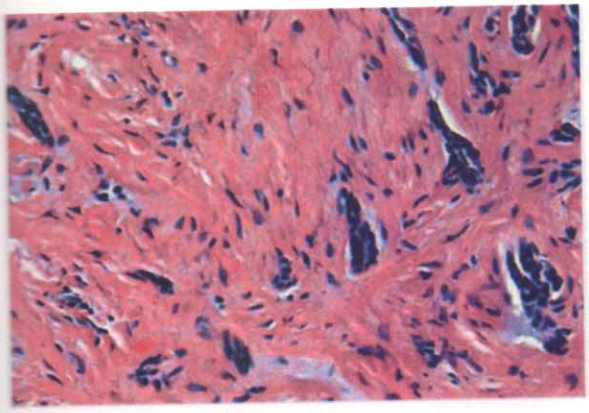


FIG 14: MORPHEAFORM BCC

MALIGNANT MELANOMA:

Most malignant melanomas arise in the epidermis and these may be in situ (entirely within the epidermis), or may be invasive (extending from the epidermis into the dermis). Occasional invasive melanomas are entirely dermal at presentation. The incidence of melanoma and mortality vary considerably, with high rates in Caucasians and low in individuals of Asian or African origin.[11] The most common sites affected in melanoma in both sexes is the face. Ear, head and neck are affected commonly in males and lower limbs in females. Risk factors for the development of melanoma include skin and hair color, numerous freckles, tendency to burn and tan properly, blistering sunburns, PUVA therapy, tanning salons, presence of nevi, genetic factors, xeroderma pigmentosum, immunosuppression, exposure to chemicals, petroleum and printing products, airline personnel and telecommunication workers and trauma.[11]

The most useful clinical criteria for diagnosis of malignant melanoma is the ABCD rule.ABCD is the acronym for asymmetry, irregular border, uneven contour and diameter >6mm.ABCD is not specific for malignant melanoma as few melanomas less than 5mm in diameter, referred to as small melanomas have been reported in the literature. And few benign melanocytic nevi may show asymmetry and uneven contours.[11]

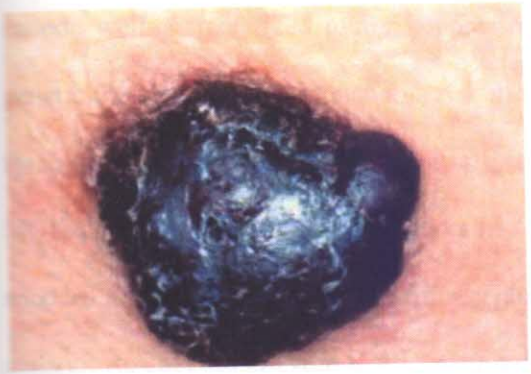


FIG 15: NODULAR MELANOMA

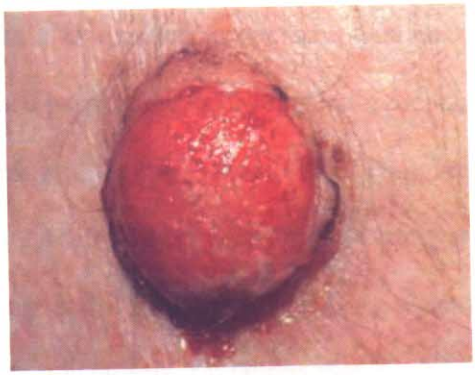


FIG 16: ULCERATED MELANOMA

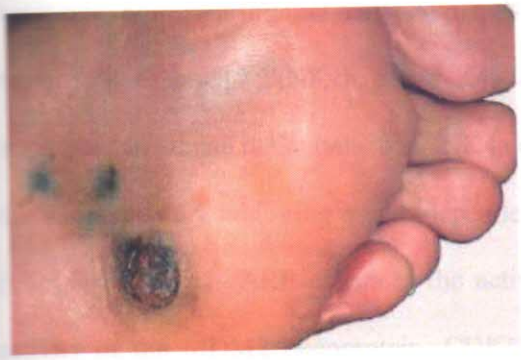


FIG 17: ACRAL MELANOMA

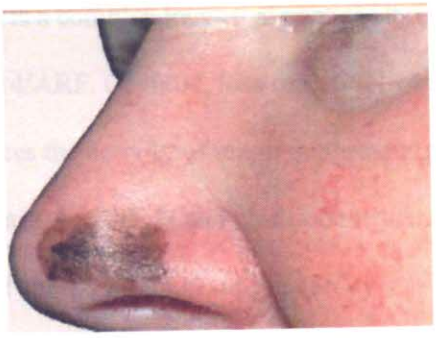


FIG 18: NASAL MELANOMA

Pathogenesis of malignant melanoma:

It is estimated that 10% to 15% of melanomas are familial, and many of those with familial melanoma also have dysplastic nevi. Several of the genes responsible for familial melanoma encode well-characterized tumor suppressors and are also mutated in sporadic tumors. Other genetic variants linked to melanoma risk in fair-skinned populations control melanin production; these pigmentation genes have weak effects, conferring a slightly elevated risk. They include MC1R, which encodes the melanocortin-1 receptor; ASIP (agouti signaling protein), which encodes a regulator of melanocortin receptor signaling, and TYR, which encodes tyrosinase, a melanocyte-specific enzyme that is required for melanin synthesis. Mutations that diminish the activity of the retinoblastoma (RB) tumor suppressor proteins are common in both familial and sporadic melanomas. The CDKN2A gene is mutated in approximately 40% of pedigrees with autosomal dominant familial melanoma. CDKN2A is a complex locus that encodes three different tumor suppressors, p15/INK4b, p16/INK4a, and p14/ARF. Of these, loss of p16/INK4a is clearly implicated in human melanoma. p16/INK4a enhances the activity of tumor suppressor proteins of the RB family by inhibiting cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6), while p14/ARF enhances the activity of the p53 tumor suppressor by inhibiting the activity of the MDM2 oncoprotein. CDKN2A is mutated in approximately 10% of sporadic melanomas, and these mutations uniformly abolish the production of p16/INK4a and more variably affect p14/ARF. The net effect of all of these alterations is the same; increased melanocytic proliferation and escape from oncogene-induced cellular senescence.[11]

The second common group of molecular lesions in sporadic melanoma leads to aberrant increases in RAS and PI-3K/AKT signaling, which you will recall are pathways that promote cell growth and survival. Activating mutations in BRAF, which encodes a serine/threonine kinase that is

downstream of RAS, are seen in 60% to 70% of melanomas, while activating mutations in NRAS (which is upstream of BRAF) occur in additional 10% to 15% of tumors. Melanomas arising in non-sun exposed sites are much more likely to have activating mutations in the c-KIT receptor tyrosine kinase, which sits upstream of both RAS and PI-3K/AKT, than in NRAS or BRAF. PTEN, a tumor suppressor that acts by down regulating PI-3K/AKT signaling, is epigenetically silenced in another 20% of melanomas.[18]

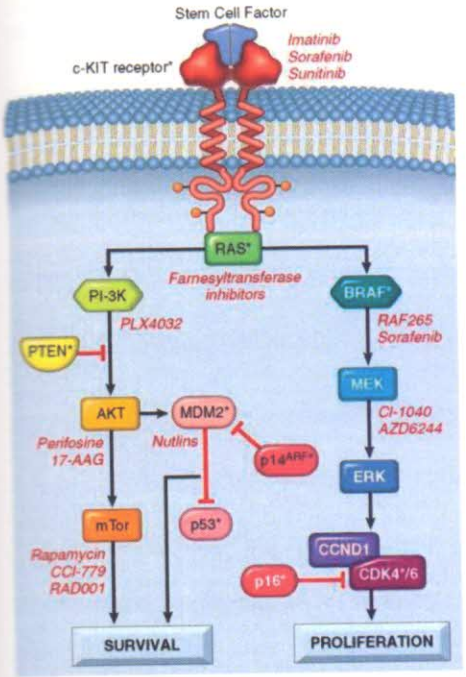


FIG 19: PATHOGENESIS OF MALIGNANT MELANOMA

These molecular insights have spawned attempts to treat melanoma with new therapeutic agents that target the RAS and PI-3K/AKT pathways. Such approaches are urgently needed, as metastatic melanoma is resistant to both conventional chemotherapy and radiation treatment. Ultimately, it is likely that these types of targeted therapies will be used in combinations tailored to the oncogenic leions found in individual tumors. For example, a tumor with an activating mutation in c-KIT will require treatment with different inhibitors than a tumor with an activating mutation in RAS or

BRAF. Immunotherapeutic approaches for melanoma in which host lymphocytes are "trained" to recognize and kill melanoma cells have also generated considerable interest, sparked in part by the recognition that spontaneous remissions of metastatic melanoma occur sporadically that are presumably mediated by the host immune response. Excellent responses are obtained on occasion, but the general applicability of such treatments has yet to be demonstrated.[11]

CLASSIFICATION OF MALIGNANT MELANOMA: [2]

There are two major categories of melanoma, which represent sequential stages of stepwise tumor progression. In the non tumorigenic radial or horizontal growth phase, the neoplastic melanocytes (melanoma cells) are confined to the epidermis (melanoma in situ), or to the epidermis and papillary dermis without formation of an expansile tumor mass (micro invasive melanoma). This phase may be followed after varying lengths of time by the focal appearance of the tumorigenic vertical growth phase, or a phase of dermal invasion with expansile tumor formation.

Melanoma is classified as

1. Radial growth phase(Non tumorigenic melanoma)
2. In situ melanoma or micro invasive melanoma
    - Superficial spreading melanoma
    - Lentigo maligna melanoma
    - Acral-lentiginous melanoma
    - Unclassified radial growth phase
3. Vertical growth phase(Tumorigenic melanoma)
4. No radial growth phase compartment: Nodular melanoma
5. Radial growth phase compartment present:
    - Usual vertical growth phase
    - Desmoplastic melanoma
    - Neurotropic melanoma

Clinically, the tumorigenic vertical growth phase is qualitatively different from the plaque-like radial growth phase. The tumor appears as an expanding papule within a previously indolent

plaque lesion, and grows in three dimensions in a balloon-like fashion to form a nodule. Typically, the ABCD criteria do not apply to the tumor nodule itself, which is commonly symmetrical, with smooth borders. The color is often quite uniform, and may be pink rather than blue-black, and the diameter of the tumor nodule itself is often <6 mm, even in a quite high-risk lesion. For these reasons, clinical diagnosis of melanoma may be subtle in a nodular melanoma that lacks an adjacent non tumorigenic compartment.[1]

HISTOPATHOLOGY OF MALIGNANT MELANOMA: [2]

The major histologic feature that distinguishes a tumorigenic melanoma is the capacity for proliferation of melanoma cells in the extracellular matrix of the dermis to form an expansile mass. In contrast, non-tumorigenic melanoma cells may proliferate inexorably in the epidermal compartment, and may invade the dermis but do not proliferate there. The lack of metastatic capacity in non-tumorigenic melanomas may be explained by considering that cell proliferation in the extracellular matrix of a distant site is essential to the development of a metastasis.

Tumorigenic melanoma:

A mass of melanoma cells is present in the dermis, defined as at least one cluster (nest) in the dermis that is larger than the largest intraepidermal cluster (indicative of a tumor with capacity for expansile growth in the dermis).

Nontumorigenic melanoma:

No mass of melanoma cells is present in the dermis (there is no cluster larger than the largest intraepidermal cluster).

Vertical growth phase (VGP):

A lesion is classified as VGP if it is tumorigenic, or if there are any dermal mitoses. The presence of any mitoses in the dermal component of the melanoma is indicative of a tumor with capacity for expansile growth in the dermis and defines the concept of typical VGP even in the absence of a frank tumor mass

Radial growth phase (RGP)

A lesion is classified as RGP only if it is nontumorigenic and there are no dermal mitoses. Alternatively, the RGP may be present as a complex primary melanoma in which the above histologic criteria apply only to that portion of the melanoma adjacent to the VGP. RGP melanoma may be defined as absence of VGP in a primary melanoma.

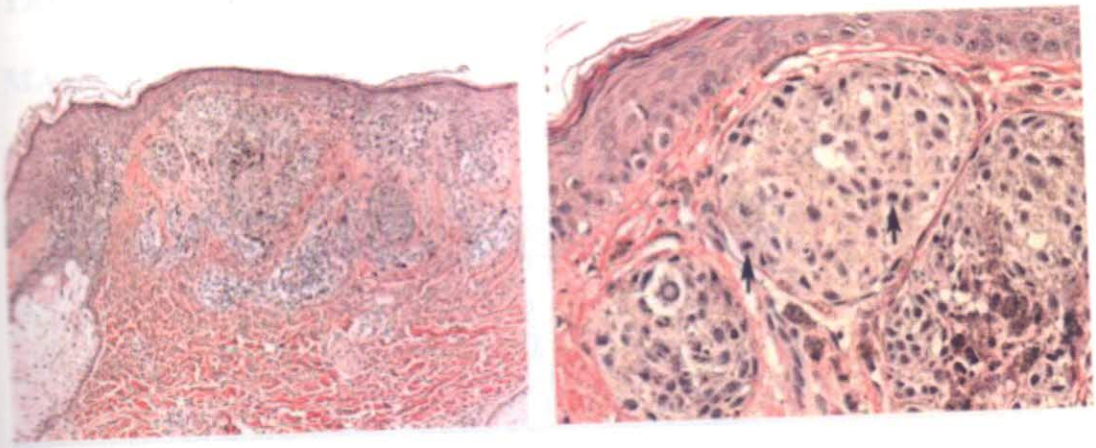


FIG 20: Melanoma with early tumorigenic vertical growth phase

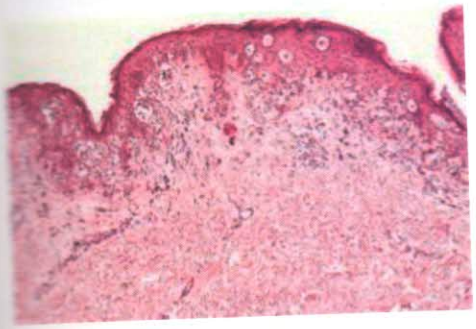


FIG 21: Superficial spreading melanoma –micro invasive

TABLE 2: HISTOLOGICAL CRITERIA FOR THE DIAGNOSIS OF MALIGNANT MELANOMA: [1]

Architectural criteria
Asymmetry Poor circumscription Consumption of the epidermis Epidermal nests of melanocytes showing: • confluence • variability in size and shape • haphazard interval and array Solitary epidermal melanocytes showing: • predominance over nests • pagetoid spread • haphazard arrangement Dermal nests showing: • variability in size and shape • confluence • lack of maturation in depth • variability in melanin distribution Melanocytes within lymphovascular spaces
Cytological criteria
Nuclear pleomorphism Nucleolar variability Mitoses: • even deep • sometimes atypical Apoptosis increased

HISTOPATHOLOGICAL VARIANTS OF MALIGNANT MELANOMA: [1,2,11]

SUPERFICIAL SPREADING MELANOMA:

The epidermis is irregularly thickened and thinned. Rather uniformly rounded, large melanocytes are scattered in a pagetoid pattern throughout the epidermis. The large cells lie predominantly in nests in the lower epidermis and singly in the upper epidermis. The nests vary in size and shape, and become confluent. Cytologically, the cells are rather uniform and have abundant cytoplasm containing varying amounts of melanin that often consists of small, dusty particles. They are almost entirely devoid of readily visible dendrites. The nuclei tend to be large and hyperchromatic, with irregular nuclear membranes and irregularly clumped chromatin .This uniform cytologic atypia is of considerable diagnostic importance.

LENTIGO MALIGNA MELANOMA:

Lentigo maligna may show at its periphery only hyperpigmentation with slight melanocytic proliferation, mainly in the basal cell layer. Toward the center of the lesion, there is a more pronounced increase in the concentration of basal melanocytes and some irregularity in their arrangement. The epidermis is frequently flattened, in contrast to SSM, where it is irregularly thickened and thinned. Some nesting of melanocytes in the basal layer may be seen. The lesional melanocytes in the epidermis show a marked increase in concentration, so that they come to lie in contiguity with one another, and their number in some areas exceeds that of the basal keratinocytes. Many of them are elongated and spindle-shaped. Their nuclei appear atypical, being enlarged,

hyperchromatic, and pleomorphic. However, the chromatin pattern is often not as open or vesicular as that seen in the more epithelioid cells of superficial spreading melanoma in its radial phase of growth. Frequently, atypical melanocytes extend along the basal cell layer of hair follicles.

ACRAL LENTIGINOUS MELANOMA:

Acral melanoma occurs on the hairless skin of the palms and soles and in the ungual and periungual regions, the soles being the most common site. The lesions are termed lentiginous because the majority of the lesional cells are single and located near the dermal-epidermal junction, especially at the periphery of the lesion. Early in situ or microinvasive lesions may show, especially at the periphery, a deceptively subtle histologic picture consisting of an increase in basal melanocytes and hyperpigmentation with only focal atypia of the melanocytes. However, in the center of the lesions, there is usually readily evident uniform, severe cytologic atypia. In most of the lesions, both spindle-shaped and round pagetoid tumor cells are seen, and, in many cases, pigmented dendritic cells are prominent. Pigmentation is often pronounced, resulting in the presence of melanophages in the upper dermis and of large aggregates of melanin in the broad stratum corneum.

NODULAR MELANOMA:

In a typical tumorigenic melanoma, there is contiguous proliferation of neoplastic melanocytes in the dermis forming a tumor mass that is larger than the largest nest in the overlying epidermis. The tumor mass is comprised of uniformly atypical Cytologically fully malignant mitotically active cells usually growing in confluent nests or in sheets. The tumor mass fills and expands the papillary dermis, or invades between coarse collagen fibers of the reticular dermis. The epidermis is

frequently ulcerated, or there is an adherent scale-crust. It is often stretched and attenuated, or alternatively may be irregularly hyperplastic and even pseudoepitheliomatous. The amount of inflammatory infiltrate in tumorigenic melanomas varies. Early invasive malignant and many in situ melanomas show a band-like inflammatory infiltrate, often intermingled with melanophages, at the base of the tumor. In tumors that extend deep into the dermis, the inflammatory infiltrate is quite variable, but it is often only slight to moderate rather than pronounced. These tumor-infiltrating lymphocytes (TILs) have been shown to have independent favorable prognostic significance. The lymphocytic infiltrate around melanomas is a T-cell response. TILs extracted from melanomas may be cytotoxic and may be directed against immunogenic melanoma-associated antigens.

DESMOPLASTIC AND NEUROTROPIC MELANOMA:

Desmoplastic melanomas present attributes of melanocytic, fibroblastic, and schwannian differentiation, often mixed within a single lesion. The lesions occur on chronically sun-damaged skin, usually in elderly patients. The lower lip is a relatively common site, sometimes in younger patients. All the desmoplastic melanomas exceed 1.5 mm in thickness and are Clark's level IV or V. There is a significant increase in local recurrence when neurotropism is present. The collagen in desmoplastic melanomas is arranged as delicate fibrils that extend between the tumor cells and separate them from one another. In desmoplastic melanoma, scanning magnification usually demonstrates an alteration of the architecture of the dermis, which may be subtle or more prominent. Frequently, this alteration extends throughout the full thickness of the reticular dermis into the fat. Typically, nodular clusters of lymphocytes and occasional plasma cells are present in

the tumor or at its periphery. At higher magnification, the melanoma cells are usually elongated and amelanotic and are embedded in a markedly fibrotic stroma, so that it is often difficult to decide which are fibroblasts and which are melanoma cells. This problem is enhanced by the relative absence of nuclear atypia in many of the neoplastic spindle cells, although close scrutiny will usually reveal cells with nuclear hyperchromasia and contour irregularities not typical of resting or activated benign mesenchymal cells. Because of the frequent absence of melanin, differentiation from a fibrohistiocytic or a neural lesion may be difficult. Staining with S-100 protein antibody usually marks many of the spindle-shaped cells, indicating that they are not fibroblastic, but nevi, neurofibromas, and neurogenic sarcomas are also typically S-100 positive. The HMB-45 or Melan-A antigens are usually not demonstrable in the spindle-cell compartment of desmoplastic melanomas, but they may be focally demonstrable in superficial dermal or in situ epithelioid melanocytes. Desmoplastic melanomas have been shown to produce fibrogenic cytokines, neurotrophins, and neurotrophin receptors, which together can account for their desmoplastic and neurotropic propensities.

Neurotropic melanoma is often a variant of desmoplastic melanoma. There are fascicles of desmoplastic melanoma that have invaded cutaneous nerves, usually in a spindle-cell vertical component with fibrosis. However, some neurotropic melanomas lack these latter features of desmoplastic melanoma. Many of these are spindle-cell tumorigenic melanomas of acral-lentiginous or lentigo maligna type, but some are composed of epithelioid cells. Neurotropism in a primary melanoma is associated with increased risk for local recurrence, even after standard definitive therapy, and may also be associated with increased mortality.

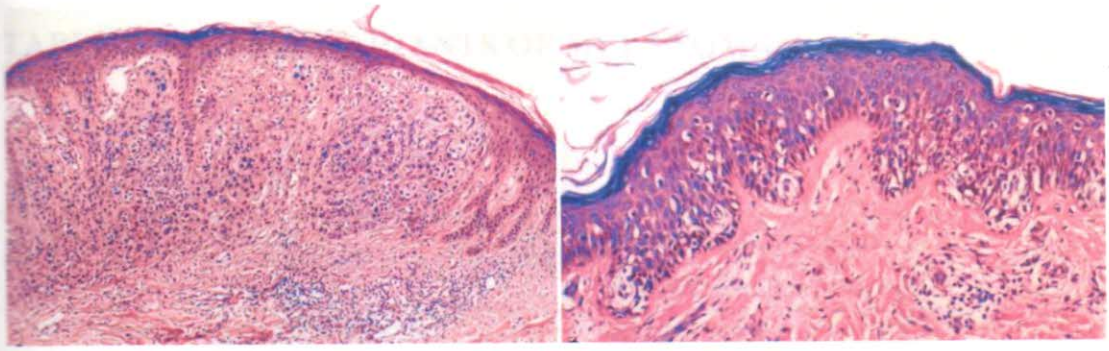


FIG 22: Superficial spreading melanoma- Low power

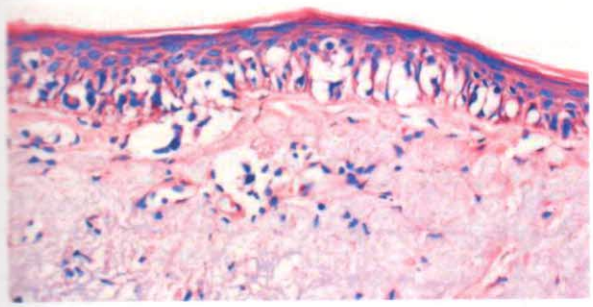


FIG 23: LENTIGO MALIGNA- SEVERE NUCLEAR PLEOMORPHISM

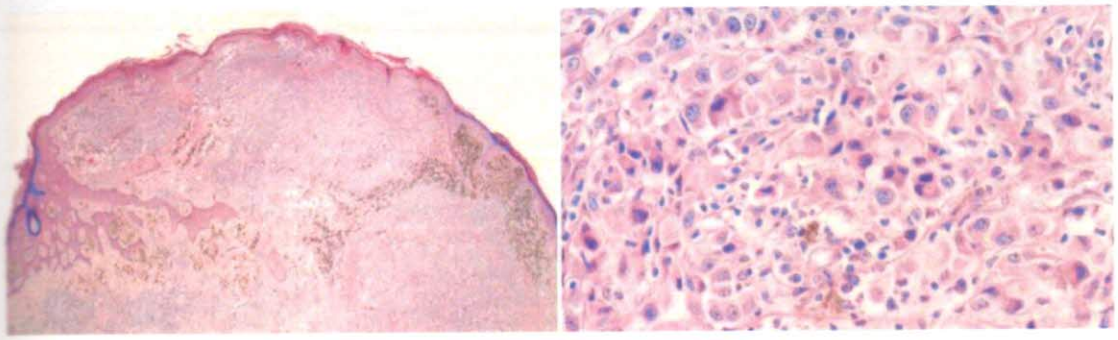


FIG 24: NODULAR MELANOMA – ASYMMETRICAL DISTRIBUTION OF CELLS, DERMAL MELANOPHAGES AND LYMPHOCYTES. TUMOR IS COMPOSED OF CELLS WITH LARGE, PLEOMORPHIC NUCLEI WITH MITOSES

TABLE 3: SPECIAL VARIANTS OF MELANOMA: [1]

Angiomatoid melanoma	Melanoma with rosettes
Angiotropic melanoma	Melanoma with sebocytes
Animal-type melanoma (pigmented epithelioid melanocytoma)	Myxoid melanoma
Balloon-cell melanoma	Neuroendocrine melanoma
Bullous melanoma	Nevoid melanoma
Chondroid melanoma	Osteogenic melanoma
Clear cell sarcoma (melanoma of soft parts)	Plasmacytoid melanoma
Cystic (adenoid cystic) melanoma	Pseudoglandular melanoma
Dermal melanoma	Pseudolipoblastic melanoma
Follicular melanoma	Rhabdoid melanoma
Ganglioneuroblastic melanoma	Sarcomatoid melanoma
Lentiginous melanoma	Schwannoid melanoma
Melanocarcinoma	Signet-ring cell melanoma
Melanoma mimicking Merkel cell carcinoma	Small cell melanoma
Melanoma resembling MPNST	Small-diameter melanoma
Melanoma with monster cells	Spitzoid melanoma
Melanoma with psammoma bodies	Vitiligo-like melanoma

TABLE 4: PROGNOSTIC FACTORS OF MALIGNANT MELANOMA: [1,2,11]

Morphological
Increasing Breslow thickness (A)
Ulceration (A)
Mitotic rate/mitotic index (A)
Increased nuclear volume (A)
Satellite deposits (A)
Hemangiolymphatic invasion (A)
Advanced clinical stage (A)
'Occult' metastasis (A)
Local recurrence (A)
Clark level (C)
Site (C)
Histological subtype (C)
Coexisting nevus (C)
Lymphocytic infiltrate (C/F)
Absence of regression (C/F)
Clinical
Female (F)
Vitiligo (F)

TUMOR THICKNESS (BRESLOW'S THICKNESS):

It is now known that tumor thickness is the single most important predictor of survival in clinical stage I melanomas. The depth of invasion is measured from the top of the granular layer to the deepest extension of the tumor; in ulcerated lesions, measurement is from the ulcer base overlying the deepest point of invasion. Melanoma < 0.5mm thick at diagnosis has better prognosis.

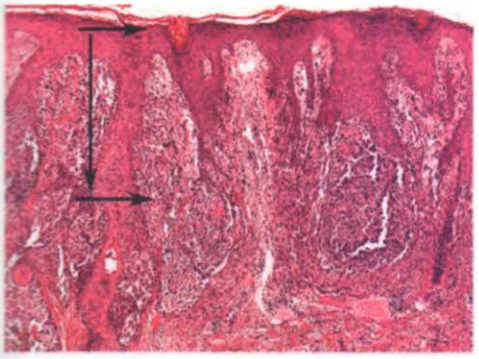


FIG 25: MEASUREMENT OF BRESLOW'S THICKNESS

In determining the depth of penetration, whether by level or by measurement, the following rules apply:

- Melanocytes in junctional nests are not considered invasive, even though they may push into the papillary dermis.
- If deep nests of melanoma cells arise from the epithelium of cutaneous appendages, they are not used in measurement from the surface.
- A column of melanocytes extending from the lower border of the lesion into the deep dermis at nearly a right angle is not measured, because it is likely that the column arises from an appendage; this supposition can usually be verified by serial sections or keratin stains. Observer agreement for Clark level has been found to be excellent.

CLARKE'S LEVEL OF INVASION:

Although tumor thickness is now considered to be the single most important prognostic attribute, the levels of invasion have prognostic value, at least in certain subsets of cases.in level I, the melanoma is confined to epidermis (melanoma in situ). In level II, melanoma cells are present in the papillary dermis which may expand but not completely fill the papillary dermis. In level III, the tumor cells expand and fill the papillary dermis completely.in level IV, the tumor cells infiltrate the collagen fibres of reticular dermis. In level V, the tumor cells infiltrate the subcutaneous tissue.

ULCERATION:

Ulceration is defined by the loss of continuity of the epithelium over the surface of the tumor. For any given thickness level. Prognosis is worse when ulceration is present.

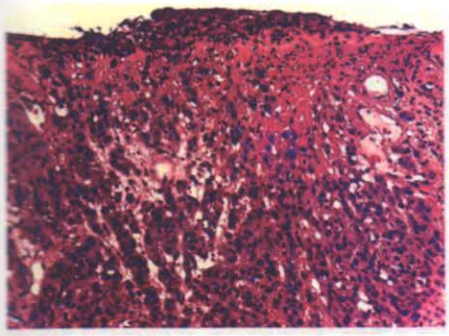


FIG 26: ULCERATED MELANOMA

MITOTIC RATE:

Patients with mitotic rate >6 were at 12 times greater risk of developing metastasis. In addition, presence of any number of mitoses in the dermis not only predicts survival, but also sentinel lymph node positivity.

VASCULAR OR LYMHATIC INVASION:

Although not considered an independent prognostic factor, lymphovascular invasion when present suggests poor prognosis.

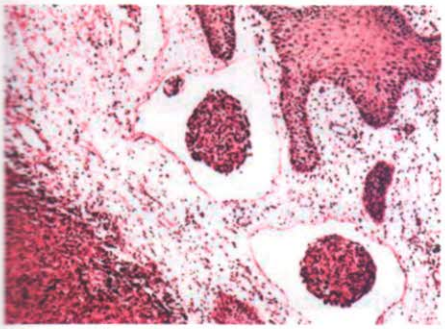


FIG 27: LYMPHATIC INVASION

TUMOR INFILTARTING LYMPHOCYTES:

The prognosis is best for tumors with a brisk TIL response, defined as lymphocytes forming a continuous band beneath the tumor or diffusely throughout its substance. Tumors with absent TILs have the worst prognosis, and a non brisk response (discontinuous band) is associated with an intermediate prognosis. The presence of a non infiltrative lymphocytic infiltrate around the tumor, usually at its base, is not associated with prognosis.

SATELLITE DEPOSITS:

The presence of microscopic satellite deposits separated from the main body of the tumor has adverse prognostic significance.

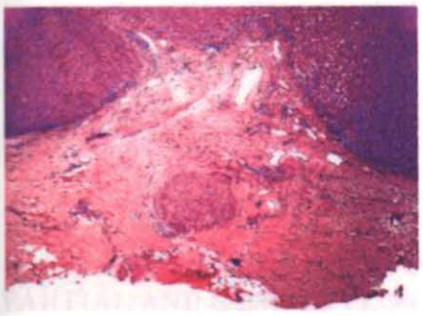


FIG 28: MICROSCOPIC SATELLITES

PATIENT GENDER AND LESIONAL CELL LOCATION;

Female patients and patients who have lesions on the limbs have better prognosis than males and lesions on trunk or extremities.

HISTOLOGICAL SUBTYPE:

Nodular melanomas, on average, are thicker than superficial spreading melanomas, and thus have a worse prognosis overall. However, the prognosis is the same for nodular and other types of melanoma of similar thickness, and in multivariable analyses, nodular type is not an independent predictor. The scarcity or absence of melanin in the tumor cells amelanotic melanoma, indicative of poor differentiation, affect the prognosis adversely.

SENTINEL LYMPH NODE DISSECTION:

The sentinel lymph node is identified by injections into the region of the melanoma of radioactive colloid and a vegetable dye. The sentinel node is identified as the first in the regional lymph node

basis to contain these markers. If the sentinel node is negative, the probability of metastases being found in the remainder of the lymph node basin is very low. It is recommended that small nodes be submitted in their entirety or that larger nodes be sections at 3- to 4-mm intervals and entirely submitted. It is currently the practice to perform S-100 (more sensitive, less specific), and either HMB-45 or Melan-A (more specific, less sensitive) staining on one profile from each submitted sentinel node.

PARTIAL AND COMPLETE REGRESSION OF MELANOMA:

Partial regression is common in melanomas. Usually, it is observed in the nontumorigenic compartment. Regression is defined as a focal area in which there is delicate fibroplasia of the papillary dermis, often accompanied by proliferation of dilated blood vessels, and usually with a sprinkling of melanophages and lymphocytes, with melanoma present in the epidermis and/or papillary dermis to one or both sides, but not within the area of regression. Paradoxically, partial regression of the RGP regression has been associated in some series with poorer prognosis. Because a more significant dermal component had been present and had metastasized before it regressed.

UNUSUAL VARIANTS OF MELANOMA: [22]

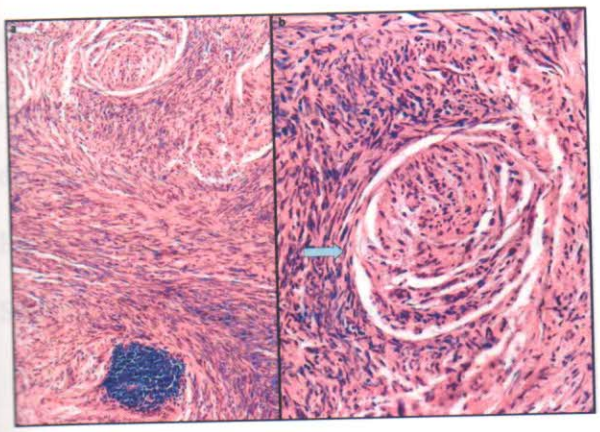


FIG 29: DESMOPLASTIC MELANOMA

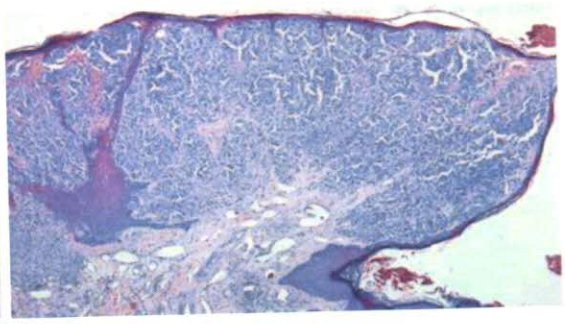


FIG 30: NEVOID MELANOMA

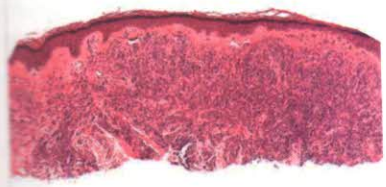


FIG 31: MINIMAL DEVIATION MELANOMA

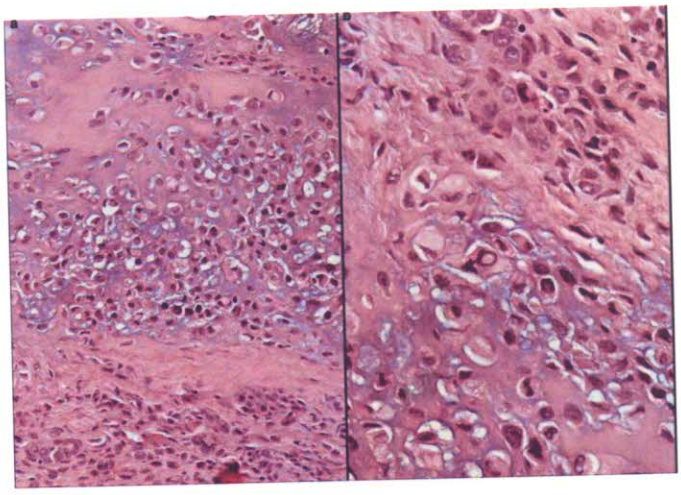


FIG 32: METAPLASTIC MELANOMA

IMMUNOHISTOCHEMISTRY IN MALIGNANT MELANOMA:

Malignant melanoma has a wide spectrum of histological features that mimic epithelial, mesenchymal and neural tumors. Therefore, immunohistochemistry plays an important role in the diagnosis of malignant melanoma.S-100 is the most sensitive marker for detection of melanoma. HMB-45, MART-1/Melan A, tyrosinase and MITF have good specificity, but not as sensitive as S-100 for the diagnosis of melanoma.[2]

C KIT: ROLE IN TUMORIGENESIS: [24]

The c-kit receptor (CD117) is a transmembrane protein with tyrosine kinase activity encoded by the oncogene c-kit. It is an important member of type III receptor tyrosine kinase family; other tyrosine kinase receptor molecules include macrophage colony-stimulating factor receptor (M-CSFR), platelet-derived growth factor (PDGF) and flk2/flk3 receptor and so on. The ligand for c-kit is stem cell factor (SCF), a hematopoietic cytokine, which plays an important role in maintaining the survival of hematopoietic cells, promoting hematopoietic cell proliferation and differentiation, and regulating growth and development of hematopoietic cells. SCF dimer forms complexes with two molecules of the extracellular domain of c-kit to activate downstream signal transduction and then regulate a variety of cells biological behavior, such as normal cells proliferation and differentiation, tumor occurrence, development, migration and recurrence. The abnormality of SCF/c-kit signaling pathway is closely related to some certain tumors.

STRUCTURE OF C KIT RECEPTOR: [24]

Human c-kit proto-oncogene is located on chromosome 4q11～12 and has a total length of 90 kb. The coded product c-kit receptor (CD117) is a type I transmembrane glycoprotein of relative molecular mass of 145 kDa and belongs to the type III receptor tyrosine kinase family. The c-kit receptor is composed of 976 amino acids (aa) divided into an extra-cellular domain with 519 aa, a trans-membrane domain with 23 aa, an intracellular tail of 433 aa consisting of a juxta-membrane domain and a tyrosine kinase domain inserted by about 80 amino acid residues.

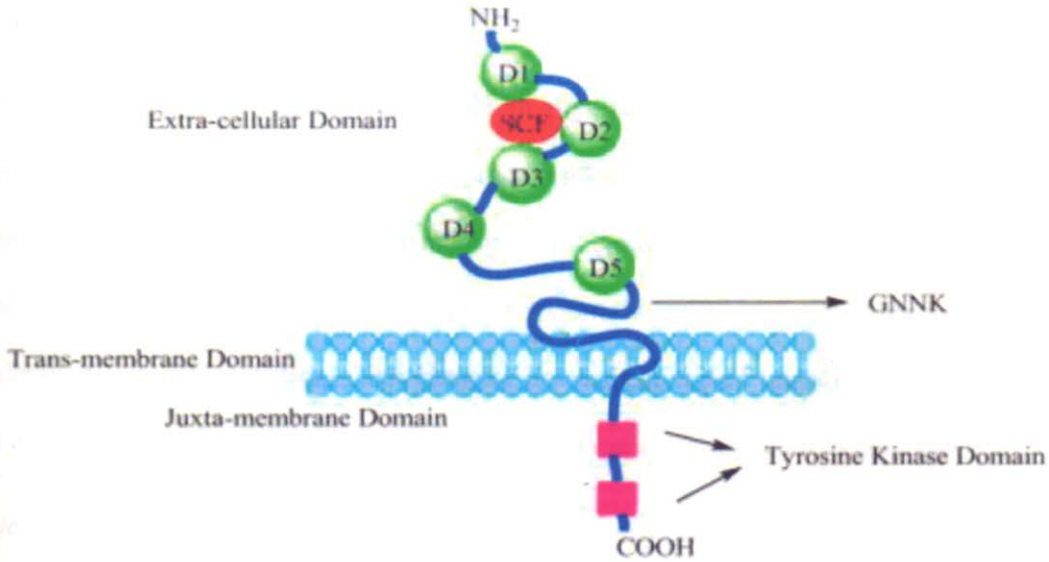


FIG 33: STRUCTURE OF C KIT RECEPTOR

DISTRIBUTION OF C KIT RECEPTORS:

C-kit receptor is widely distributed in hematopoietic cells and other tissue cells. In healthy people, approximately 1% to 4% of bone marrow stem cells and 60% to 75% of CD34+ positive hematopoietic cells express c-kit receptor. C-kit-dependent cell types include hematopoietic cells, germ cells, mast cells, melanoma cells and the gastrointestinal tract cajal cells. 66.3% of the germ cells and the sperm express c-kit receptor, which is in the fact that each type of germ cells can be isolated from human semen by using anti-CD117 monoclonal antibody. C-kit receptor is also found in skin appendages, the breast epithelial cells and the small neurons in the brain.

THE SIGNALLING PATHWAY OF C KIT RECEPTOR: [24]

C-kit signaling plays a very important role in regulation of the red blood cell production, lymphocyte proliferation, mast cell development and function, melanin formation, and gamete formation. Specific binding of SCF can induce homologous dimerization, and drives downstream signal transduction pathway. It subsequently regulates gene expression and cell growth, proliferation and differentiation. C-kit receptor downstream signal transduction pathways have been described in different cell lines. Mast cells are the most commonly used cell type.

SCF/c-kit downstream signal transduction pathways are very complex and currently known signal transduction pathways are as follows:

Ras/Erk signal transduction pathway: [24]

Ras/Erk pathway plays a very important role in cell differentiation and survival. That RTK collaborates with GDP/GTP exchange factor Sos can activate Ras. Activated Ras can activate the Ser/Thr kinase Raf-1 which can then activate the dual specificity kinases Mek1 and Mek2. Phosphorylation of Mek1 and Mek2 can re-activate Erk1 and Erk2. Subsequently, dimerization Erks move to the nucleus and regulate gene activities.

PI3K signal transduction pathway: [24]

After c-kit receptor activation, PI3K dimerizes through SH2 and then recruits to membrane. SH2 which contains an adapter protein of 85 kDa connects 110 kDa esterase subunits to c-kit receptor. As PI3K produces a series of biological signals on the membrane, signal transduction molecules downstream of PI3K are activated and cell survival and angiogenesis are regulated.

PLC-γ signaling transduction pathway: [24]

Phospholipase (PLC) PLC-γ contains two SH2 domains, a SH3 domain, a PH domain and a catalytic domain. PLC-γ can catalyze phosphoinositide PIP2 to generate second messenger diacylglycerol (DAG) and soluble inositol 1,4,5-trisphosphate (IP3). DAG can activate PKC; IP3 then binds to the endoplasmic reticulum to stimulate the release of Ca+.

JAK/STAT signaling pathway: [24]

AKs are cytoplasmic tyrosine kinases. The c-kit receptor can be fast and transient to activate JAK2.Then, activated JAKs can make the transcription factor STATs phosphorylation and dimerization. Finally, dimerized STATs transfer to the nucleus and regulate cell proliferation, differentiation and apoptosis.

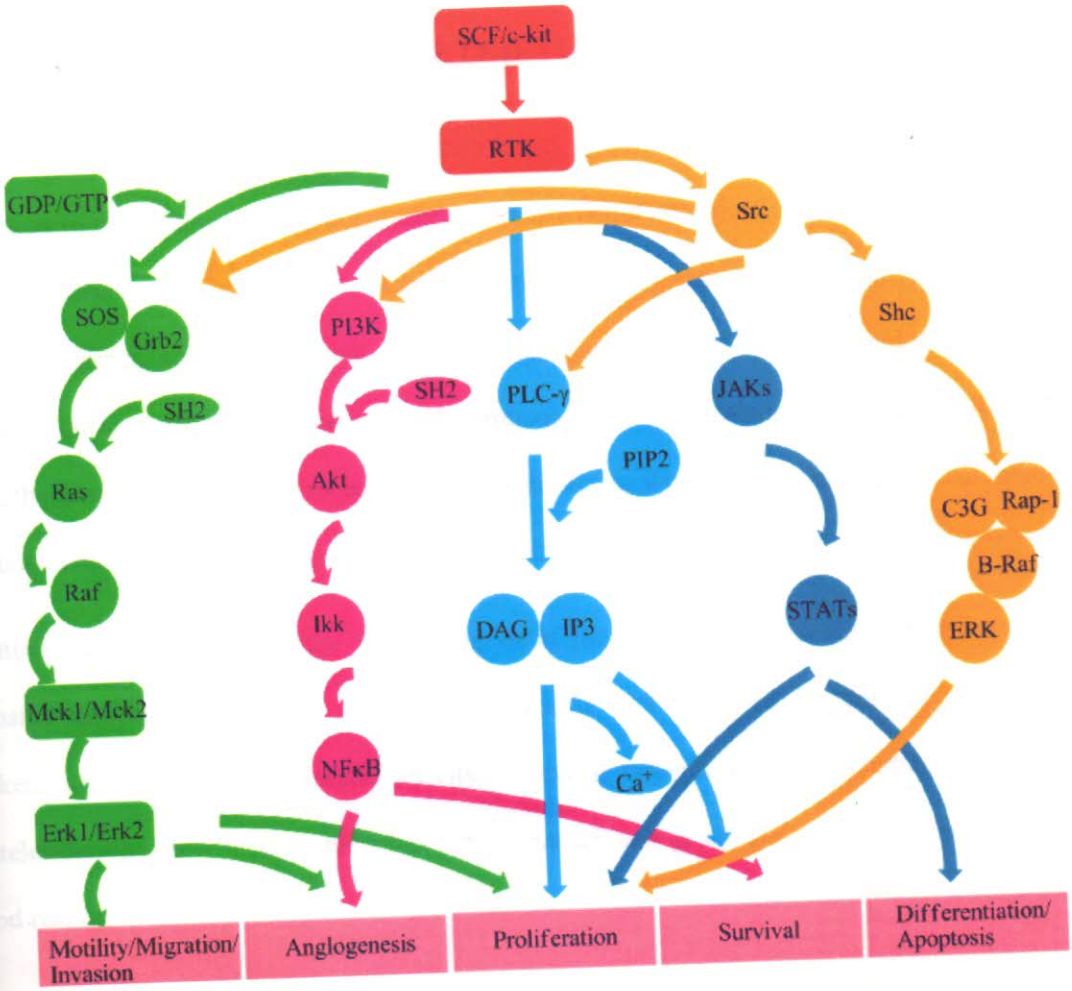


FIG 34: C-KIT SIGNALLING PATHWAYS

C KIT RECEPTOR AND TARGETED THERAPIES: [25]

Cancer is a major disease threatening human health and the whole world has been paying close attention to its treatment. Traditional chemotherapy is not completely effective for many tumor treatment because of their nonspecific blocking tumor cells division and damage to normal cells. . The appearance and development of c-kit antibodies, especially the monoclonal antibodies, promote the research of c-kit receptor for continuous activation mechanism. It is generally believed that, the dimerization of c-kit receptor is closely related to its continuous activation and thereby it is considered to be c-kit receptor-related tumor pathogenesis.

Imatinib mesylate is one of the first examples of the successful solid tumor treatment with a tyrosine kinase-targeted drug in humans, which is first designed for chronic myelogenous leukemia. It can inhibit enzymatic activity of several tyrosine kinases including c-kit and the platelet-derived growth factor receptor. Later Imatinib was gradually applied to treat GIST with good results.

Sunitinib is the second generation of multi-target tyrosine kinase receptor inhibitor approved by the FDA in 2006 and the second-line drugs for imatinib-resistant GIST patients that may extend survival. The sensitivity of sunitinib is also related to the state of c-kit gene.

C-kit is a suitable target for future drug development for the treatment of a multitude of human malignancies related to c-kit. Although the multi-target drugs have made great progress in cancer therapy, many kinase inhibitors also inhibit the normal signal transduction pathways. Collectively, the low toxicity and multi-target anti-cancer drugs are the trend to study. Moreover G-rich region of c-kit receptor promoter has become an important potential target. As there exists the differential resistance for different cancer patients, besides efficiency and low toxicity target drugs we should

further need to consider about other characteristics of tumors to implement joint and individualized treatment programs.

Maia et al [6], in his study of 139 Vulvar squamous cell carcinoma found c-Kit positivity in 70.5% cases which was associated with a higher recurrence free and global survival. They found an absence of lymph node metastasis and HPV infections suggesting C-kit to be a good prognostic marker.

Ongkeko WM et al [7] found C-kit to be positive in 71% cases of squamous cell carcinoma of head and neck.

Fan et al [29] studied C- kit expression in esophageal squamous cell carcinomas. They concluded that C-kit expression in esophageal squamous cell carcinoma is associated with worse outcome.

Pelosi et al [30] evaluated 201 cases of adenocarcinoma and squamous cell carcinoma of lung for CD 117 immunoreactivity, documenting as positive or negative if >5% or <5% tumor cells are stained. They concluded that CD 117 expression identifies adenocarcinoma and squamous cell carcinoma with highly proliferative tumors and may have prognostic relevance. Targeting CD 117 pathway could be a novel therapeutic option in a subset of pulmonary carcinomas.

In basal cell carcinoma, Leon et al [26] used CD117(C-kit) as a marker to identify the mast cells in the stroma earlier. However, recently Terada T[27] in an immunohistochemical study of 66 consecutive cases of basal cell carcinoma using various markers found C-kit expression to be high in 93% of cases compared to other markers which showed low expression.

C-Kit expression is also detected in melanomas and in nevi. Rivera et al [27] and Alexis et al [28] found an increased expression of C-kit in 88.8% and 80% of melanoma cases respectively. These authors reported an increased therapeutic value rather than a prognostic value of C-kit over expression in melanomas. Studies have shown that C-Kit could be a useful marker to differentiate primary melanoma from compound nevi.

Beadling et al [31] screened 89 melanoma patients for Kit mutations in exons 11, 13 and 17. Kit copy number was assessed by PCR. IHC was done to evaluate Kit expression. They found that Kit mutations are common in acral and mucosal melanomas and screening for kit mutations may open up new treatment options for melanoma patients.

MATERIALS AND METHODS:

SOURCE OF DATA:

The study was done on paraffin embedded tissue blocks with a confirmed histopathological diagnosis of malignant cutaneous epidermal tumors obtained from Department of Pathology, SDUMC, Kolar from the year 2007-2014.

INCLUSION CRITERIA:

All cases of surgically resected primary epidermal cutaneous tumors like squamous cell carcinoma, basal cell carcinoma and malignant melanoma were included in the study.

EXCLUSION CRITERIA:

Biopsy specimens, dysplastic lesions, benign cutaneous tumors and metastatic malignant melanomas were excluded from the study.

METHOD OF COLLECTION OF DATA:

Fifty three formalin fixed, paraffin embedded tissue sections with a confirmed histopathological diagnosis of primary malignant epidermal cutaneous tumors were retrieved from archives of Department of Pathology. Medical records were reviewed to assess the age and sex of the patient, gross appearance and dimensions of the tumor, histopathological diagnosis and margins and lymph node involvement.

Hematoxylin & Eosin stained slides were reviewed to validate the final histopathological diagnosis and to record the histopathological subtypes in basal cell carcinoma and malignant melanoma and

degree of differentiation in squamous cell carcinoma. Also, Clarke's level of invasion was reviewed in cases of malignant melanoma.

Immunohistochemistry was performed using C-kit antibody (Rabbit monoclonal antibody, Biogenex) using peroxidase-antiperoxidase method.

Sections were cut 4-5 micro meter thickness, floated on to organosialine coated slide and left on hot plate at 60° over night

Deparaffinization using Xylene I and II for 15 min each

Dexylinisation using absolute alcohol I and II for1 min each

Dealcoholisation using 90% and 70% alcohol for 1 min each

Washing with distilled water.

Antigen Retrieval technique: Microwave power 10 for 6 minutes in TRIS EDTA buffer of pH-9.0 for 2 cycles.

Distilled water rinsing for 5 minutes. Transfer to TBS (Tris buffer solution pH- 7.6) - 5minutes x 2 times-wash.

Peroxidase block- 15-20minutes to block endogenous peroxidase enzyme. TBS buffer for 5 minutes washing for 3 times.

Power block- 15-20 minutes to block non- specific reaction with other tissue Antigen.

Cover sections with targeted antibody (primary) for 1hr

TBS buffer- 5min x 3Times.

Super Enhancer- 45 minutes to enhance the reaction between primary and secondary antibodies.

TBS buffer- 5min x 3 times

Super sensitive poly- HRP (secondary antibody) for 30 min

TBS buffer- 5min x 3 times

Color development with working color development solution (DAB) for 5-8 min

All the slides were examined for color development

TBS wash- 5min x 3 times

Counter stain with hematoxylin for 3 sec

Tap water wash for 5 minutes.

Dehydrate, clear and mount

Mount with DPX

INTERPRETATION OF IMMUNOHISTOCHEMISTRY RESULTS:

The percentage and the intensity of C-kit immunoreactivity in the tumor cells was evaluated according to Pilloni et al as follows:

Percentage of positive cases was recorded as

0 - negative

1 - <5% of tumor cells staining

2 - 5-50% of tumor cells staining

3 - 51-95% of tumor cells staining

4 - >95% of tumor cells staining

Intensity was scored as

0 - Negative

1+ -Weak

2+ -Moderate

3+ -Strong

The product of the percentage and intensity was used as the final measure of staining.

Positivity cut-off was taken as at least 5% of tumor cells showing weak intensity. (Score >1)

RESULTS:

A total of 53 cases were included in the study. Out of 53 cases, 58% were SCC, 21% of cases were each of BCC and MM.

FIG 35: DISTRIBUTION OF SCC, BCC AND MM

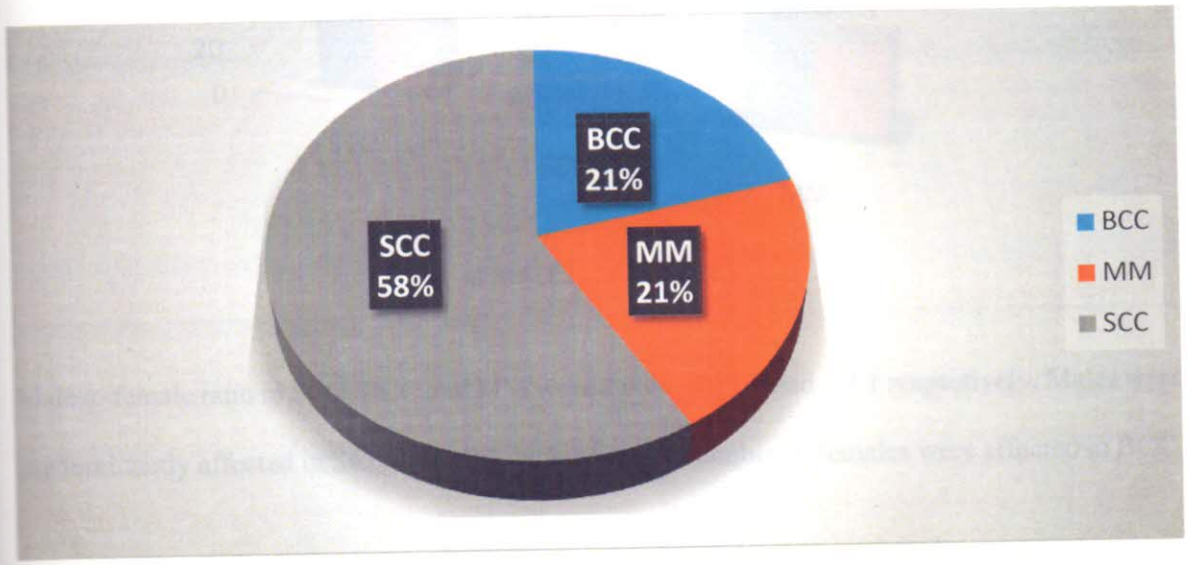
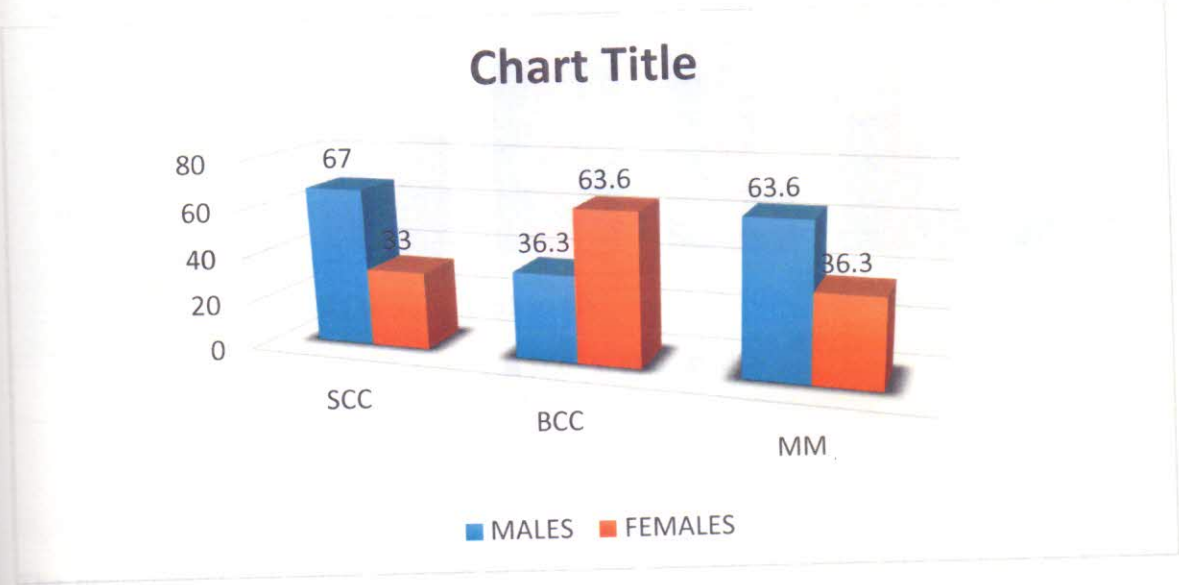
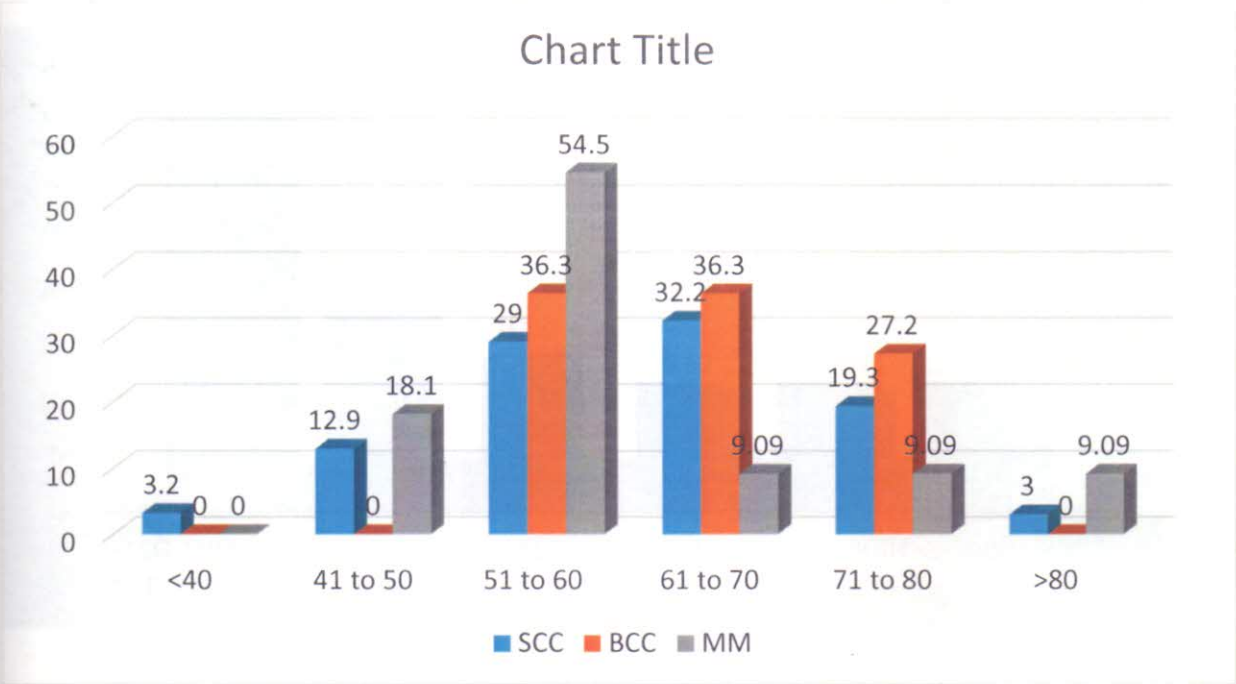


FIG 36: SEX DISTRIBUTION IN SCC, BCC AND MM



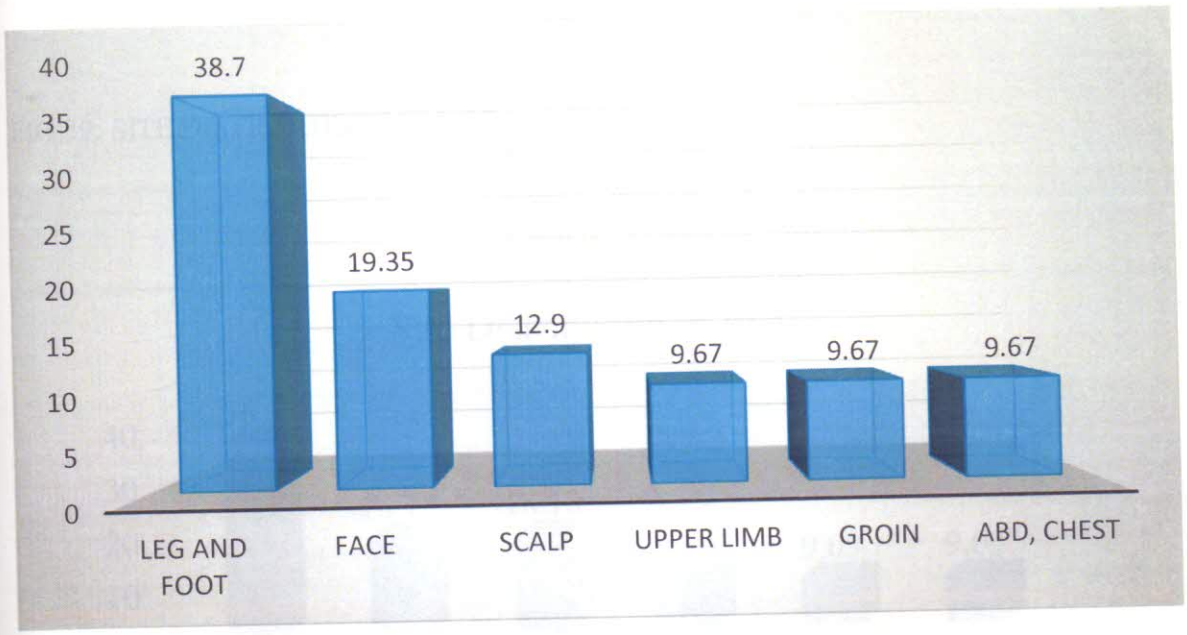
Male to female ratio in SCC, BCC and MM were 2.03:1, 0.57:1 and 1.7:1 respectively. Males were predominantly affected in SCC and MM, whereas more number of females were affected in BCC.

FIG 37: AGE DISTRIBUTION IN SCC,BCC, AND MM



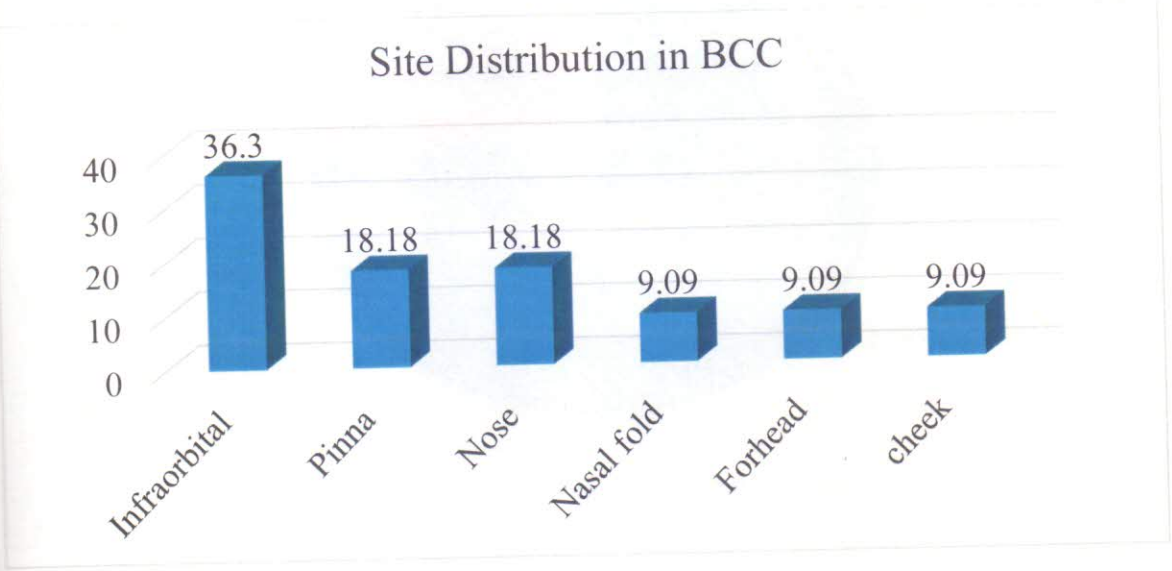
The predominant age group affected in SCC was between 61-70yrs (32.2%). In BCC, most commonly 50-60yrs and 61-70yrs were affected with 36.3% each. And in MM, 54.5% of cases were seen in the age group between 51-60yrs.

FIG 38: SITE DISTRIBUTION IN SCC



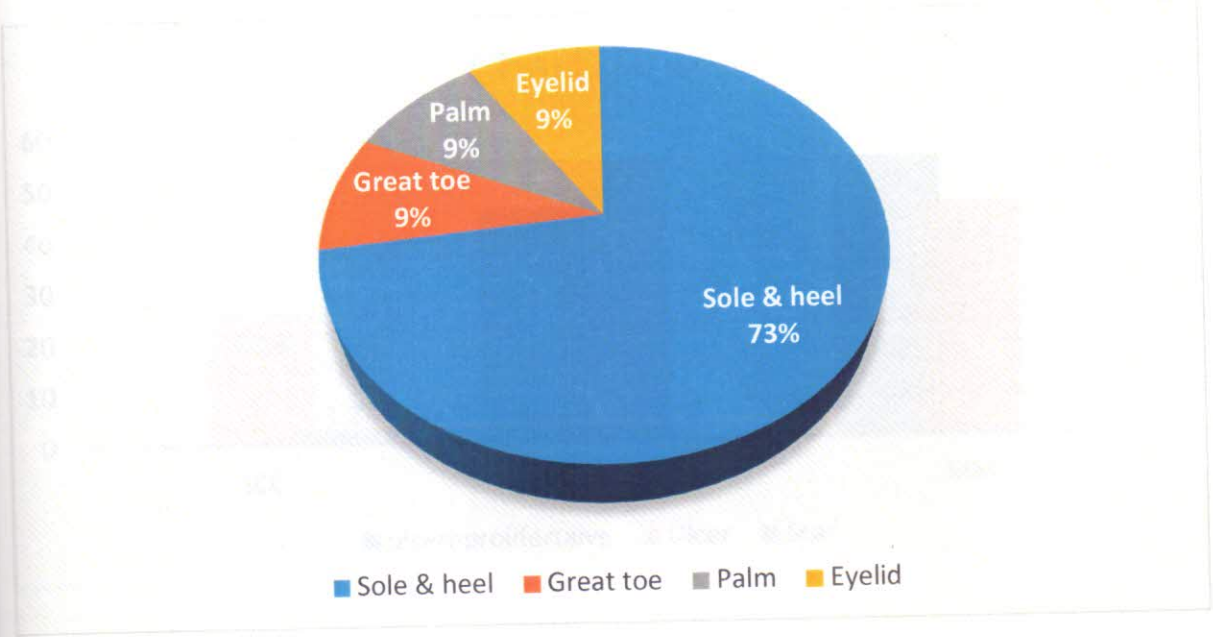
SCC was seen in leg and foot in 39% of cases followed by 19% of cases involving the face and 13% in scalp.

FIG 39: SITE DISTRIBUTION IN BCC



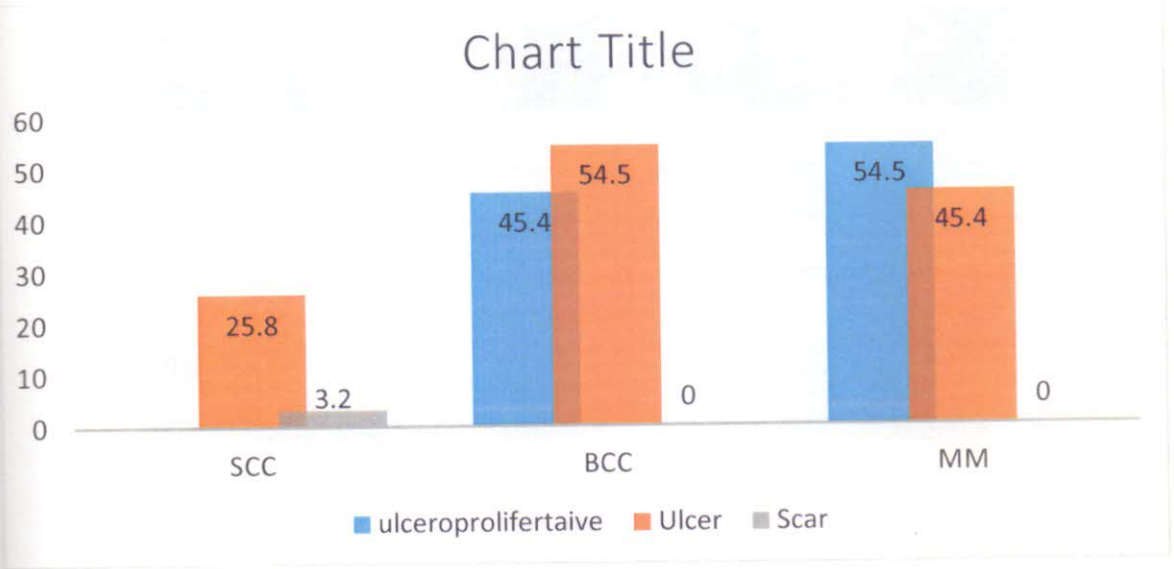
In BCC, infra orbital region was affected in majority of cases (37%), followed by nose and pinna (18% each) and cheek, nasal fold and forehead (9% each).

FIG 40: SITE DISTRIBUTION IN MM



Lower limb(sole and heel) was involved predominantly in 73% cases of MM, followed by 9% cases involving eyelid, palm and great toe.

FIG 41: GROSS FEATURES OF SCC, BCC, AND MM



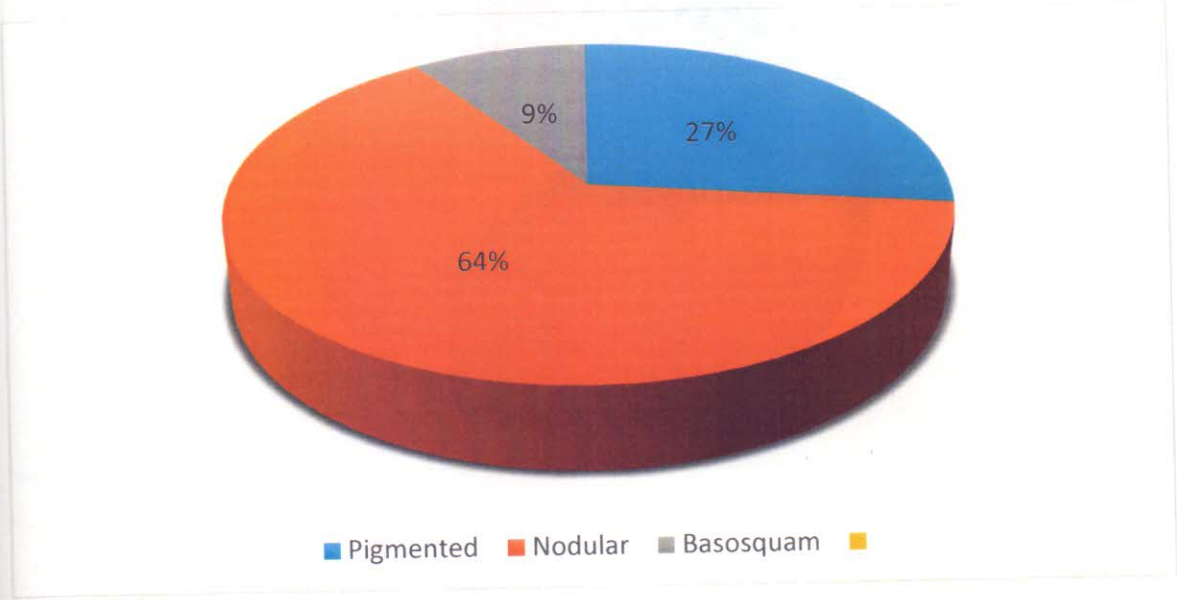
Majority of SCC presented clinically as ulceroproliferative growth (61.2%), followed by 25.8% of cases presenting as ulcerative lesions. BCC presented as ulcerative lesions in 54.5% of cases and ulceroproliferative growths in 45.4% of cases. Ulceroproliferative growth (54.5% and ulcerative lesions (45.4%) were the most common presentations in MM.

FIG 42: HISTOLOGICAL DIFFERNTIATION IN SCC

DIFFERENTIATION	PERCENTAGE
WDSCC	77.4%
MDSCC	16.1%
PDSCC	6.4%

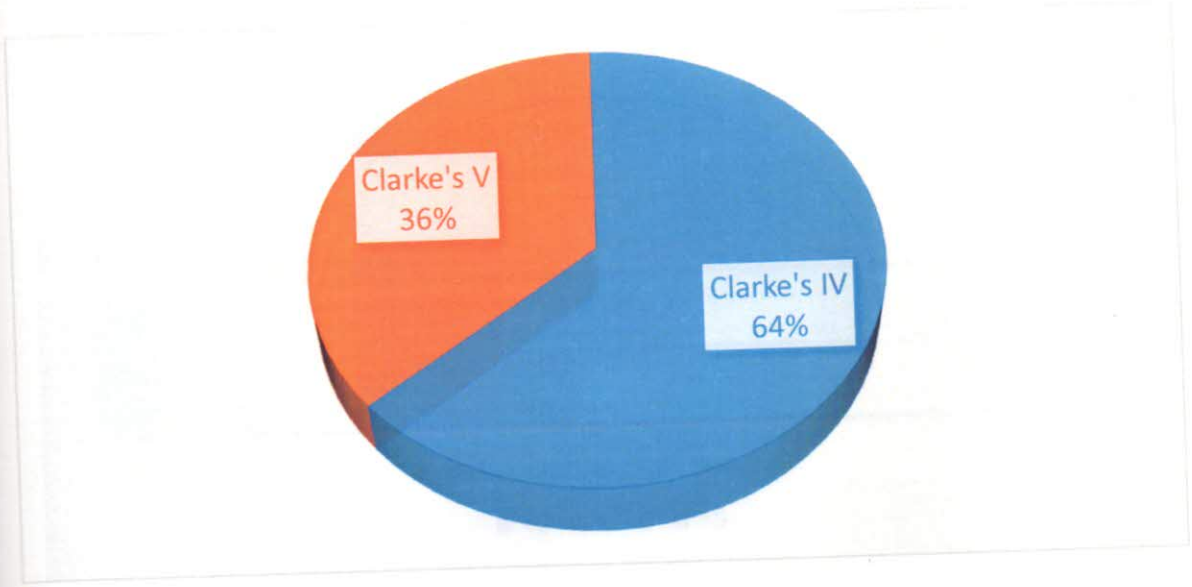
Histologically, most of SCC were well differentiated constituting about 77.4% of cases, followed by 16.1% cases of moderately differentiated SCC.

FIG 43: HISTOLOGICAL TYPES OF BCC



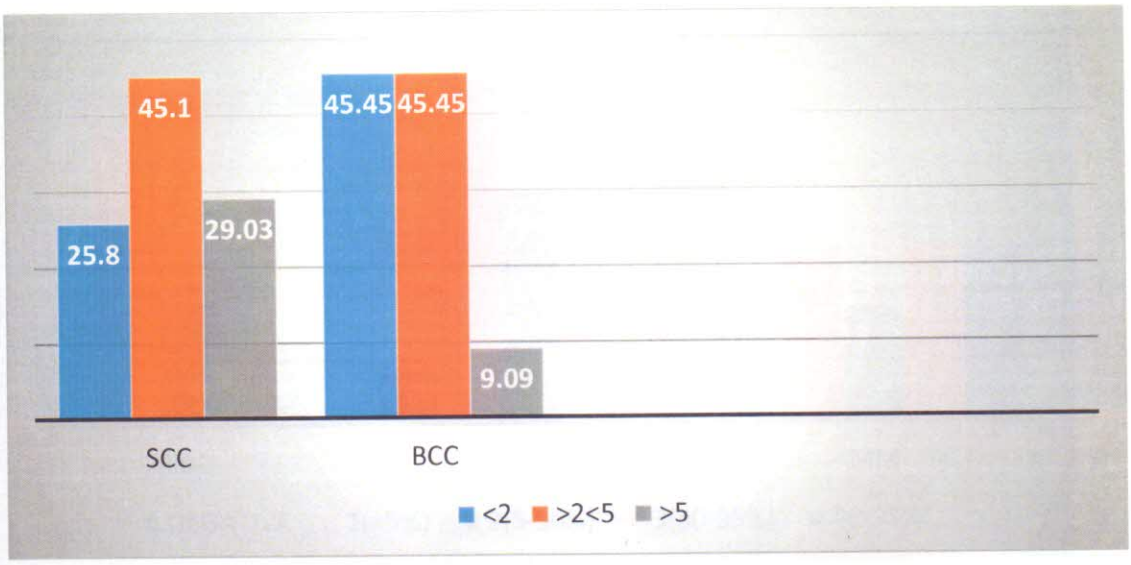
Nodular BCC was seen in majority of BCC cases (64%), followed by pigmented BCC (27%) and basosquamous carcinoma (9%).

FIG 44: CLARKE'S LEVEL OF INVASION IN MM



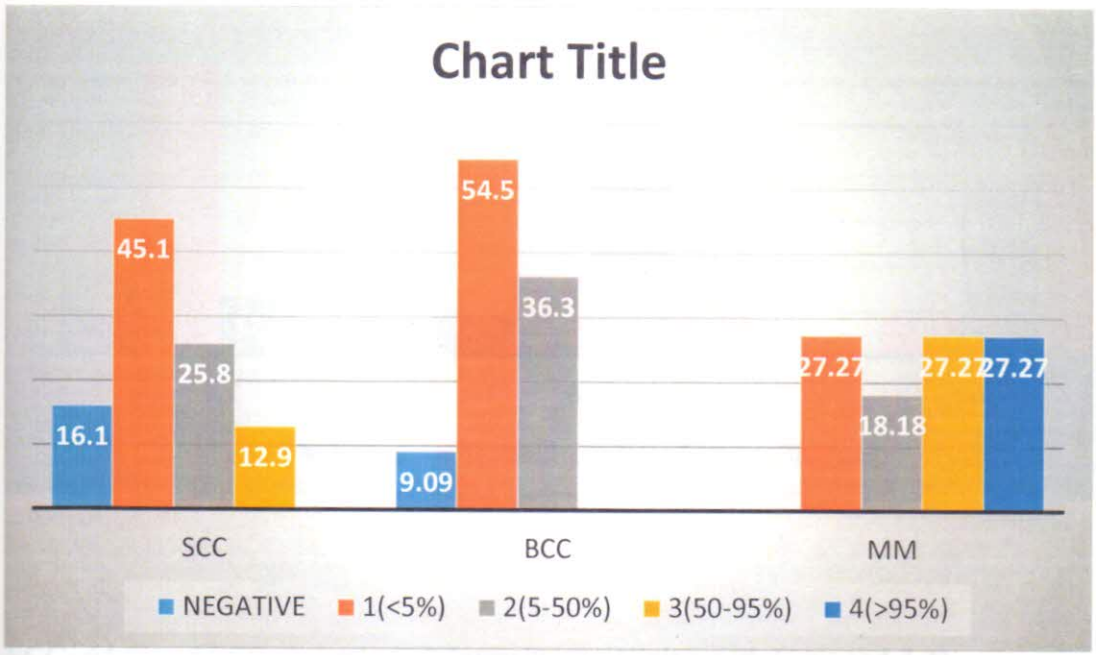
64% of MM showed Clarke's level of invasion-IV, whereas Clarke's level of invasion V was seen in 36% of cases.

FIG 45: SIZE OF THE TUMOR IN SCC AND BCC



Most of the SCC were between 2-5cm in size (45%), whereas in BCC, 45.45% of cases were between <2cm and 2-5cm in size. Size was not considered in MM, as Clarke's level of invasion is a better prognostic indicator than size of the tumor.

FIG 46: PERCENTAGE OF TUMOR CELLS EXPRESSING C-KIT IN SCC, BCC AND MM

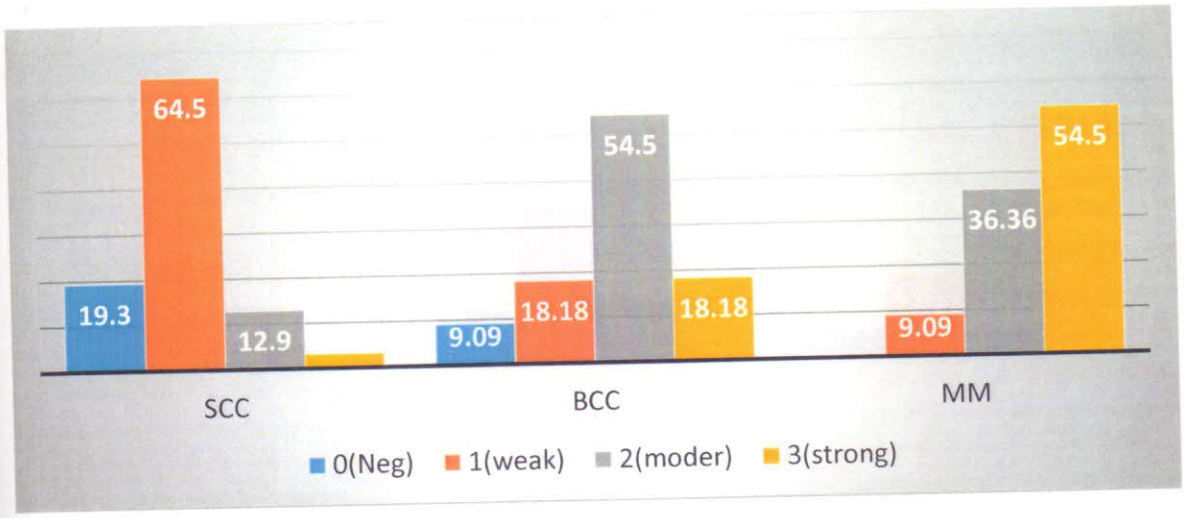


SCC: Majority of the tumor cells in SCC expressed C-kit in <5% of cells (Score 1). 25.8% and 12.9% of cases expressed C-kit in 5-50 %(score 2) and 50-95 %(Score 3) of cells respectively. None of the tumor cells showed >95% of tumor cell positivity. 16.1% of tumors were negative for C-kit immunostaining in SCC.

BCC: 54.5% of cases of BCC showed positive C-kit staining in <5% of tumor cells (score 1) and 36.3% of cases showed positivity in 5-50% of cases (score 2). None of the cases showed positivity in 50-95% of cells or >95% of cells. Negative staining was seen in 9.09% of cases.

MM: 27.27% cases of MM showed tumor cell positivity in <5% (Score 1), 50-95% (score 3) and >95% (score 4) of cells, followed by 18.18% of cases showing a score of 2. None of the cases of MM were negative for C-kit immunostaining.

FIG 47: INTENSITY OF STAINING OF C-KIT IN SCC, BCC AND MM.

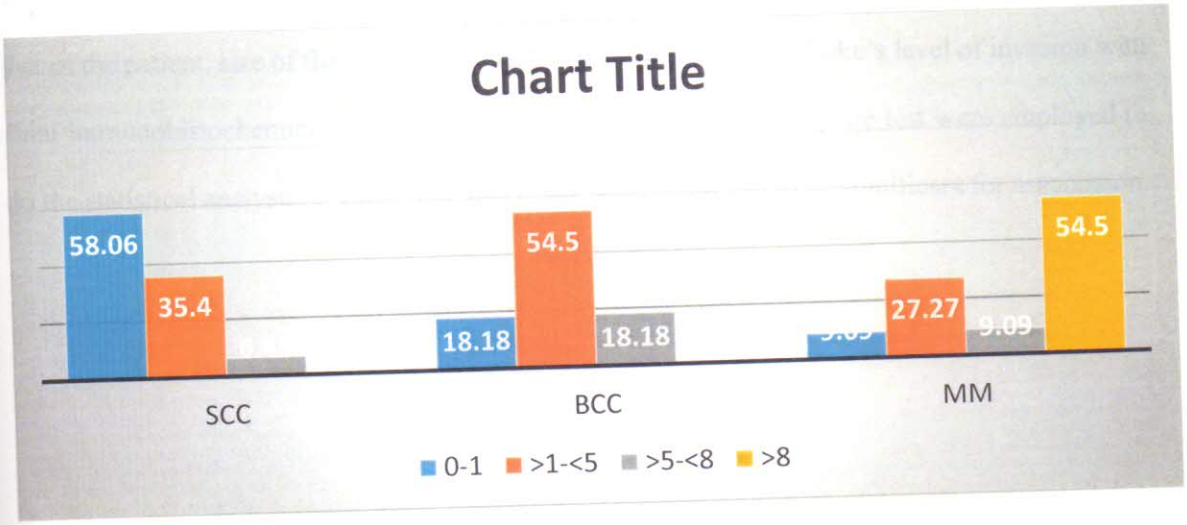


SCC: 64.5% of SCC showed weak intensity on staining with C-kit and 3.2% of cases showed strong intensity.

BCC: 54.5% of cases of BCC showed moderate intensity of staining.

MM: 54.5% of cases of MM showed strong intensity, followed by 36.6% of cases showing moderate intensity of staining.

FIG 48: FINAL SCORE OF IHC FOR C-KIT IN SCC, BCC AND MM



Positivity cut off was taken as >5% of tumor cells staining with at least weak or moderate intensity i.e. final score of >1.

SCC: 58.6% of cases of SCC were negative for C-kit immunostaining and 35.45% of cases had a final score between 1-5(Weak staining).None of the cases of SCC showed a score of >8.

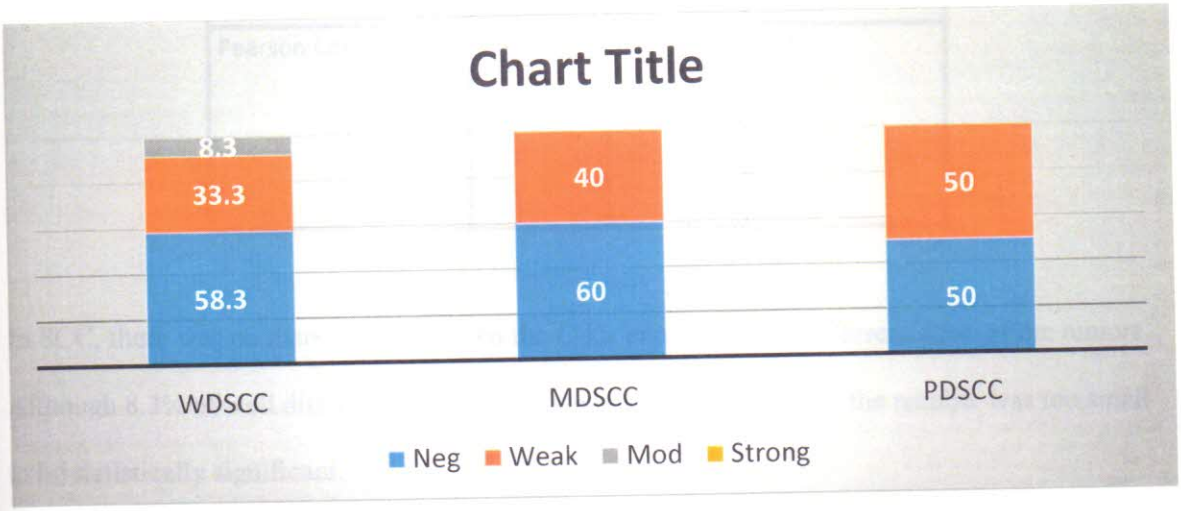
BCC: 54.5% cases of BCC showed weak positivity (Final score between 1-5) and 18.18% of cases showed moderate positivity. None of the cells showed strong positivity.

MM: 54.5% of case of MM showed strong positivity with a final score of >8. 27.27% of cases showed weak staining and 9% of cases were negative for C-kit immunostaining.

STATISTICAL ANALYSIS:

Statistical analysis was done to evaluate the association between various parameters like age and sex of the patient, size of the tumor, histopathological subtype and Clarke's level of invasion with final immunohistochemical score. Pearson correlation test and chi square test were employed to do the statistical analysis. *P* value less than 0.001 was considered to be significant for association.

FIG 49: CORRELATION OF FINAL IHC SCORE WITH TUMOR DIFFERENTIATION IN SCC

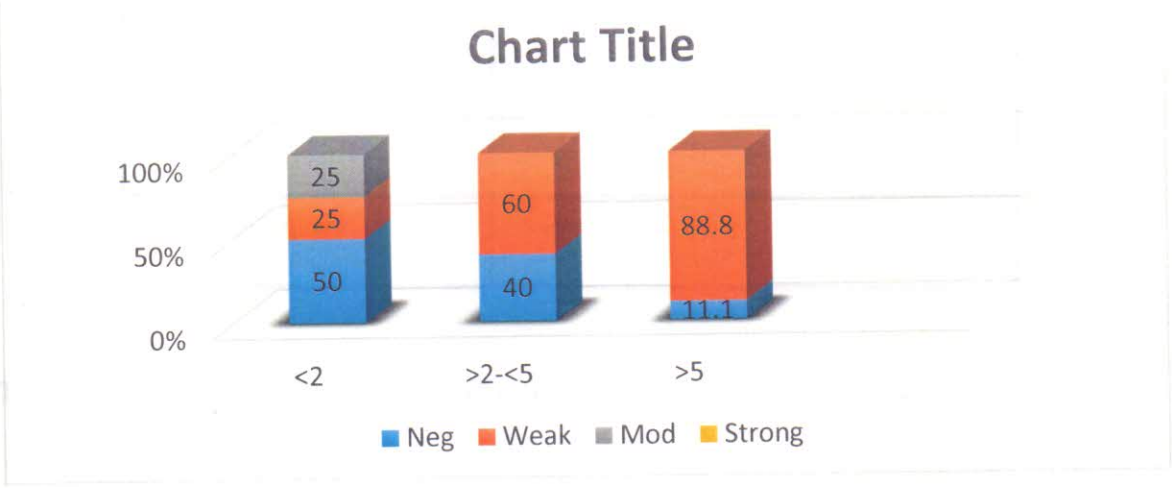


			Final score recoded			Total
			0 to 1 negative	2 to 4 Weak	5 to 7 Moderate	
SCCvariants	MDSCC	Count	3	2	0	5
		% within SCCvariants	60.0%	40.0%	0.0%	100.0%
	PDSCC	Count	1	1	0	2
		% within SCCvariants	50.0%	50.0%	0.0%	100.0%
	WDSCC	Count	14	8	2	24
		% within SCCvariants	58.3%	33.3%	8.3%	100.0%
Total		Count	18	11	2	31
		% within SCCvariants	58.1%	35.5%	6.5%	100.0%

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.788[b]	4	.940

In SCC, there was no association between the C-kit expression and differentiation of the tumors. Although 8.3% of well differentiated SCC showed moderate positivity, the number was too small to be statistically significant.

FIG 50: CORRELATION OF FINAL IHC SCORE WITH TUMOR SIZE IN SCC



			Final score recoded			Total
			0 to 1 negative	2 to 4 Weak	5 to 7 Moderate	
Dimensions recoded	< 2	Count	3	2	0	5
		% within Dimensionsrecoded	60.0%	40.0%	0.0%	100.0%
	2 to 5	Count	5	2	2	9
		% within Dimensionsrecoded	55.6%	22.2%	22.2%	100.0%
	> 5	Count	10	7	0	17
		% within Dimensionsrecoded	58.8%	41.2%	0.0%	100.0%
Total		Count	18	11	2	31
		% within Dimensionsrecoded	58.1%	35.5%	6.5%	100.0%

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.534[b]	4	.237

There was no association seen between C-kit expression and size of the tumor in SCC.

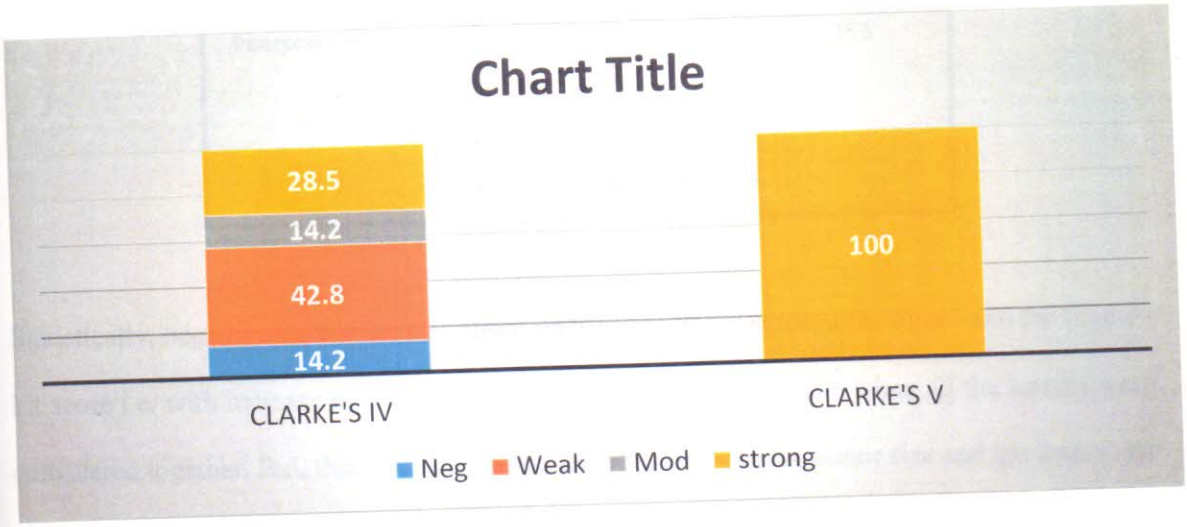
FIG 51: CORRELATION OF FINAL IHC SCORE WITH TUMOR SIZE IN BCC

			Final score recoded			Total
			0 to 1 negative	2 to 4 Weak	5 to 7 Moderate	
Dimensionsrecoded	< 2	Count	0	4	1	5
		% within Dimensionsrecoded	0.0%	80.0%	20.0%	100.0%
	2 to 5	Count	1	1	0	2
		% within Dimensionsrecoded	50.0%	50.0%	0.0%	100.0%
	> 5	Count	1	2	1	4
		% within Dimensionsrecoded	25.0%	50.0%	25.0%	100.0%
Total		Count	2	7	2	11
		% within Dimensionsrecoded	18.2%	63.6%	18.2%	100.0%

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.986[b]	4	.560

There was no association between C-kit expression and size of the tumor in BCC.

FIG 52: CORRELATION OF FINAL IHC SCORE WITH CLARKE'S LEVEL OF INVASION IN MM



			Final score recoded				Total
			0 to 1 negative	2 to 4 Weak	5 to 7 Moderate	> 8 Strong	
Clarkes level	IV	Count	1	3	1	2	7
		% within Clarkeslevel	14.3%	42.9%	14.3%	28.6%	100.0%
	V	Count	0	0	0	4	4
		% within Clarkeslevel	0.0%	0.0%	0.0%	100.0%	100.0%
Total		Count	1	3	1	6	11
		% within Clarkeslevel	9.1%	27.3%	9.1%	54.5%	100.0%

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.238[b]	3	.155

Statistically, negative correlation was observed between dimensions of the tumor and the final C-kit score i.e. with increase in size there was decrease in the final score when all the tumors were considered together. But, there was no correlation seen between the tumor size and the final C-kit score when individual tumors were considered. Statistically significant association was seen only between the age of the patient and BCC. No statistically significant association was seen between differentiation in SCC, histopathological types, sex in BCC and Clarke's level of invasion in MM and the C-kit expression.

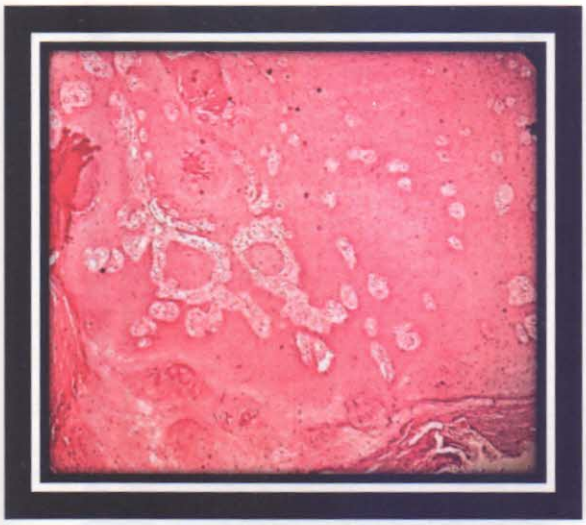


Fig 53: Microphotograph of Squamous cell carcinoma – Low power (B/1963/11)

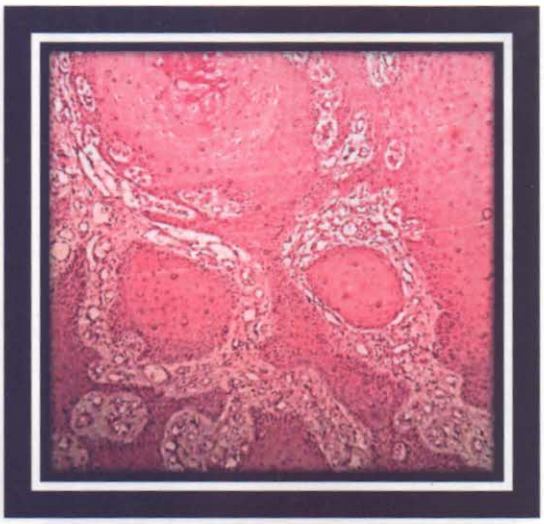


Fig 54: Microphotograph of squamous cell carcinoma – High Power (B/1963/11)

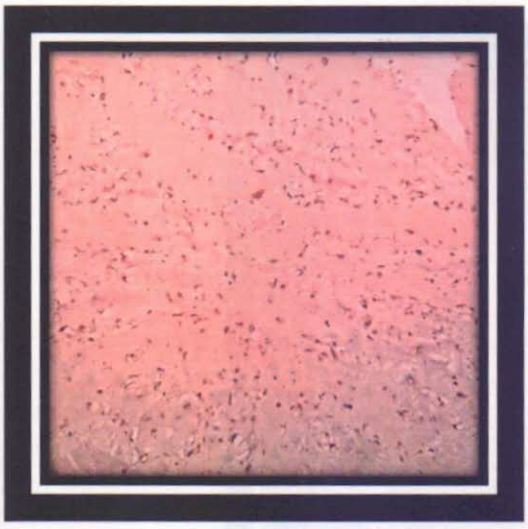


Fig 55: C-kit expression in SCC (Score 6) - Low power

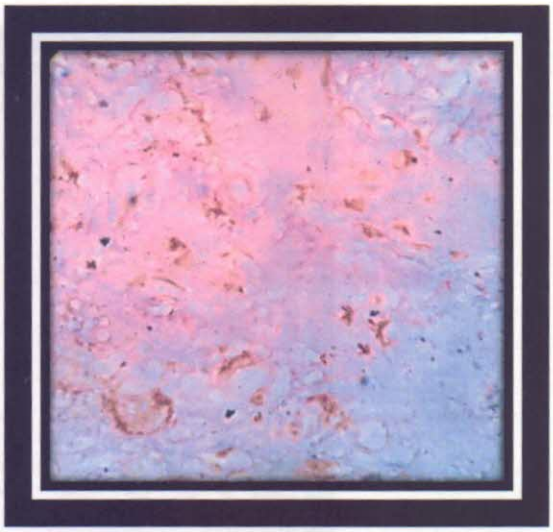


Fig 56: C-kit expression in SCC (Score 6) – High power

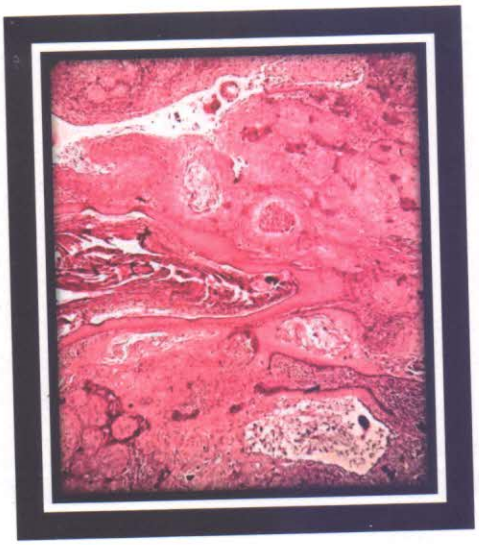


Fig 57: SCC – scanner view (B/1050/15)

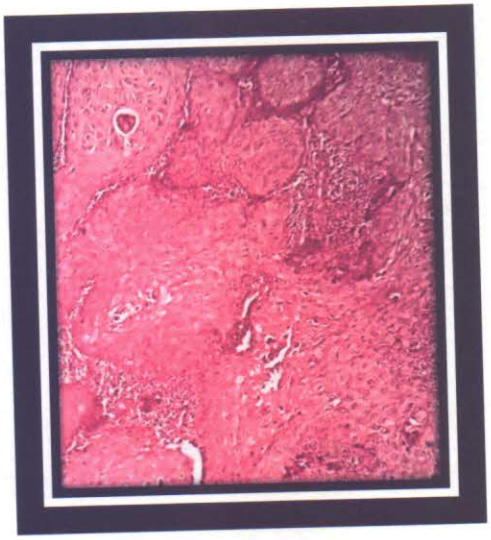


Fig 58: SCC – Low power (B/1050/15)

Fig 59: C-kit in SCC – Negative (B/1050/15)

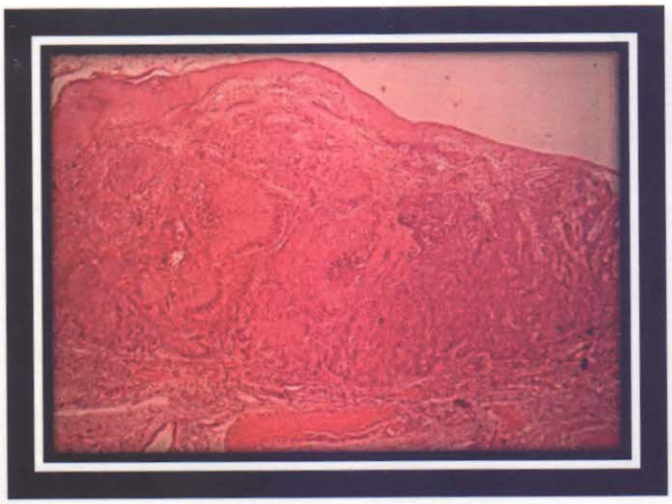


Fig 60: BCC – Scanner view (B/296/11)

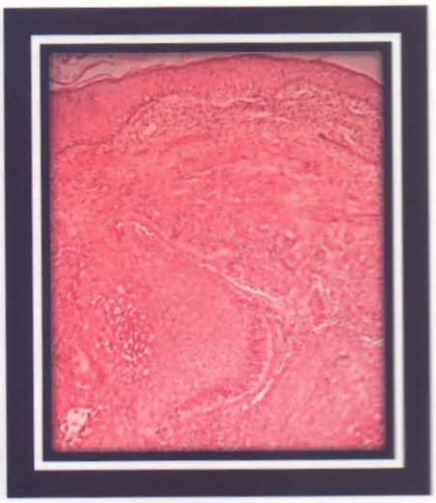


Fig 61: BCC – Low power (B/296/11)

Fig 62: BCC – High power (B/296/11)

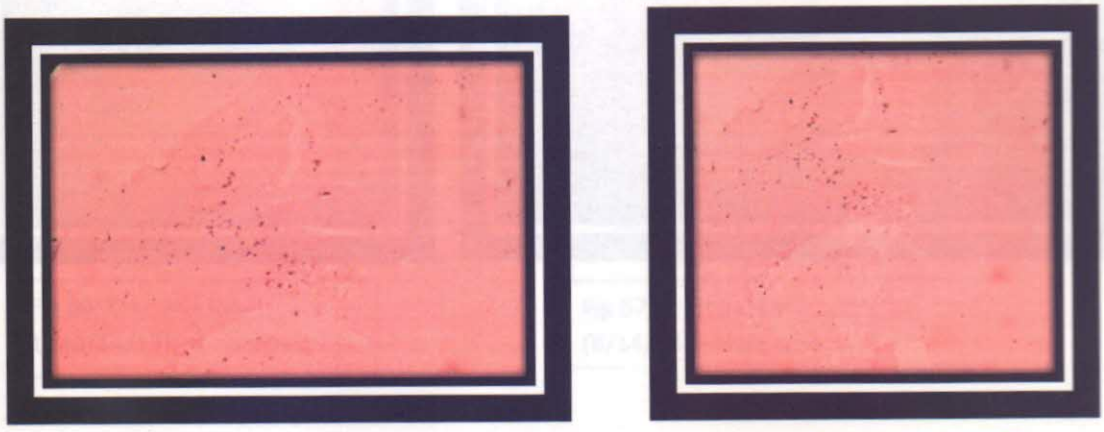


Fig 63: C- Kit in BCC – Score 6 (B/296/11) – Low power

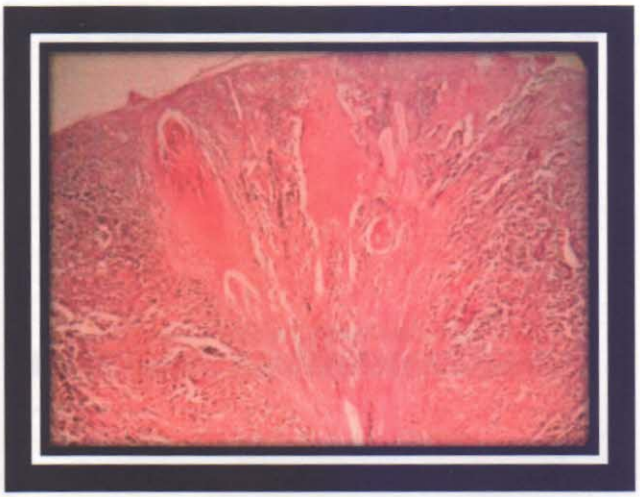


Fig 64: MM – scanner view (B/14/13)

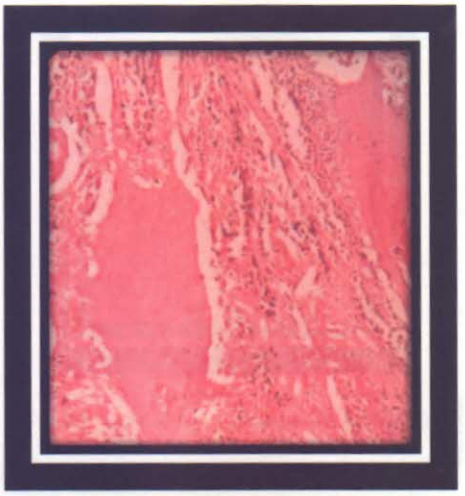


Fig 65: MM – Low power (B/14/13)

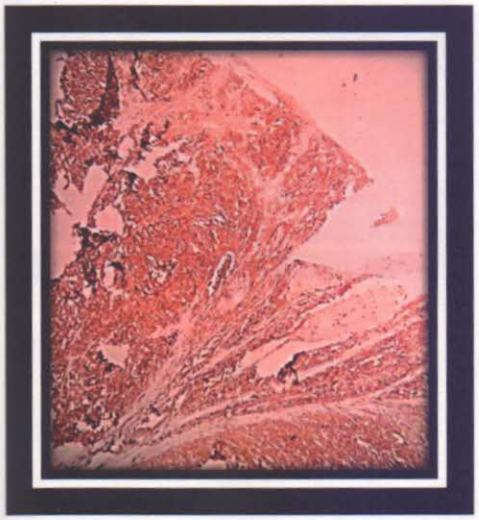


Fig 66: C-kit in MM – score 12 (B/14/13) – Low power

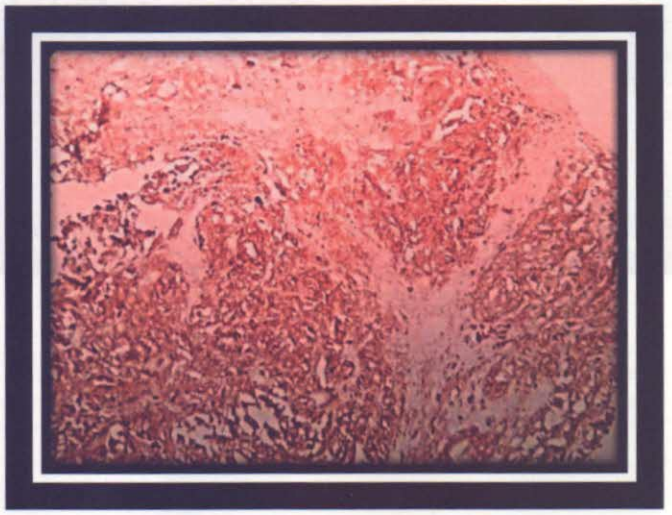


Fig 67: C-kit in MM – score 12 (B/14/13) – High power

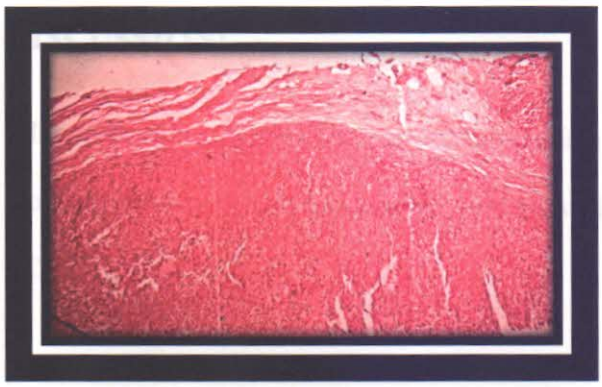


Fig 68: MM – scanner view (B/486/07)

Fig 69: MM – Low power (B/486/07)

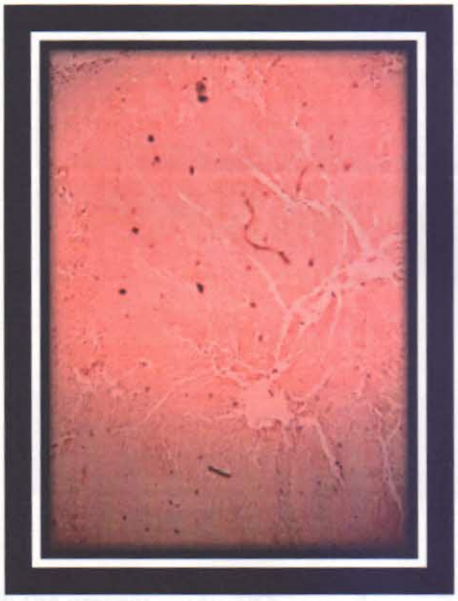


Fig 70: C Kit in MM – score 9 (B/486/07) – Low power

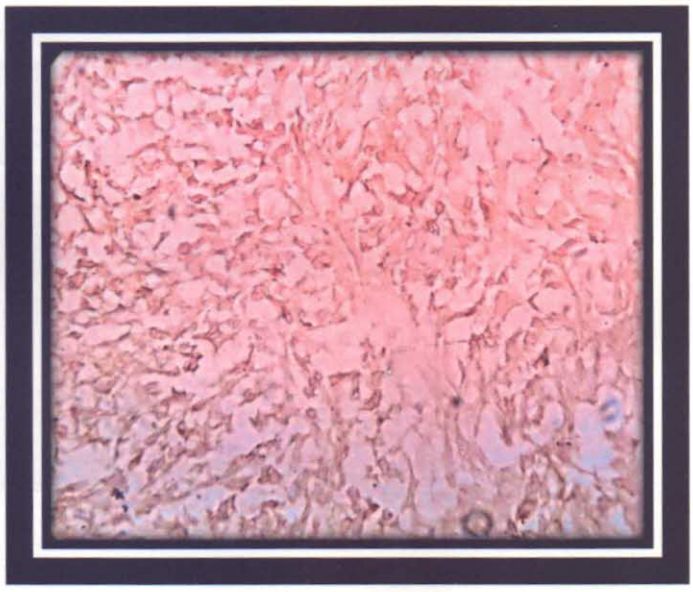


Fig 71: C-kit in MM – score 9 (B/486/07) – High power

DISCUSSION:

Malignant epidermal tumors constitute a very small subset of epithelial malignancies. BCC is the most common malignant tumor in the western world whereas in India, SCC is more common than BCC.[10]

Table 5: COMPARISON OF PREVALANCE OF SCC, BCC AND MM

	SCC	BCC	MM
Present study	58.4%	20.7%	20.7%
Deo et al[32]	55.8%	18.1%	26.1%
Laishram et al[33]	43.6%	32.6%	14.2%

In the present study, SCC was the most common tumor in concordance with other studies done in Manipur and New Delhi, India. Keratinocytic tumors are more common in fair skinned individuals than in Blacks/Asians due to protection offered by melanin pigment against UV radiation induced DNA damage which leads to carcinogenesis.

Sex ratio in SCC:

Table 6: COMPARISON OF SEX RATIO IN SCC

	M:F ratio
Present study	2.03:1
Schmults et al[34]	1.13:1
Robsahm et al[35]	1.12:1
Hollestein et al[36]	1.49:1

M:F ratio in the present study was 2.03:1. There was a male preponderance in the present study when compared to other studies.[2,3,5] This may be due to more number of men working outdoors in India and especially in the rural part. Women are mostly housewives and work indoors when compared to men.

Table 7: COMMON AGE GROUP AFFECTED IN SCC

	AGE GROUP
Present study	61-70yrs (32.2%)
Khullar et al[37]	40-60yrs(median)
Schmults et al[34]	71yrs(Median)
Baruah et al[10]	40-50yrs (54.1%)
Laishram et al[31]	61-70yrs (28.3%)

Most common age group affected in SCC in the present study was 61-70yrs. SCC is seen in the sun exposed skin of elderly people. This was in concordance to a study done in North India [33] where the most common age group affected was between 61-70yrs (28.3%). In another study done in Sikkim[10], most common age group affected was 40-50yrs. This may be because of temperate climate seen in Sikkim throughout the year. Therefore, the etiology of SCC may be different from other areas where sun exposure plays a major role. The SCC arising in the setting other than sun exposure affects younger age group and have a variety of other etiological factors like immunocompromised individuals.

Table 8: MOST COMMON SITE INVOLVED IN SCC

	SITE(%)
Present study	Lower limb(38.7)
Schmults et al[34]	Head and neck(28.7)
Khullar et al[37]	Head and neck(50)
Hollestein et al[36]	Head and neck(41.1)
Baruah et al[10]	Upper lip(75)

The most common site involved in the present study was lower limb (38.7%). But this was in contrast to other studies, [10, 34, 36, 37], where head and neck was the most common site affected by SCC. In rural parts of India, especially in South India, people wear shorts and work in the fields, which may predispose the farmers for the development of SCC in the lower limb.

Table 9: MOST COMMON HISTOPATHOLOGICAL TYPE IN SCC

HISTOPATHOLOGICAL TYPE	GRADE(%)
Present study	WDSCC(83.8)
Baruah et al[10]	WDSCC(66.7)
Alakloby et al[38]	WDSCC(45.1)

The most common histopathological type in SCC was the well differentiated SCC which was in concordance to other studies.

Table 10: COMPARISON OF M: F RATIO IN BCC

	M:F RATIO
Present study	0.57:1
Kumar et al[39]	0.57:1
Malhotra et al[40]	1.6:1
Chow et al[41]	0.7:1

The M: F ratio in the present study was 0.57:1. This was in concordance to other studies done in India[39, 40] and China [41].

Table 11: MOST COMMONLY AFFECTED AGE GROUP IN BCC

	AGE GROUP
Present study	50-60,61-70yrs(36.3%)
Khullar et al[37]	75yrs(mean)
Kumar et al[39]	60-85yrs(47.2%)
Chow et al[41]	73.1yrs(Mean)
Baruah et al[10]	61-70yrs(46.1%)

Most commonly affected age group in BCC in the present study was 50-70yrs (36.3%) which was in concordance with other studies conducted in India and China[37, 39, 41, 10].

BCC is commonly seen in sun damaged skin in fair skinned individuals in adult patients[11]. Age over 60yrs is associated with recurrent BCC and poor prognosis.

Table 12: MOST COMMON SITE INVOLVED IN BCC

	SITE (%)
Present study	Infra orbital (36.3%)
Chow et al[41]	Nose (31.6%)
Christenson et al[42]	Nose (13.3%)
Baruah et al[10]	Upper lip (84.6%)

BCC affects head and neck region predominantly due to maximum exposure to sun. In the present study, all the cases (100%) involved head and neck in concordance with a study done by Baruah et al [10]. In another study done in North India, Head and neck was involved in 80% of cases. In the head and neck region, nose is affected in most of the cases in a study done by Chow et al[41] However, in the present study, infra orbital region was affected in majority of cases (36.3%).

Table 13: PERCENTAGE OF BCC IN HEAD AND NECK REGION

	HEAD AND NECK
Present study	100%
Khullar et al[37]	80%
Christenson et al[42]	57.8%
Baruah et al[10]	100%

Table 14: MOST COMMON HISTOLOGICAL PATTERN IN BCC

	HISTOLOGICAL TYPE
Present study	Nodular (63.6%)
Alakloby et al[38]	Nodular (84.3%)
Malhotra et al[40]	Nodular (64.7%)
Kumar et al[39]	Nodular (77.8%)

Nodular BCC was the most predominant histopathological type in the present study which is in concordance with other studies[38, 39, 40]. Nodular BCC has a better prognosis than other histologic subtypes like micro nodular, morphoeic and infiltrative BCC.

Table 15: COMPARISON OF M: F RATIO IN MALIGNANT MELANOMA

	M:F RATIO
Present study	1.7:1
Sharma et al[43]	1.2:1
Mukhopadhyay et al[44]	3:1
Lee et al[45]	1.3:1

MM is most common in men than women. M: F ratio in the present study was 1.7:1 which was in concordance with other studies done by Sharma et al and Lee et al. In an article published by Mukhopadhyay et al, the M: F ratio was 3:1.

Table 16: MOST COMMON AGE GROUP AFFECTED IN MM

	AGE GROUP
Present study	51-60yrs (54.5%)
Lee et al[45]	61-70yrs (25%)
Mukhopadhyay et al[44]	51-60yrs (31.25%)
Sharma et al[43]	50-60yrs (55.5%)

MM affects elderly people with a peak incidence around 60yrs[11].The present study also showed majority of cases around the age of 50-60yrs. Other studies done by Mukhopadhyay and Sharma also showed that most of the cases in the range of 50-60yrs.

Table 17: MOST COMMON SITE INVOLVED IN MM

	SITE
Present study	Lower limb (81.7%)
Sharma et al[43]	Eye (31.9%)
Laishram et al[33]	Lower limb (38.4%)

Most commonly affected site in MM is the face and head & neck region. The lower limbs are affected in females more than males[11]. In the present study, the lower limbs were affected predominantly in 81.7% of cases. Face or head and neck region were involved in only 9% of cases. This was in contrast to a study done by Sharma et al, where the eye was the most predominant site involved. However, in a study published by Laishram et al [33], lower limb was the most common site involved, similar to the present study. But, the number affecting the lower limbs were less compared to the current study.

C-kit expression in SCC:

TABLE 18: COMPARISON OF C-KIT EXPRESSION IN SCC

SCC	Site of SCC	Percentage of positive cases
Present study	SCC-Skin	41.85%
Ongkeko et al[7]	SCC-Head and Neck	86%- Pharynx 50%- Larynx
Maia et al[6]	SCC-Vulva	70.5%
Shang et al[46]	SCC-Esophagus	55.9%

In the present study, 41.85% of cutaneous SCC showed C-kit expression, of which 35.45% had a weak expression and 6.45% showed moderate expression of C-kit. None of the cases showed strong C-kit expression. Though, C-kit expression in SCC of various sites have been studied, extensive review of literature revealed no studies of C-kit expression in cutaneous SCC.

Ongkeko et al studied expression of protein kinases in head and neck squamous cell carcinomas.[7] IHC expression was considered positive if the tumor cells showed moderate intensity or if >10% of cells stained more than the negative background cells. The study showed 86% of pharyngeal tumors and 50% of laryngeal tumors expressed C-kit protein. This difference in expression of C-kit in pharyngeal and laryngeal tumors was thought to be related to the different embryologic

derivatives of pharynx and larynx. They also suggested that these C-kit expressing tumors may benefit from targeted therapy by Imatinib as normal squamous epithelium of pharynx and larynx did not show C-kit expression. C-kit expression was seen exclusively in carcinoma cases. However, in the present study, positivity cut off was taken as >5% of tumor cells showing at least weak intensity. This method evaluation of C-kit was employed in a study done by Pilloni et al in melanocytic lesions. As we considered all the malignant epidermal tumors, we followed the same scoring system for all the tumors to have uniformity and eliminating the bias that can occur because of different scoring system.

In another study by Maia et al, C-kit expression was seen in 70.5% of cases of vulvar squamous cell carcinoma. Expression of C-kit was considered positive when tumor cells showed moderate or strong intensity in either >10% or 50% of tumor cells. They observed that increased C-kit expression was associated with higher global survival and disease free survival. As the vulvar SCC expressing C-kit have better prognosis and higher global survival, C-kit inhibitors and other chemotherapeutic agents may not be useful in improving the survival in C-kit expressing vulvar SCC.[6]

C-kit binds to stem cell factor and plays a critical role in stimulating invasion and metastasis in various cancers. Also, SCF-CD117 pathway plays an important role in promoting angiogenesis. Studies have shown that C-kit over expression may be correlated with chemo resistance through activation of wnt-beta catenin-ATP binding cassette G2 signaling.[48]

In a study done by Shang et al, over expression of C-kit was seen in 55.9% of esophageal squamous cell carcinomas, but was not significantly correlated with poor survival.[46] In contrast, Fan et al[29]

showed C-kit expression in 29.9% of esophageal squamous cell carcinoma and amongst the positive tumors, 10.8% of tumors were strongly positive for C-kit. In this study, significant association was seen between C-kit expression and T stage, lymph node metastasis and distant metastasis. They also showed that C-Kit positive tumors had decreased progression free survival and overall survival. In the present study, we could not assess the correlation between C-kit expression and overall survival as many patients were lost to follow-up. Also, none of the SCC cases showed strong C-kit expression. Statistically no significant association was seen between the differentiation and size of the tumor with the final C-kit expression.

The fact that C-kit exerts divergent functions depending on its modulation by environmental factors, its interaction with several different intracellular effector pathways and alternative splicing of its mRNA, may be the cause for contradictory results seen in different tumors.[47]

However, more studies have to be done to evaluate the correlation between C-kit expression and prognosis and disease free survival in squamous cell carcinoma.

C-KIT IN BCC:

Table 19: Comparison of C-kit score in BCC

BCC	PERCENTAGE OF TUMOR CELLS POSITIVE FOR C-KIT
Present study	72.68%
Terada et al[9]	92%

The number of BCC cases positive for C-kit in the present study was 72.68%. But, none of the tumor cells in BCC showed strong positivity i.e. score >8. This high percentage of tumor cells positive for BCC was in concordance to a study done by Terada T.[9] Terada was the first person to demonstrate C-kit expression in basal cell carcinoma. In his study, he showed C-kit to be positive in 92% of cases (61/66 cases). He also studied other makers like NCAM, PDGFRA, chromogranin and synaptophysin in BCC. But, high expression was seen only in respect to NCAM and C-kit.

The other studies showed consistently lower c-kit expression in BCC. [48, 49, 50] However, in these studies, C-kit expression was studied in all solid tumors and the number of BCC cases included in the study was minimal. This may be the possible reason for low expression of C-kit in BCC in these studies.

Mino et al[48] studied C-kit expression in neoplasms of head and neck and showed C-kit could be an ancillary marker for adenoid cystic carcinoma. In this study, 11 cases of BCC were included and all of them showed negative C-Kit expression.

In various other studies, they have evaluated the expression of C-kit in the stroma of basal cell carcinoma[26, 51]. Mast cells in the stroma stain positive for C-kit. Studies have shown that increased mast cells in the stroma correlates with poor prognosis as mast cells may have a role in tumor progression and metastasis. However, in these studies, they have not mentioned about the C-kit positivity in the tumor cells. Mast cells were not assessed in the present study.

There was no significant association seen between histological types, age and sex of the patient and size of the tumor with the final C-kit expression in BCC in the present study.

This conflicting results regarding expression of C-kit in BCC should be validated using bigger sample size and evaluating for association between C-kit expression and prognosis/overall survival. This would help in categorizing the patients who would benefit from targeted therapy with Imatinib.

C-KIT EXPRESSION IN MM:

TABLE 20: C-KIT IN MALIGNANT MELANOMA

MM	PERCENTAGE OF POSITIVE CASES
Present study	91.29%
Guerriere-Kovach et al[52]	48.8%
Ericsson et al[53]	62.6%
Sheikh et al[54]	36%
Potti et al[55]	22.8%

In the present study, C-kit expression was seen in 91.29% of cases of MM. 54.5% of cases showed strong expression, 9.09% of cases showed moderate expression and 27.27% showed weak expression of C-kit.

The positivity of C-kit expression in primary MM among various studies is highly variable.

Potti et al[55] studied C-kit expression in 202 melanoma cases. Only 22.8% of cases showed C-kit expression. In the present study, 91.29% of cases were positive for C-kit expression. This discordance may be because of more number of cases included in the former study, whereas only 11 cases of malignant melanomas were included in the present study.

Pilloni et al[8] studied c-kit expression in nevi and malignant melanoma. In this study, they evaluated C-kit expression in epidermal and dermal melanocytes separately using the same scoring system as the present study. 18 MM cases were included in the study, out of which 3 were melanoma in situ, 1 was acral melanoma and 14 were superficial spreading melanomas. 1 acral melanoma included in the study showed strong C-kit expression with a score of 12 in the epidermal component and moderate expression in the dermal component. Out of the 14 SSM, 50% showed moderate and 35% showed strong reaction to C-kit in epidermal component whereas 28.5% showed moderate and only 7% showed strong intensity in the dermal component.

Sheikh et al evaluated the expression of C-kit in 12 malignant melanomas, out of which 10 were cutaneous and 2 were oral mucosal melanoma. Out of the 10 cutaneous melanomas, 6 showed positive C-kit expression (60%). Strong expression was seen in 4 cases66.6%) and moderate expression in 2 cases. This was in concordance to the present study where strong expression of c-kit was seen in 54.5% of cases.

Lin et al[56] evaluated C-kit expression in malignant melanomas and its association with clinicopathological parameters. 111 cases of primary and metastatic melanomas were included in the study along with benign nevi and dysplastic nevi. The average C-kit score in malignant melanoma was 89.5+/- 22.6. The scoring system employed for evaluation of C-kit expression was different from the present study. They considered the intensity of staining (0-4) and percentage of tumor cells positive for C-kit. They multiplied the intensity and percentage of tumor cells positive for C-kit to get the final score. The range was between 0-400. However, they have not mentioned the percentage of MM cases which were positive for C-kit in this study.

Studies have also shown variable C-kit expression in melanomas depending on the anatomic location.

Tariq et al[57] studied anorectal melanomas and clinicopathological features along with C-kit expression. Out of 61 cases of anorectal melanomas, immunohistochemistry for C-kit was performed in 4 cases and positive expression was seen in only one case (25%). The low percentage of tumors expressing C-kit may be because only 4 cases were evaluated for the expression of C-kit.

In another study done by Chute et al[58], C-kit expression was seen in 75% of anorectal malignant melanoma. In the present study, we did not have any case of anorectal melanoma.

Few studies have shown that acral and mucosal melanomas are the most common subtype of melanomas harboring Kit mutation and expressing high C-kit expression[31, 59] than the other subtypes. Beadling et al[31] studied kit mutations in melanomas affecting various anatomical sites. He showed 23% of acral melanomas, 15.6% of mucosal melanomas, 7.7% of conjunctival melanomas and 1.7% of cutaneous melanomas had Kit gene mutations. C-kit immunohistochemical staining was done in 105 melanoma cases which showed positivity in 39% of cases. C-kit expression did not correlate with the Kit mutation status. But, Contradicting results were obtained in another study done in China where acral and mucosal melanomas showed a low level of C-kit expression.[59] In this study, 502 cases of MM were studied for Kit gene mutations and C-kit expression immunohistochemically.11.9% of acral and mucosal melanomas showed kit gene mutation. C-kit expression was seen in 37.3% of acral melanomas. He also analyzed the correlation of Kit mutation and C-kit IHC expression. The data indicated that Kit mutation may not necessarily lead to increased C-kit expression immunohistochemically. These results suggest that C-kit expression is not a reliable marker for Kit aberration analysis and should not be used as a screening test to identify Kit genetic alterations.

Studies have shown C-kit to be positive in dysplastic nevi more than in MM. In a study done by Pilloni et al [8], they assessed the intensity and percentage of tumor cells stained with C-kit in the epidermis and dermis. Out of 14 superficial spreading melanoma, they found that intensity and percentage of tumor cells positive for C-kit is more in the junctional component than the tumor cells in the dermis. They concluded that C-kit expression gradually decreases from dysplastic nevi to malignant melanoma and almost negative in metastatic melanomas.

Shen et al[60] analyzed protein tyrosine kinases in melanocytic lesions comprising of benign nevi, dysplastic nevi, malignant melanoma and metastatic melanoma. The study showed 55% expression in benign nevi, 100% in dysplastic nevi, 96% in malignant melanoma and 45% in metastatic melanoma. In concordance with Pilloni et al, they too showed the loss of c-kit expression in metastatic melanoma compared to primary malignant melanomas

Lin et al[56] showed higher C-kit score was seen in dysplastic nevi than in malignant melanoma. Dysplastic nevi showed maximum score of 275.1, whereas primary melanomas showed decreased expression of C-Kit with a score of 89.5.This implies that C-kit protein is involved in the mid step of the transformation between common melanocytic nevi and malignant melanoma.

In a study done by Huang[61], he demonstrated that melanoma cells expressing C-kit binds to the kit ligand i.e. SCF (stem cell factor) and induces apoptosis. In tumors not expressing C-kit, the SCF does not bind to C-kit and the tumor cells continue to survive and result in metastasis. This may explain the loss of C-kit expression from dysplastic nevi to metastatic melanoma.

Although, C-kit may be used as a diagnostic marker in MM, GIST and leukemias and to differentiate MM from clear cell sarcoma, which is C-kit negative, it can also be used to assess the prognosis of various tumors.

Potti et al[55] did a multivariate analysis to evaluate the correlation between difference in survival in kit negative and kit positive groups. Though, C-kit overexpression was seen in only 22.8% of cases in MM. However, 53.7% of cases of superficial spreading melanoma showed C-kit over expression, indicating C-kit is expressed in the early stage of the disease and down regulated in primary and metastatic melanomas. This study also showed C-kit overexpression has no prognostic value in malignant melanomas. There was no statistically significant association between survival and Kit expression.

Zhao et al[62] did a systematic review and meta-analysis to assess the prognostic significance of C-kit expression in various tumors. No association was found between C-kit expression and overall survival in malignant melanomas as they had limited number of studies included in meta-analysis. Also, the method of detection of C-kit expression and scoring system used to evaluate C-kit expression was different in all the studies and provided potential source of bias.

In the present study, the higher percentage of C-kit expression in MM may be because of limited number of cases of MM (11). Also most of the cases (81.7%), were seen in the lower limb (Acral melanomas). High number of acral melanoma cases in the present study may be a source of bias. This difference in C-kit expression may be attributed to various Kit gene mutations that may be present according to the ethnicity of the population studied. More studies have to be done to evaluate the Kit gene mutations in Indians and its correlation with Kit overexpression so as to device an accurate method to determine c-kit expression and also to identify the subset of patients who may benefit from tyrosine kinase inhibitor therapy.

Recent data have shown that response to Imatinib may not be entirely dependent on Kit expression level. The response rate may depend on the presence of Kit mutations in the tumor and also on the location and type of mutation.[54] Kit mutations represent a spectrum of changes such as point mutations, in frame deletions and internal tandem duplications. Some of the C-kit mutations have specific clinical connotations and differ in inhibitor sensitivity.

Although numerous studies have been done to evaluate C-kit expression in various tumors, data regarding the usefulness of C-kit expression and targeted therapy with Imatinib and other drugs have not been studied extensively in malignant epidermal cutaneous tumors. Very few studies have been done to correlate C-kit expression with prognosis/overall survival/disease free survival. C-kit expression has different implications in various tumors. For example, C-kit expression was associated with higher global survival and recurrence free survival in vulvar squamous cell carcinoma, where targeted therapy may not be beneficial as these patients have a better prognosis than C-kit negative tumors. Where as in malignant melanoma, C-kit expression decreases as the tumor progresses from dysplastic nevi to malignant melanoma to almost negative C-kit expression in metastatic melanoma.

The present, retrospective study is a pilot study done to evaluate the C-kit expression in malignant epidermal cutaneous tumors. However, more studies have to be done to validate the usefulness of C-kit as a prognostic marker and also to implement C-kit expression as a site for targeted therapy.

SUMMARY:

A retrospective study was undertaken to evaluate the C-kit expression in malignant epidermal cutaneous tumors i.e. squamous cell carcinoma, basal cell carcinoma and malignant melanoma in the Department of Pathology, SDUMC, Kolar.

Following were the salient features of the study:

1. A total of 53 cases were selected, out of which 31 were SCC, 11 each of BCC and MM.
2. Male to female ratio in SCC, BCC and MM was 2.03:1, 0.57:1 and 1.7:1 respectively.
3. Common age group affected in SCC was 61-70yrs, 50-70yrs in BCC and 50-60yrs in MM.
4. Most common site involved in SCC was lower limb, head and neck(Infra orbital region) in BCC and lower limb in MM
5. Most common histopathological type in SCC was the conventional SCC and majority were well differentiated SCC. Majority of the BCC's were of nodular subtype.
6. C-kit positivity was seen in 41.85% of SCC, 91.29% of MM and 76.68% of BCC.
7. Statistically, negative correlation was observed between dimensions of the tumor and the final IHC score.
8. No association was observed between C-kit expression and age and sex of the patient, tumor differentiation and histological type in SCC and BCC and Clarke's level of invasion in MM.

CONCLUSION:

C-kit expression was detected in 41.85% of SCC, 72.68% of BCC and 91.29% of MM. Weak and moderate C-kit expression was seen in SCC and BCC. Strong C-kit expression was not seen in SCC and BCC. 54.5% of MM showed strong C-kit expression and 9.09% showed weak and moderate expression. There was no correlation between the different grades of differentiation, age and sex of the patient, size of the tumor and C-kit expression in squamous cell carcinoma. There was no significant association between histological type, sex of the patient and size of the tumor and C-kit expression in basal cell carcinoma. There was no significant association between Clarke's level of invasion and C-kit expression in malignant melanoma.

FURTHER SCOPE FOR THE STUDY:

C-kit expression is varied in malignant epidermal tumors. C-kit expression in BCC is relatively a new finding. More studies have to be done to evaluate the usefulness of C-kit as a prognostic marker. Identification of C-kit in a significant proportion of patients could possibly have important therapeutic implications and in evaluating the role of site specific therapies in squamous cell carcinoma, basal cell carcinoma and malignant melanoma.

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EXPRESSION OF C-KIT IN MALIGNANT EPIDERMAL CUTANEOUS TUMORS

PROFORMA

Biopsy number

Patient name

Hospital number

Age

Sex

History

Site of the lesion

Clinical diagnosis

HISTOPATHOLOGY:

Gross :

Size

Type of growth Ulcer Ulceroproliferative growth Nodular Others

Appearance Hyperpigmented Hypopigmented

Distance of tumor from margin

No of lymph nodes retrieved

MICROSCOPY:

1. Epidermis

2. Dermis

3. Diagnosis

4. Tumor thickness

5. Margins Involved Free

6. Clarke's grading

7. Breslow's thickness

IMMUNOHISTOCHEMISTRY: C-Kit

1. No. of cells staining

0 1 2 3 4

2. Intensity of staining

0 1+ 2+ 3+ 4+

KEYS TO MASTER CHART:

Sl no	Serial number
Biopsy number	Biopsy number
Name	Patient's name
Site	Site of the tumor
Gross	Gross appearance of the tumor
Dimension	Size of the tumor
Margins	Margins of the tumor
Lymph node	Lymph node involved/not
HPE	Histopathological diagnosis
Clarke's level	Clarke's level of invasion in MM
Percentage of cells	Percentage of cells staining for C-kit
Intensity	Intensity of staining with C-kit
Final score	Final c-kit score

sl no	Biopsy no	Name	Age	sex	Clinical Diagnosis	site	gross	Dimensions	Margins	Lymph nodes	HPE	clarke's level	% cells	Intensity	Final score
4	1488/13	Lakshmamma	65	F	BCC	Nose	ulcer	1X0.5	free		Basosquamous Ca		1	2	2
2	1354/07	Narayanaswamy	50	M	SCC	Left cheek	Grey brown stm	3X2X0.5	free		BCC		1	2	2
5	788/05	parvathamma	80	F		Forehead	Pigmented Growth	1.5X1	free		BCC		2	2	4
10	296/11	Muniyamma	80	F	?BCC	lower eyelid	ulcer	1X0.5	free		BCC		2	3	6
38	710/11	Sunandamma	55	F	BCC	L infraorbital	UP growth	1X1	Involved		BCC		1	2	2
42	922/15	Varalakshmamma	75	F	?SCC	Left pinna	ulcer	3X2X1	Involved		BCC		1	1	1
47	472/11	Rangappa	70	M	BCC	L Lower eyelid	UP growth	3X2	Free		BCC		1	2	2
8	113/10	Raghupathy	55	M	?BCC	lower eyelid	UP growth	6X5	DRM involved		BCC		2	2	4
29	0008/10	Syed hussain	75	M	Infected corn	R foot	ulcer	2X2	Free		BCC- solid pig variant		1	1	1
32	71/11	Mangamma	65	F	SCC	R parietotemp	warty growth	6.5X5.5	free		MDSCC	IV	2	2	4
39	120/11	Kakanna	75	M	SCC	L foot	fungating	5X4	free		MDSCC	IV	3	1	3
6	743/09	Rangappa	60	M	?MM	Great toe	ulcer	2.5X2.5	DRM involved		MDSCC	V	1	2	2
7	1039/09	Obalamma	75	F	?MM	R Heal	ulcer	2X1.5	Free		MM	IV	2	2	4
11	486/07	Krishna Reddy	87	M	SCC	L foot	nodular growth	2.5X2.5	free		MM	IV	3	3	9
12	1329/06	Venkateshappa	40	M		R foot	G/W stb	1X0.5	free		MM	V	1	1	1
13	80/06	Subbamma	60	F	MM	R palm	G/W nodule	1.5X0.5	free		MM	IV	1	1	1
14	129/06	Varadegowda	70	M	SCC	R eyelid	Exophytic growth	1.5X0.5	Free		MM	IV	1	2	2
15	14/13	Venkataramappa	60	M	MM	R sole	G/W ulcer	3X3	free		MM	IV	3	3	9
16	1897/13	Jayappa	60	M	MM	sole	G/W tumor	5X4	free		MM	IV	4	3	12
43	1043/15	Krishna shetty	60	M	MM	Left heel	UP growth	3X2	free	Three	MM	V	4	2	8
45	1045/15	Narasamma	60	F	?MM	Left leg	ulcer	3X2	Free		MM	IV	2	3	6
17	1961/11	puttamma	40	F	Sebaceous cyst	Sole	ulcer	2.5X2.5	free		MM	V	3	3	9
3	15/13	Vinayakamma	65	F	BCC	L Pinna	ulcer with rolled out mar	2.5X1.5	free		MM	V	4	3	12
40	888/08	Adilakshmi	60	F	incisional hernia	ant abd wall	scar	8X7X3	DRM involved		MM-spindle cell varian	V	0	0	0
9	976/11	Rathnamma	55	M	?BCC ?MM	L nasal fold	ulcer	0.7X0.5	free		Nodular BCC		2	1	2
1	289/07	Susheela	62	F	BCC	Nose	Hypopigmented	3.5X3X0.5	free		Non keratinizing SCC	IV	1	2	2
18	642/07	Thayamma	80	F	SCC	Cheek	G/W growth	1.5X1	free		Pig BCC		2	3	6
34	1796/11	Revaiah	77	M	Non healing ulcer	L leg	ulcer	1X1	free		Pigmented BCC		2	2	4
37	1963/11	Anwar	50	M	?metastasis	Groin	STM	2X2	free		PDSCC	V	1	1	1
44	1044/15	Ramegowda	55	M	SCC	Chest wall	UP growth	2X1X1	free		WDSCC	IV	3	2	6
46	1046/15	Beeramma	65	F	?BCC	Left lower lip	UP growth	4X3	Free		WDSCC	IV	1	1	1
48	1047/15	Ramappa	65	M	?MM	Right heel	UP growth	4X3	Free		WDSCC	IV	1	1	1
49	1048/15	Maramma	75	F	SCC	Left forearm	ulcer	3X1	Free		WDSCC	V	0	0	0
50	1049/15	Venkatappa	60	M	?BCC ?SCC	right cheek	UP growth	3X3	Free		MDSCC	V	1	1	1
51	1050/15	Sultan	80	M	SCC	Chin	UP growth	3X1	Free		WDSCC	V	1	1	1
52	1051/15	Mahadev	70	M	SCC	R Shoulder	UP growth	4X2	Free		WDSCC	IV	0	0	0
53	1052/15	Rathnamma	65	F	SCC	Scalp	UP growth	3X2	free		WDSCC	IV	1	1	1
27	2720/10	Mangamma	65	F	sebacous cyst	scalp	STM	2X1	free		WDSCC	IV	1	1	1
41	2700/10	Gurappa	60	M	SCC	Left heel	ulcer	2.5	Free		MDSCC	IV	2	1	2
19	1229/07	Venkatappa	85	M	SCC	Groin	G/W CF growth	6X5	DRM involved	0/2	WDSCC	IV	3	1	3
20	1690/06	Dhan Singh	55	M	SCC	R leg	Growth with everted edges	3X1	free		WD SCC	III	1	1	1
21	578/13	Rajamma	65	F	SCC	Sternum	Nodular growth	5X2	free		WDSCC	III	0	0	0
22	260/05	Chalapathi	35	M	Pilonidal sinus	anus	G/W area with sinus tract	4X1.5	free		WDSCC	IV	1	1	1
23	1851/09	Venkateshappa	55	M	SCC	Cheek	STM	2.5X1.5	free		WDSCC	III	0	0	0
24	732/08	Haneef	44	M	SCC	R foot	ulcer	5X1.5	free		WDSCC	V	1	1	1
25	133/08	Bhagyamma	50	F	Marjolin's ulcer	Popliteal fossa	ulcer	5X3	free		PDSCC	IV	1	1	1
26	2048/10	Sridhar Murthy	57	M	SCC	L leg	UP growth	5X6	free		WDSCC	IV	1	1	1
28	1616//10	Krishna Reddy	68	M	pilomatricoma	R thigh	fungating ulcer	4.5X4	free		WDSCC	V	2	1	2
30	250/10	Chandrashekhar	52	M	Trophic ulcer	R foot	up growth	2.5X1.5	free		WDSCC	II	2	1	2
31	380/10	Venkatappa	52	M		R foot	fungating growth	8X6	free		WDSCC	III	2	1	2
33	1456/11	Subbamma	45	F	Marjolin's ulcer	L upper limb	ulcer	8X7	free	One	WDSCC	V	3	1	3
35	2291/11	Lakshmana	70	M	SCC	scalp	ulcer	1X0.5	free		WDSCC	IV	1	2	2
36	1866/11	Shaikh	67	M	Infected sebaceous cyst	Nose	Nodule	2X1.5	Free		WDSCC	III	0	0	0
												IV	2	3	6