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Original Article

Pathology Section

The Importance of Cytokeratin 19 Expression in the Differentiation of Basal Cell Carcinoma and Trichoepithelioma

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ABSTRACT

Introduction: Basal cell carcinoma (BCC) is the most common skin neoplasm reported in human. On the other hand, trichoepithelioma (TE) is a rare, benign tumour of skin adnexa. The differentiation of BCC and TE may be difficult since their morphological findings are similar. In a few studies, it has been determined that undifferentiated basaloid cells are highly positively stained with cytokeratin 19.

Aim: The aim of this study was the comparison of cytokeratin 19 expression in cases of BCC and TE.

Materials and Methods: Sections of skin tissues of 17 TE, 25 BCC and 12 non-neoplastic cases were used for cytokeratin 19 (CK19) immunohistochemical staining.

Results: Staining with CK19 of the BCC cases gave 15(60%) diffuse, 7 (28%) focal and 3 (12%) negative staining. On the other hand, among TE cases, 2 (12%) gave diffuse, 5 (29%) focal and 10 (59%) negative staining with CK19. In the non-neoplastic skin tissue samples, while positive staining with cytokeratin 19 in the outer root sheath of hair follicles and sweat glands were observed, there was no staining in basal layers.

Conclusion: CK19 expression may be helpful in the differential diagnosis of BCC and TE especially in small skin biopsy samples in which morphologic differentiation is difficult.

Keywords: Differential diagnosis, Follicular germinative cells, Hair buds, Immunohistochemistry, Skin tumour

INTRODUCTION

Basal cell-carcinoma (BCC) is the most common skin tumour constituting approximately 70% of all skin malignancies. It occurs mostly in the elderly especially in the head and neck regions in sun- exposed areas. It is a locally aggressive tumour with very rare metastatic rates. With immunohistochemical studies, it has been shown that BCC originates from follicular bulge stem cells and basaloid epithelia of follicular projections of the anagen hair buds [1,2]. On the other hand, trichoepithelioma (TE) is a rare, benign tumour of skin adnexa originating from follicular germinative cells. They are commonly located on the face and hairy skin. There are three subtypes of TE: desmoplastic, solitary and multiple. Lesions are generally solitary and sporadic papules or nodules in skin color. They show similarities with BCC since they are formed from basaloid islands and cordons with peripheral palisading in fibrous stroma. Abortive hair follicles or hair papillae may be seen that mimic epithelial structures in the tumour. Small keratinous cysts are quite common in the dermis [3]. In small skin biopsies, if morphological findings of BCC and TE are overlapping, differential diagnosis may be especially difficult. In previous studies, for the differentiation of these two tumours, some immunohistochemical markers such as CD10, bcl2, CK15, and Ber-EP4 have been used [2,4, 5]. In several studies, cytokeratin 19 (CK19) has been determined to have a high specificity for undifferentiated basaloid cells. CK19 is a small (40 kDa) acidic keratin that is expressed in germinative basaloid cells. The CK19 gene has been mapped on chromosome 17, which is homologous to the murine and bovine CK19 genes. In immunohistochemical evaluations, many different cell types such as human oval cells, or putative hepatic stem cells, cholangiocytes and human corneal epithelial basal stem cells have been shown to express CK19. CK19 in the skin is expressed by basal cells on the external root sheath of hair follicles [6]. In the study of Stoll et al., [7] for the differentiation of odontogenic keratocysts and dentigerous or radicular cysts, CK19 expression has been determined to be beneficial. They did not determine the expression of CK19 in odontogenic keratocysts but found a positive expression in dentigerous and radicular cysts. In the study of Aslan et al., [8] for the subtyping of intraepidermal malignancies with epithelial origin, CK19 expression has been shown to be different. They denoted a widespread positive reaction with CK19, a helpful feature in the differentiation of Paget's disease from Bowen's disease and bowenoid actinic keratosis (BAK).The aim of this study is to investigate CK19 expression in the differentiation of BCC and TE.

MATERIALS AND METHODS

Skin sections of 25 BCC and 17 TE cases (Department of Pathology, Recep Tayyip Erdogan University Medical Faculty, Rize, Turkey) collected between 2010 and 2013 were investigated. The diagnoses of all cases were confirmed by the re-evaluation of haematoxylin-eosine stained specimens by two different investigators. Twelve non-neoplastic skin tissue samples were used as negative control. Age, gender, and location of the lesions were recorded in all cases. None of the cases selected for this study had been previously stained with the antibody to CK19. The age of the paraffin embedded blocks was less than four years. The samples consisted of punch biopsy with adequate tumour tissue and excisional biopsy of the tumour. Normal skin biopsy samples were used for control. Very tiny punch biopsies, poorly fixed samples and cases with diagnostic confusion were excluded.

3-4 μm sections were obtained from the paraffin embedded blocks, fixed with formalin, and stained immunohistochemically with a monoclonal mouse antibody to human CK19 (clone A53-B/A2-26, A00122-007, ScyTek, USA) at 1:100 dilution. The biotin-free, HRP multimer-based, hydrogen peroxide substrate and 3, 3'-diaminobenzidine tetrahydrochloride (DAB) chromogen containing ultraView™ Universal DAB Detection Kit (Catalog number 760-091, Ventana Medical Systems, Tucson, AZ, USA) and a fully automated immunohistochemistry (IHC) staining device (Ventana Bench Mark XT, Ventana Medical Systems, Tucson, AZ, USA) were used for the IHC staining system. The sections were counterstained with Mayer's hematoxylin and bluing solution in the device was followed by dehydration after which the sections were made transparent

Patient No	Age	Sex	Localization	Immunoreactivity
1	64	F	Cheek	Diffuse positive
2	76	M	Right auricula	Diffuse positive
3	82	F	Nasal dorsum	Focal positive
4	59	F	Lomber bölge	Focal positive
5	63	M	Right eyelid	Diffuse positive
6	78	F	Right eyelid	Focal positive
7	85	M	Scalp	Diffuse positive
8	76	M	Nasal dorsum	Focal positive
9	62	F	Upper lip	Diffuse positive
10	78	M	Right postauricula	Focal positive
11	79	F	Right postauricula	Negative
12	75	M	Leftalanasi	Focal positive
13	67	M	Nasal dorsum	Diffuse positive
14	76	F	Nasal dorsum	Diffuse positive
15	81	F	Right malar site	Diffuse positive
16	77	F	Right auricula	Focal positive
17	52	M	Cheek	Diffuse positive
18	66	F	Gluteal region	Diffuse positive
19	75	M	Right leg	Diffuse positive
20	57	M	Left shoulder	Negative
21	84	F	Right eyelid	Diffuse positive
22	78	M	Scalp	Negative
23	60	M	Nasal dorsum	Diffuse positive
24	48	M	Cheek	Diffuse positive
25	78	F	Scalp	Diffuse positive

[Table/Fig-1]: Clinicopathologic findings of BCCs

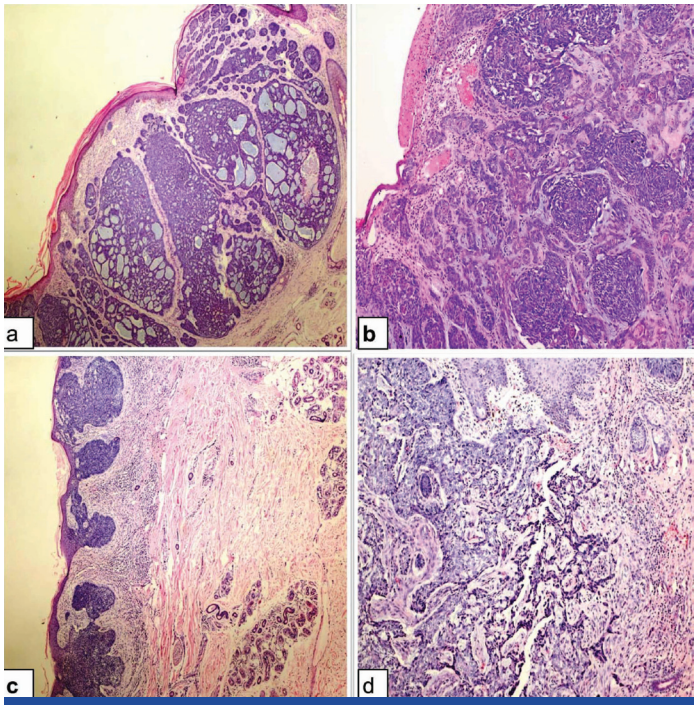
with xylene and the process was concluded with manual cover slip closure.

EVALUATION OF IMMUNOREACTIVITY

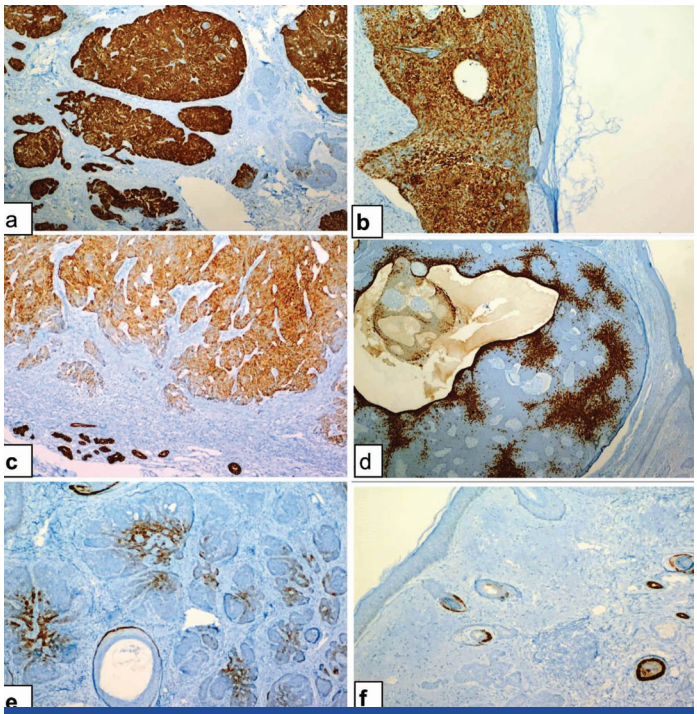
The degree of immunoreactivity with CK19 was classified into the following three categories: negative (no positive cells in the tumour), + (<50% focal positive in the tumour) and ++ (>50% diffuse positive cells in the tumour). Reactivity of the tumour cells was analyzed for central and/or peripheral staining pattern. CK19 expression was compared with the positive control outer root sheaths of hair follicles and sweat glands [9].

RESULTS

Of the 25 BCC cases 12 were women, while 13 were men. The mean age was 71 y (range 43-94 y). The most common location of BCC was the face and the most common subtype was the nodular type (16 cases). Others included superficial type (3 cases), morphea-like type (3 cases) and keratotic type (3 cases). Age, gender and location of BCC cases are summarized in [Table/Fig-1]. In the histopathological investigation of BCC cases, solid islands in the dermis and peripheral palisading basaloid cells around the islands that showed infiltration in cordons were observed. Retraction artefacts between the tumour islands and the stroma and in the interconnections of basaloid islands with epidermis were detected. In the nodular subtype, a peripheral palisading pattern in basaloid cells forming nodular aggregates was observed [Table/Fig-2a]. Keratotic type BCCs showed keratinized cells forming glob cornea mixed with basaloid islands [Table/Fig-2b]. On the other hand, in the superficial subtype, tumour islands were interconnecting with the inferior layers of the epidermis, which infiltrated the superficial layers of the dermis [Table/Fig-2c]. In morphea-like type BCCs, tumour infiltration producing thin layers embedded in the dense fibrous stroma were observed [Table/Fig-2d].



[Table/Fig-2]: a) Nodular type BCC forming nodular aggregates (H&E, X40), b) Keratotic type BCC with keratinized cells forming glob cornea mixed with basaloid islands (H&E, X100), c) Superficial type BCC with peripheral palisading through the basal layer of epidermis and proliferating through the upper dermis (H&E, X40), d) Morphea-like type BCC with tumoural infiltration producing thin layers embedded in the dense fibrous stroma (H&E, X100)



[Table/Fig-3]: a) Nodular type BCC with diffuse positive staining for CK19 (x40), b) Superficial type BCC with diffuse positive staining for CK19 (x100), c) BCC with focal positive staining for CK19(x100), d) TE with central positive staining for CK19 (x40), e) TE with focal positive staining for CK19 (x40),
f) Non-neoplastic skin tissue with positive staining with cytokeratin 19 in the outer root sheath of hair follicles and sweat glands (x100)

Of all BCC cases [Table/Fig-3a-f], 15 (60%) had diffuse positive staining and 7 (28%) had focal positive staining, while 3 (12%) were negatively stained with CK19 [Table/Fig-3a-c]. The BCC cases did not show central and peripheral staining pattern. The CK19 staining ratios of BCC cases and subtypes are summarized in [Table/Fig-1,4].

Of the 17 TE cases 12 were women and, five were men. The mean age was 50 y (range 21-82 y). The most common location of TE was the face. The age, gender and location of the TE cases are

BCC subtype	CK19 (-)	CK19 (+)	CK19 (++)
Nodular	2/25	4/25	10/25
Morfea-like	1/25	2/25	0/25
Superficial	0/25	1/25	2/25
Keratotic	0/25	0/25	3/25

[Table/Fig-4]: CK19 results of BCC subtype
- negative (no positive cells in the tumor), + focal positive (<50% focal positive in the tumor), ++ diffuse positive (>50% diffuse positive cells in the tumor)

Patient No	Age	Sex	Localisation	Immunoreactivity
1	32	F	Scalp	Diffuse positive
2	82	F	Right inguinal region	Negative
3	67	F	Nasal dorsum	Focal positive
4	79	M	Nasal dorsum	Negative
5	47	F	Cheek	Negative
6	65	F	Gluteal region	Diffuse positive
7	46	F	Leftleg	Negative
8	66	F	Perianal region	Focal positive
9	55	F	Upper lip	Negative
10	40	M	Left auricula	Negative
11	35	F	Scalp	Negative
12	42	F	Cheek	Negative
13	21	F	Right alanasi	Focal positive
14	34	F	Vulva	Focal positive
15	38	M	Face	Focal positive
16	50	M	Left alanasi	Negative
17	62	M	Right malar site	Negative

[Table/Fig-5]: Clinicopathologic findings of TEs

summarized in [Table/Fig-5]. In the histopathological evaluation of the TE, tumour originated from basaloid cells forming solid islands and cordons in dermis. In contrast to BCC cases, retraction artefacts between the tumour islands and the stroma or the interconnections of basaloid islands with epidermis were not detected in the TE cases. Among the TE cases, 2 (12%) had diffuse positive staining while 5 (29%) had focal positive staining and 10 (59%) were negatively stained with CK19. Central staining pattern was observed in one case only [Table/Fig-3d,e].

In non-neoplastic skin tissue samples, while positive staining with cytokeratin 19 in the outer root sheath of hair follicles and sweat glands was observed, there was no staining in the basal layers [Table/Fig-3f].

STATISTICAL ANALYSIS

Mann Whitney U-test was used to compare differences between groups using the SPSS 17.0 program. p-value of <0.05 was considered to be statistically significant.

BCC cases, 13 (52%) were men and 12 (48%) were women. TE cases, 5 (29%) were men and 12 (71%) were women. The mean age and gender distribution of the cases are summarized in [Table/Fig-6]. The mean age of the BCC cases was statistically significantly older than that of the TE cases. When the gender distribution is investigated, many of the TE cases were female. When the BCC and TE cases are compared for gender distribution, being female was statistically significantly more common among TE cases (p= 0.035). The data regarding the immunoreactivity according to the tumour type is summarized in [Table/Fig-7]. The CK19 staining pattern was different between the BCC and TE groups (p=0.001), which is statistically significant.

DISCUSSION

BCC is the most common tumour of the skin and nearly all lesions are located in hairy regions of skin. They occur most commonly

		BCC	TE	total	p
Age		71.04±10.36	50.64±17.28	63.87±16.93	0.002
Gender	Male	12	5	17	0.035
	Female	13	12	25	

[Table/Fig-6]: Gender and age distribution of cases

		tumor type	
		BCC	TE
Immuno-reactivity	negative n(%)	3 (12)	10 (58.9)
	focal n(%)	7 (28)	5(29.4)
	diffuse n(%)	15(60)	2(11.8)

[Table/Fig-7]: Comparison of CK19 expression between BCCs and TEs

on the face and sun-exposed areas. Although the tumour generally interacts with the inner layers of epidermis, it originates from hair follicles. Even though there is no consensus about the origins of BCCs, they are believed to originate from follicular germinative cells [9]. The clinical and histopathological differentiation of TE and BCC may be difficult. This is especially so in small and superficial skin biopsy specimens, since the histopathological findings are similar, and therefore, correct diagnosis may be difficult. Owing to this similarity, Ackerman et al., [10] named the BCC as "trichoblastic carcinoma". Basaloid cells in BCC have been denominated as abnormal analogues of germinative cells of the embryo. The most important histopathological findings observed in TEs are the presence of tumour islands originating from basaloid cells anastomosing with fibrocellular stroma. In the dermis, cordons and keratin-filled horn cysts may also be seen. In TE, there is no interaction of basaloid islands with epidermis and any retraction artefact between basaloid islands and stroma is not present. The stroma of TE originates from collagen forming hard clusters around the follicular sheath. In BCC, there is an interconnection between the basaloid islands and the epidermis. Also in BCC, retraction artefacts between tumour islands and the surrounding stroma are present. Ulceration in epidermis and presence of myxoid changes in the stroma favours BCC. Desmoplastic TE is accompained by extensive fibrous proliferations that surround and distort the epithelial islands. In contrast with the conventional form, this variant is usually single. Its main differential diagnosis is the morphea-like type basal cell carcinoma [10]. The differentiation of keratotic type BCC from TE may be difficult even for experienced dermatopathologists because of the keratinous structures in dermis [3]. Apoptotic bodies and mitoses are common while ruptured keratinous cysts and foreign-body granulomas are uncommon in BCCs [11]. These findings should be considered in the differential diagnosis of the two tumours.

In the literature, there are studies with some immunohistochemical markers for the differentiation of BCC and TE. Sari Aslani et al., [2] determined a difference in the staining pattern of CD10 expression in basaloid and stromal cells in their study. In TE, although a positive staining with CD10 was seen in stromal cells, basaloid cells lacked staining. On the other hand, in BCC, a denser and more positive staining with CD10 was observed in basaloid cells compared with stromal cells. For the differentiation of these two tumours, there are studies withsome cytokeratins (CK). In a previous study, TE was always positively stained with CK 1/5, CK10/4, CK5/8, CK14 and CK7; while focal staining was observed in basaloid cells with CK17 and CK19 [12]. Schirren et al., [13] concluded that for the differential diagnosis of nodular BCCs and TEs, CK expression is not beneficial. In a few studies, CK19 was shown to be highly expressed in BCCs. In the study of Heyl and Mehregan [14], the staining pattern of CK19 expression for the differential diagnosis of sebaceous tumours and BCCs was employed. In the study of Ishida et al., [9], 14 (70%) of 20 BCC cases showed focal positive staining, while Ber-EP4 showed diffuse expression in all BCCs and

CD34 showed focal expression in only two (10%) of the cases. In our study, among the BCC cases, 15 (60%) showed strong positive staining and 7 (28%) had focal positive staining while 3 (12%) were negatively stained with CK19. In the study of Swanson et al., [15], Ber-EP4 and bcl-2 were determined to be beneficial for the differentiation of squamous cell carcinoma and BCC. However, they reported that although bcl-2 and CD34 expressions were reliable for the differentiation of TEs and BCCs, they concluded that a more definitive method was not present other than conventional microscopic evaluation. Sabeti et al., [4] investigated the bcl-2 and CK15 expressions for the differentiation of these two tumours. They stated that since CK15 showed higher central staining of the TE specimens, it may be helpful for the differential diagnosis of BCC and TE. They observed similar staining patterns with Bcl-2 in both tumours. In our study, only one of the TE cases showed central staining pattern. Ishida et al., [16] used CK17, CK19 and p53for the differentiation of neoplastic and non-neoplastic basaloid cells in an immunohistochemical study of one BCC case originating from seborrheic keratosis. They determined positive staining for BCC with CK17 and CK19 while there was no staining of seborrheic keratosis. An overexpression was found with P53 in BCC but not in seborrheic keratosis. In one of our cases, in a superficial type BCC originating from seborrheic keratosis, diffuse staining with CK17 and CK19 of the neoplastic basaloid cells and over-expression of P53 were determined [17]. BCCs show greater expression of p53 than TEs [18]. Ki-67 and PCNA staining is more diffuse and stronger in BCCs than that of TEs [19]. These immunohistochemical markers may be useful for the differential diagnosis of both tumours.

CONCLUSION

Differential diagnosis of TE and BCC, especially in small skin biopsies, may be difficult owing to the similarities between histopathological findings. In these cases, CK 19 staining may be helpful for the differentiation of BCC and TE. Similarly, distinction of the small superficial type of BCCs from the non-neoplastic hair buds may not be feasible. However, CK19 may also be useful for differentiating superficial type BCCs from the non-neoplastic hair buds.

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