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Evaluation of Relevant CD Markers in Basal Cell Carcinoma Patients in Diyala Province, the Republic of Iraq

Hala Yaseen Kadhim[1,3*], Ali Hafedh Abbas[2], Thekra Atta Ibrahim[3]
[1] *Department of Pathology and forensic, College of Medicine. University of Diyala*
[2] *Tropical – Biological Research Unit, College of Science, University of Baghdad*
[3] *Department of Biology, College of Education for Pure Sciences.*

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Abstract

Basal Cell Carcinoma (BCC) is the most highly prevalent type of skin cancer in the world, where BCC incidence has increased significantly in the last few years. There were many components of the immune system that were reported to play a remarkable role in BCC appearance and development. Among them, three specific clusters of differentiation (CD) biomarkers (CD25, CD68 and CD10, also termed as MME) were studied as prognostic markers with increased levels in the sera of immune-related cancers. The current investigation aimed to evaluate the levels of these biomarkers in the sera of 33 patients who have BCC compared with 55 healthy individuals (control). The results demonstrated significantly increased levels of CD25, CD68 and CD10/MME in the BCC group compared to the controls, suggesting a potential role as biomarkers, in addition to their correlation with BCC aggressiveness. The present investigation could be the first in Iraq that tried to evaluate the levels of these CDs in BCC patients. The present investigation suggests potential implications of these CDs for the development of immunotherapeutic strategies and as prognostic markers for BCC, along with their possibility of predicting the risk of new BCC tumors.

Keywords: Cluster of Differentiation biomarkers, CD25, CD 68, MME, skin cancer, basal cell carcinoma.

تقييم بعض عناقيد التمايز لدى مرضى سرطان الخلايا القاعدية في محافظة ديالى–العراق

حلا ياسين كاظم [1، 3*] ، علي حافظ عباس[2]، ذكرى عطا ابراهيم[3]
[1] فرع الامراض والطب العدلي، كلية الطب، جامعة ديالى
[2] وحدة الأبحاث البايولوجية للمناطق الحارة، كلية العلوم، جامعة بغداد
[3] قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة ديالى

الخلاصة

يعتبر سرطان الخلايا القاعدية النوع الأكثر شيوعا من سرطانات الجلد، حيث زادت معدلات الإصابة بسرطان الخلايا القاعدية بشكل ملحوظ في السنوات القليلة الماضية، هناك العديد من مكونات الجهاز المناعي التي لها دوراً ملحوظاً في ظهور وتطورأورام سرطان الخلايا القاعدية. من بينها، درست ثلاثة من عناقيد التمايز

* **Email**: hala@uodiyala.edu.iq

CD25 وCD68 وMME كمؤشرات حيوية لتوضيح علاقتها بالعديد من السرطانات وزيادة مستوياتها في المصول للسرطانات المرتبطة بالمناعة. هدفت الدراسة الحالية تقييم مستويات هذه المؤشرات الحيوية في مصل مرضى سرطان الخلايا القاعدية (شملت الدراسة 33 مريض بسرطان الخلايا القاعدية) ومقارنتها بـ 55 شخص سليم كمجموعة سيطرة صحية. أظهرت النتائج زيادة معنوية في مستويات CD25 وCD68 وMME في مصول مجموعة مرضى سرطان الخلايا القاعدية مقارنة بمجموعة السيطرة. مما يشير إلى إمكانية اعتمادها كمؤشرات حيوية، وارتباطها بعدوانية الورم. كانت هذه الدراسة هي الأولى في العراق التي تناولت تقييم مستويات هذه المؤشرات الحيوية لعناقيد التمايز لدى مرضى سرطان الخلايا القاعدية، يمكن الاستنتاج من النتائج الحالية أن عناقيد التمايز قد يكون لها آثار محتملة في تطوير استراتيجيات العلاج المناعي وكعلامات إنذار لسرطان الخلايا القاعدية، والتنبؤ بخطر ظهور أورام سرطان خلايا قاعدية جديدة.

Introduction

Basal Cell Carcinoma (BCC) is the most common type of skin cancer, accounting for approximately 80% of all diagnosed cases [1, 2, 3]. It arises from the basal cells of the epidermis, which are responsible for producing new skin cells [4]. BCC typically occurs in sunlight-exposed areas, particularly the head and neck region, because this region is more exposed to sunlight than other areas [5]. Although it rarely metastasizes or spreads to other parts of the body, BCC can cause local tissue destruction if left untreated [6]. Sunlight includes different wavelengths of radiation, some of which are useful for living organisms and some are harmful. Among those harmful solar rays are ultraviolet (UV) rays, of which UVA and UVB are the most important that lead to human skin diseases, including skin cancer. UV rays at any wavelength are considered a class I carcinogen to humans. UVB rays with the wavelength of 280 - 320 nm are harmful to humans, causing skin carcinomas, such as BCC. These radiations have mutagenic effects depending on the DNA damage resulting from the direct absorption by nuclear acids [7, 8]. In recent years, there has been increasing interest in understanding the immune response and expression of immune markers in BCC. The immune system is essential for recognizing and eliminating cancer cells, and its dysfunction can facilitate tumor growth and evasion. The immune expression has been studied, focusing on specific immune biomarkers, including particles called clusters of differentiation (CD) that have an important role in investigating the immune system in BCC [9, 10 – 12]. CD25, also known as the alpha chain of interleukin-2 receptor, is a biomarker expressed on some immune cells like the regulatory T cells (Tregs). Tregs are essential for immune regulation and they can suppress the activity of other immune cells, thereby inhibiting the receptors to the tumor [13]. In BCC, the presence of CD25+ Tregs in the tumor microenvironment has been studied to evaluate their activity on disease progression. A previous study suggested that increased infiltration of CD25+ Tregs in BCC tumors may ensure immune evasion and resistance to therapy [14]. In contrast, CD68 is a marker expressed on macrophages (the key of immune cells involved in tumor immune response). Macrophages show the pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes on their surfaces. Therefore, the presence of macrophages in the tumor microenvironment has a main role in BCC's development [15]. Previous studies examined the infiltration and polarization state of CD68+ macrophages in BCC, where M2-like CD68+ macrophages were associated with BCC aggressiveness and prognosis [16-19]. Also, the immune expression of CD25 and CD68 were investigated for its clinical significance. The high levels of CD25+ Tregs were correlated with advanced BCC stages, larger tumor size, and increased recurrence rates. The elevated M2-like CD68+ macrophages also were correlated with BCC progression, invasion, and metastasis [20]. The understanding of the immune expression of CD25 and CD68 in BCC has a significant impact on the progression of immunotherapeutic strategies. Targeting CD25+ Tregs or changing the polarization state of CD68+ macrophages may offer possibly therapeutic avenues to encourage the anti-tumor immune responses in BCC. Additionally, evaluating CD25 and

CD68 levels as prognostic markers could help predict disease outcomes and guide treatment decisions [21]. CD10, also known as neutral endopeptidase (NEP) or membrane metallo-endopeptidase (MME), is a cell surface enzyme with endopeptidase activity [22- 24]. CD10 is expressed in various cell types, including certain immune and some epithelial cells. It is involved in numerous physiological processes, including cell growth, differentiation, and signal transduction. CD10 also functions to degrade a variety of signaling peptides, which can potentially impact several pathophysiological processes [23, 24].

The relationship between CD10 protein and BCC is not yet fully established. CD10/MME/NEP is a cell surface enzyme expressed in various cell types and is involved in several biological processes, including cell growth, differentiation, and signal transduction [16]. It can degrade a variety of signaling peptides, potentially affecting various pathophysiological processes. However, its specific role in BCC is not thoroughly understood [23 – 25].

Research has suggested that CD10 is often expressed in the surrounding stroma of BCC tumors. CD10's presence in the stroma is thought to be related to the aggressiveness of BCC [25, 26, 27, 28]. However, whether MME expression contributes directly to the formation of BCC, or if it is a result of the tumor environment, is not clearly determined. From the aforementioned information, the present study aimed to evaluate the serum level of CD25, CD68 and MME in the sera of the BCC patients as compared to healthy individuals.

Materials and Methods

The present study included 33 patients (23 males and 10 females) with BCC diagnosed by dermatologists, compared to 55 healthy individuals (30 males and 25 females). The included samples were obtianed from the primary care hospital at Baquba Teaching Hospital, Diyala province, Iraq, from March 2022 to December 2022. Three milliliters of venous blood were withdrawn from each participant, placed in a gel tube, and allowed to clot at room temperature. Subsequently, the tubes were placed in a centrifuge and spun at a speed of 6000 rpm for 10 minutes to separate the serum. The serum was divided into two Eppendorf tubes, then frozen at -20 °C until further immunological tests could be conducted. The levels of three CD biomarkers (CD25, CD68, and MME) were measured using an ELISA technique. The experimental procedure followed the instructions provided by the manufacturer (Al-Shukairate Company, Jordan).

The study was approved by the Scientific Research Ethics Committee, College of Science, University of Baghdad (CSEC/0322/0153).

In the current study, the IBM SPSS version 27.0 was used to analyze the data statistically. The homogeneity, linearity, and normality were calculated. The median and percentiles were adopted to test significant differences when comparing the infected group with the control, in the cases when normal distribution was absent. The differences were considered significant when the p-value was < 0.05.

Results

The homogeneity, linearity, and randomization were tested in the studied groups using the Kolmogorov-Smirnov and the Shapiro-Wilk tests, to determine the type of the present results. Based on the results presented in Table 1, the current data were observed to be nonparametric. Therefore, the median and 25% and 75% percentiles were calculated. The results showed that 69.70% of BCC patients were males and 30.30% were females, with no significant sex differences, as shown in Table 2. The age median (25% - 75% percentile) was 66.0 years

(60.0 - 71.0 years), and no significant differences appeared when compared to the control groups' age (Table 3).

Table 1: Normality test for the cluster of differentiation in the studied groups.

CD markers	Groups	Kolmogorov-Smirnov test			Shapiro-Wilk test		
		Statistic	DF	Probability	Statistic	DF	Probability
CD25	Patients	0.200	33	P < 0.05	0.869	33	P < 0.001
	Control	0.191	55	P < 0.001	0.775	55	P < 0.001
CD68	Patients	0.230	33	P < 0.001	0.789	33	P < 0.001
	Control	0.268	55	P < 0.001	0.691	55	P < 0.001
MME	Patients	0.224	33	P < 0.001	0.881	33	P < 0.001
	Control	0.358	55	P < 0.001	0.378	55	P < 0.001

Table 2: The median age frequency values of the studied groups.

	Patients group	Control group	Probability
Age median (Years)	66.0	59	0.096
Percentile 25%	60	35	
Percentile 75%	71	75	

Table 3: The sex frequency values in the studied groups.

Sexes	Frequency (%)		Probability
Males	23 (69.7)	30 (54.55)	0.160
Females	10 (30.3)	25 (45.45)	
Total	33 (100.0)	55 (100.0)	

The results of CD levels in the BCC and control groups indicated significant differences. In the BCC group, the median level of CD25 was significantly increased compared to the controls (1826.67 *vs.* 682.22 pg/ml), as presented in Table 4.
Also, the findings revealed that the median level of CD68 in the BCC group was significantly increased compared to the controls (26.89 *vs.* 3.42 pg/ml). Additionally, the median level of MME was significantly increased in the BCC group compared to the control group (17.23 *vs.* 1.61 ng/ml), as shown in Table 4.

Table 4: Cluster of differentiation levels in the studied groups.

Mean ± SE or median		Patients group	Control group	Probability
CD25 (pg/ml)	Median	1826.67	682.22	0.002
	Percentile 25%	493.33	148.89	
	Percentile 75%	3648.89	1282.22	
CD68 (pg/ml)	Median	26.89	3.42	9.27×10^{-13}
	Percentile 25%	25.29	1.31	
	Percentile 75%	27.85	7.17	
MEM (ng/ml)	Median	17.23	1.61	7.0×10^{-6}
	Percentile 25%	13.44	0.22	
	Percentile 75%	18.51	6.83	

Discussion

Basal Cell Carcinoma (BCC) is the most common type of skin cancer, and its incidence has been steadily increasing, doubling every 14 years [20]. Following the initial tumor, there is a significant risk of developing additional BCCs, with 44% of individuals experiencing further lesions within three years [25]. Risk factors for the first occurrence of BCC include age, sunlight exposure, fair skin, and male gender. However, these variables do not consistently predict subsequent tumor development [25,29]. The immune system plays a crucial role in both the appearance and progression of BCC tumors [30]. In the context of BCC, tumors are characterized by disrupted borders, lymphocytic infiltration surrounding and penetrating the tumor, and histologic evidence of regression [15, 31]. Studies have suggested that immune responses induced by BCC tumors may contribute to tumor disruption [15, 32]. In this study, the levels of these three CD biomarkers, namely CD25, CD68, and MME, were investigated. To improve the understanding of BCC and its relationship with the immune system, several studies have been conducted to examine the incidence and trends of BCC over a longer period of time, aiming to reveal any changes or patterns. Further investigations have attempted to identify additional risk factors for BCC developing beyond age, sun exposure, fair skin, and male gender [33, 34, 35].

Similarly, researchers have investigated the immune system and its role in BCC tumor onset based on the type of immune marker examined and the impact that it has on host tumor features [36, 37]. In addition, other investigations also examined possible therapeutic options for influencing the immune response in BCC. These investigations collectively contribute to a holistic view of BCC and pave the way for its diagnosis and treatment [30, 38]. Several studies on BCC were carried out on biomarkers that are related to CD25. All these studies show that the expression level of CD25 on B and T cells in BCC and other related tumors significantly differs from that in normal B and T cells, suggesting it as an exceptional marker for BCC patients [32 – 40]. For instance, Kaporis and colleagues examined CD25 + FoxP3 + cells, dominant phenotypic T-regs, and found their substantial presence in the vicinity of BCC sites [33]. Understanding the immune microenvironment, as shown by these investigations, would develop more effective immune-based treatments, not only for BCC but also for other human carcinomas, through understanding the mechanisms of these immune microenvironments [39]. Kleina and her team examined CD68 levels before and after cryotherapy and imiquimod cream treatment [40]. Their findings confirmed the presence of CD68-positive cells primarily in the direct vicinity of BCC tumor as well as the surrounding area, corroborating previous research. Furthermore, Zhang and colleagues investigated the correlation between CD68 expression and immune infiltrates in the tumor microenvironment among 33 different tumor types [41]. Additionally, another study revealed that CD25 and CD68 levels in BCC biopsies could serve as predictive indicators for the risk of new BCC tumors [42].

To expand our knowledge in this field, it is crucial to conduct further studies that build upon the existing research and explore additional biomarkers and their implications in various aspects of BCC and other carcinomas. These endeavors will undoubtedly contribute to advancements in immune-based therapies and enhance our understanding of the complex immune microenvironment in cancer.

A multitude of studies have investigated BCC tumors, revealing significant findings regarding the level of MME. IN our study, in the BCC group, the median level of MME was 17.23 ng/ml, whereas the control group exhibited a level of 1.61. Interestingly, the majority (75%) of BCC patients demonstrated an elevated level of MME, reaching 18.5 compared to 6.83 in the control group. Conversely, the remaining 25% of BCC group had a level of 13.44,

while the control group showed a level of 0.22. Importantly, statistical analysis indicated highly significant differences between the studied groups at a probability level of 7×10^{-6}, as presented in Table 4. MME (CD 10) is a metallopeptidase; forty-seven distinct evolutionary families of metallopeptidases were identified in the last years [21]. In the context of carcinogenesis, CD10 plays a dual role, acting as a double-edged sword in cancer progression. Its behavior depends on the peptides present in the tumor microenvironment, which can either promote or inhibit cancer progression. High expression of MME has been associated with cancer progression and migration in esophageal squamous carcinoma. Conversely, MME can also exhibit tumor-suppressive properties in certain tumors by inhibiting various events involved in tumor progression. Remarkably, the weak expression of MME in the deeper regions of BCC is correlated with increased tumor aggressiveness, further highlighting its involvement in BCC pathogenesis [21, 43].

To gain a comprehensive understanding of these phenomena, it is crucial to conduct additional studies exploring the level of MME and the intricate role of MME in various types of tumors, compared to BCC. In addition, other factors that put one at risk of developing BCC beyond age, sun exposure, fair skin and male gender have been investigated. Research has extensively looked into the immune system's involvement in BCC tumors' appearance and progression by investigating various immune markers and their influence on tumor characteristics. Studies are exploring potential therapies targeting the immune response in BCC tumors. Taken together, these investigations enable a holistic comprehension of BCC and pave the way for newer diagnostic and treatment modalities. Many studies have focused on biomarkers, especially CD25 with BCC [1]. These studies found high levels of CD25 in surrounding areas of BCC tissues, among others, indicating that it could be used as a helpful biomarker for patients with this condition [2-3].

Knowledge expansion in this area will help in developing effective therapeutic strategies and provide greater insights into the mechanisms underlying tumor progression. The present study was limited by small number of BCC's patients.

It can be concluded that the levels of CD25, CD68, and MME biomarkers were significantly increased in BCC patients compared to control individuals. These findings suggest that these markers have potentials to be used as immunotherapeutic targets for BCC and predictors of new BCC cases' development. Furthermore, they may provide clues about treatment approaches as well as tumor progression mechanisms in basal cell carcinoma, along with their possible use as diagnostic and prognostic factors for this disease. However, more research should endeavor to identify additional biomarkers and their roles in BCC's as well other types of cancers' development.

Conflict of interest

The authors declare that they have no conflicts of interest.

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