THE UTILITY OF BETA 2 MICROGLOBULIN (B2M) AS AN INITIAL DIAGNOSTIC TOOL FOR ORAL SQUAMOUS CELL CARCINOMA (OSCC): EVIDENCE FROM A MALAYSIAN SAMPLE

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ABSTRAK

Latar Belakang Kajian

Barah mulut / Oral squamous cell carcinoma (OSCC) mempunya prognosa yang agak buruk kerana pesakit sering diberi diagnosis pada peringkat barah yang lanjut. Kajian terbaru telah menerokai penggunaan serum petanda tumor seperti Beta 2 Microglobulin (B2M) untuk membantu di dalam membuat diagnosis awal. Walaupun terdapat banyak kes OSCC di Asia Tenggara, namun tiada kajian yang dilaksanakan mengenai penggunaan klinikal B2M di rantau ini.

Objektif Kajian:

Mengenal pasti jikalau serum B2M dapat digunakan sebagai alat diagnostik awal, dan memberi indikasi jikalau biopsi perlu dilaksanakan. Seterusnya, kajian ini ingin mencadangkan nilai rujukan serum B2M setempat untuk mengenalpasti pesakit-pesakit OSCC.

Metodologi

Seramai dua puluh satu orang pesakit dan dua puluh satu orang sampel kawalan yang sihat telah mengambil bahagian di dalam kajian ini di Hospital Universiti Sains Malaysia (HUSM) dalam tempoh satu tahun iaitu di antara Jun 2016 sehingga Jun 2017. Selain daripada latar belakang dan sejarah pesakit, sebanyak 5ml darah telah diambil daripada saluran darah setiap pesakit pada pra-rawatan dan telah dianalisis melalui kaedah immunoturbidometry menggunakan ARCHITECT c800 Analyser. Keputusan kajian telah dianalisa menggunakan analisis ROC dan ujian Mann Whitney.

Keputusan:

Tahap serum B2M adalah signifikan ($p<0.001$) di kalangan pesakit-pesakit berbanding sampel kawalan. Ujian tersebut mempunyai peratus sensitiviti dan spesifikasi yang sama iaitu sebanyak 90.5%. Untuk membezakan kes-kes daripada sampel kawalan, tahap B2M yang telah dikenalpasti sebagai serum penanda tumor yang sensitif dan spesifik adalah pada nilai 1.57mg/l.

Kesimpulan:

B2M ialah petanda serum tumor yang sensitif dan khusus untuk membezakan kes-kes daripada sampel kawalan. Memandangkan petanda tumor ini kos efektik dan kurang invasive, maka ia berpotensi sebagai alat diagnostik tambahan yang berguna bagi kumpulan pesakit berisiko tinggi.

ABSTRACT

Background

The delay in diagnosis of oral squamous cell carcinoma (OSCC) is a factor in rendering the poor prognosis, and recent research has explored the use of serum tumour markers such Beta 2 Microglobulin (B2M), to aid early diagnosis. However, despite a high incidence of OSCC in Southeast Asia, no studies on the clinical use of B2M in the region were found.

Objectives:

To determine if serum B2M level can serve as an initial diagnostic tool to indicate if a biopsy is warranted, and if so, to propose a local B2M serum reference value to identify OSSC patients

Methodology:

Twenty-one patients diagnosed with OSCC were seen at Hospital Universiti Sains Malaysia (HUSM) over a one-year period, between June 2016 and June 2017, and an equal number of healthy controls participated in the study. Apart from patient history, venous blood of approximately 5ml volume was collected from each subject at the pre-treatment stage and analysed by an Abbot ARCHITECT c8000 analyser using the immunoturbidometry method.The results were analysed using ROC analysis and the Mann Whitney test.

Results:

Serum B2M levels showed a statistically significant increase ($p<0.001$) in patients compared to controls. The test was shown to have 90.5% sensitivity and 90.5% specificity. It was found to be a sensitive and specific serum tumour marker at a cut off value of 1.57 mg/l to differentiate cases from controls.

Conclusion:

B2M is a sensitive and specific tumour marker to differentiate OSCC cases from controls. It is cost effective and minimally invasive, making it a potentially useful adjunct diagnostic tool in a high-risk patient pool.

Chapter 1

INTRODUCTION

1.1 INTRODUCTON

Oral cancer is defined as a malignant neoplasm arising from oral cavity or oropharynx; and commonly involves sub-sites such as the anterior two thirds of the tongue, tonsils, upper and lower alveolar ridge, buccal mucosa, and the hard palate [1]. Oral cancer is the 6th most common cancer in the world but shows wide geographical variation, but a higher incidence has been noted in South and Southeast Asian countries such as India, Bangladesh, Taiwan, and Sri Lanka [2]. Squamous cell carcinoma constitutes about 90% of oral malignancies with tumours arising from minor salivary glands accounting for a further 5% [1].

Local epidemiology data from the 2007 National Cancer Registry in Malaysia reported a total of 353 cases of oral cancer; it was ranked as the as the 21st most common cancer in the general population; the 17th most common among males and the 16th most common among females. Furthermore, only 35.4% of the 205 cases reported with staging were of stages I and II [3]. Oral squamous cell carcinoma (OSCC) is characterised by a high rate of metastasis, recurrence, and second malignancy; with a poor prognosis [4, 5]. The 5-year survival rate for OSCC is approximately 50% and has remained so for the past several decades [6]. This lack of improvement is attributed to the fact that a large number of cases are diagnosed at an advanced stage. The prognosis of patients with early treatment is much better, with 5-year survival rates as high as 80% [7]. Thus, early detection becomes vital.

OSCC is a multifactorial disease, and the risk factors include smoking, alcohol consumption, betel quid chewing, poor oral hygiene and diet and nutritional deficiencies, especially in vitamin A, C, E, iron, selenium, folate and phosphate. Other factors such as human papilloma virus (HPV) infection and premalignant conditions such as leukoplakia and erythroplakia have also been shown to influence the pathogenesis of OSCC [1].

Clinical oral examination (COE) is the gold standard for the initial detection of dysplastic or malignant oral lesions at an early stage, with confirmation by biopsy and histopathological examination (HPE). However, a recent systematic review concluded that the overall performance of COE as a diagnostic method for predicting dysplasia and OSCC has been poor [8]. HPE is a time-consuming process, requiring several days to fix, embed and stain the biopsy specimen [9]. The specimen can also be affected by artefacts resulting from crushing, incorrect fixation and freezing [10]. Furthermore, the biopsy process is invasive in nature and can pose technical difficulties to the clinician when lesions are extensive, as the most representative areas must be selected to avoid diagnostic errors [11]. As such, the proportion of OSCC cases diagnosed at an early and localised stage is still <50% within a 5-year period [12, 13]. It has also been observed that the majority of patients failed to recognise the early signs and symptoms of OSCC [14]. Furthermore, 30-40% of patients with a negative nodal status at resection eventually die from metastatic disease [15]. Thus, it is clear that the current diagnostic methods on which treatment modality is based on are of insufficient sensitivity. A non-invasive, cost effective and rapid method for early diagnosis of OSCC is, therefore, a priority.

Tumour markers are substances found in blood, urine, or body tissues that can be elevated by the presence of one or more types of cancer. Recent research has explored the use of these markers to aid early diagnosis of carcinoma and they have been shown to have a wide range of potential applications, including screening, prognosis, and monitoring for recurrence or metastasis [9]. One such tumour marker is the Beta 2 Microglobulin (B2M) which was first described in 1968 [16]. B2M is a low molecular weight protein on the beta-chain of the human leukocyte antigen (HLA). It occurs physiologically in small quantities in human urine, plasma, and cerebrospinal fluid. It exists in two main forms, free or non-covalently linked to HLA antigens. The free form, which is relevant as a biomarker, is found in serum, and consists of a

single polypeptide chain with a single intrachain disulfide bridge with no carbohydrate content [16, 17].

According to a recent meta-analysis on the diagnostic accuracy of serum biomarkers for head and neck cancer, 15 biomarkers were reported as having excellent sensitivity and specificity but only B2M was reported as fulfilling the accuracy criteria twice [18]. The increased production of B2M by carcinoma cells compared to non-neoplastic cells may be due to increased cell synthesis, breakdown, or both [19]. B2M levels have been reported to be significantly increased in individuals exposed to carcinogens, having premalignant conditions, at different stages of oral cancer and histological differentiation, when compared with healthy controls [20-22]. However, it has been shown that serum B2M is a superior diagnostic tool when compared to B2M level in saliva for OSCC [23]. Upregulation of B2M expression in tumour tissue has been associated with OSCC progression, invasion and metastasis, while the suppression of B2M expression in in-vitro studies using small interfering RNA (siRNA) has been shown to decrease cell migration and invasion [24, 25].

This study had two inter-related objectives: to establish if serum B2M level can serve as an initial diagnostic tool to quickly determine if further investigation through biopsy is warranted for patients suspected of having OSCC. And if so, to propose a local reference serum B2M value that can be used to identify OSSC patients. A review of literature showed no studies on the clinical use of a B2M cut off level specific to the local Southeast Asian population, despite a high incidence of the disease in the region.

The use of serum B2M level has several strengths. First, it is an inexpensive, less invasive technique that provides rapid results. Second, it avoids inter-observer variations associated with other simpler alternatives. Finally, raised B2M levels serve to increase the index of suspicion regarding the presence of OSCC, in the case of a negative biopsy.

Chapter 2

OBJECTIVES OF THE STUDY

2.0 Objectives

2.1 General objective

To study the levels of Beta 2 Microglobulin (B2M) in OSCC patients

2.2 Specific objectives

1. To determine the diagnostic utility of the B2M test in identifying OSCC patients.

2. To determine the association of the Beta 2 Microglobulin level with the histological differentiation of OSCC.

3. To determine the association of the Beta 2 Microglobulin level with the nodal status of OSCC patients.

4. To determine the association of the Beta 2 Microglobulin level with the presence of distant metastasis.

Chapter 3

MANUSCRIPT

The utility of Beta 2 Microglobulin (B2M) as an initial diagnostic tool for oral squamous cell carcinoma (OSCC): evidence from a Malaysian sample

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Abstract:

Background

The delay in diagnosis of oral squamous cell carcinoma (OSCC) is a factor in rendering the poor prognosis, and recent research has explored the use of serum tumour markers such Beta 2 Microglobulin (B2M), to aid early diagnosis. However, despite a high incidence of OSCC in Southeast Asia, no studies on the clinical use of B2M in the region were found.

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Serum B2M levels showed a statistically significant increase ($p<0.001$) in patients compared to controls. The test was shown to have 90.5% sensitivity and 90.5% specificity. It was found to be a sensitive and specific serum tumour marker at a cut off value of 1.57 mg/l to differentiate cases from controls.

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B2M is a sensitive and specific tumour marker to differentiate OSCC cases from controls. It is cost effective and minimally invasive, making it a potentially useful adjunct diagnostic tool in a high-risk patient pool.

Introduction

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This study had two inter-related objectives: to establish if serum B2M level can serve as an initial diagnostic tool to quickly determine if further investigation through biopsy is warranted for patients suspected of having OSCC. And if so, to propose a local reference serum B2M value that can be used to identify OSSC patients. A review of literature showed no studies on the clinical use of a B2M cut off level specific to the local Southeast Asian population, despite a high incidence of the disease in the region.

The use of serum B2M level has several strengths. First, it is an inexpensive, less invasive technique that provides rapid results. Second, it avoids inter-observer variations associated with other simpler alternatives. Finally, raised B2M levels serve to increase the index of suspicion regarding the presence of OSCC, in the case of a negative biopsy.

Materials and methods

Case and control group selection

This study involved a total of 42 patients from a single centre, Hospital Universiti Sains Malaysia (HUSM), in Kelantan, Malaysia. Subjects were made up of two groups. The 'case' group was made up of OSCC patients seen at the Otorhinolaryngology-Head and Neck Surgery (ORL-HNS) and Oral and Maxillofacial (OMF) Surgery clinics of HUSM, over a period of one year, between June 2016 and June 2017. A matching number of healthy individuals were also recruited to serve as the 'control' group.

Eligible subjects for the case group were those with a clinically evident oral lesion, with HPE confirmation, and had not undergone any previous therapy/treatment for oral cancer; and were willing to provide written consent. Non-eligible subjects were those who had an autoimmune disease, who were pregnant, or were being treated for an active infection, or had an underlying kidney/liver disease, or previous diagnosis of another malignancy, or were unwilling or unable to give written consent. A total of 21 out of 30 patients satisfied the eligibility criteria to form the case group. The control group was made up of 21 healthy subjects recruited from those accompanying patients to the ORL-HNS clinic, and through advertisements placed in the ORL-HNS clinic inviting voluntary participation as control subjects. Their health status was established after a physical examination and blood screening to rule out pre-existing medical conditions or pregnancy. Written informed consent was obtained from all subjects.

The study protocol was reviewed and approved by the Human Research Ethics Committee (HREC) of USM (Study protocol code USM/JEPeM 16030139.)

Sample characteristics

The ethnic composition of the case group correctly reflects the composition of the population of the study area with 95% being Malay, and about 5% being Chinese [26]. The control group had a larger representation of Chinese. There were no Indian subjects in our sample **(Table 1)**

Both case and control groups were drawn from subjects aged between 18 to 80 years old, with the mean age among the case group being 60.6 years, with most of them within the 60-69 age range. The mean age among controls was lower (43.7 years). Males predominated in both case and control groups.

OSCC Assessment

Data regarding patient demographics, risk factors, histopathology and stage of disease were collected and recorded on a data collection sheet. All blood samples were collected according to a standard collection protocol. About 5mls of blood were collected in a test tube. The samples were centrifuged at 3000 rpm for 5 minutes after being clotted for 30 -60 minutes at room temperature. Aliquots of serum samples were stored at -80ºC until analysis was performed. Samples were processed by an Abbot Architect c8000 analyser using the immunoturbidometry method for the quantitative determination of B2M. The detection limit was 0.046 mg/l.

Statistical analyses

Statistical associations were analysed using SPSS software, version 23.0. A *p*-value of< 0.05 was taken to be statistically significant at a confidence level of 95%. All the numerical data were presented as medians (IQR) while categorical data were expressed as percentages

(%). Analytical statistics were done using the Mann-Whitney test and Receiving Characteristic Operating Curve (ROC) analysis.

Results

The 21 subjects in the case group had HPE confirmed OSCC, with a staging CT done but no prior treatment. The most common site of presentation of primary tumour was the tongue (47.6%), followed by buccal mucosa (33.3%). Smoking was the highly prevalent risk factor for OSCC (71.4%) among the case group, as compared to betel quid chewing (23.8%). All the patients were in Stage IV of the disease, with moderately differentiated squamous cell carcinoma being the most common (52.4%) histopathological presentation **(Table 2).**

The median serum B2M level in the control group was 1.37 (0.16) mg/l and 2.56 (1.04) mg/l in the case group**).** This higher serum B2M level in the case group, as compared to the control group, was statistically significant ($p<0.001$) **(Table 3).** Among the 10 cases with well differentiated OSCC, the median serum B2M level was 2.44 (1.51) mg/l, while in 11 cases of moderately differentiated OSCC, the level was 2.67 (0.78) mg/l. Increased serum B2M level was thus seen to be correlated with the degree of histological differentiation, although the difference was not statistically significant (p =0.833) (**Table 4).**

In ROC analysis, a B2M value of 1.586 mg/l was taken as a cut-off value for differentiating cases from controls. The recorded area under the ROC curve was 0.97. Thus, our study shows a 90.5% sensitivity and 90.5% specificity **(Table 5).**

Median serum B2M level was 1.85 (1.67) mg/l for patients with a nodal status of N0 (4 cases), and 2.67 (0.79) mg/l in patients with positive nodes (17 cases). However, this was not statistically significant (p = 0.244) (**Table 6**).

Median serum B2M level was 2.50 (1.19) mg/l for patients with no distant metastasis (17 cases), and 2.67 (3.80) mg/l in patients positive for distant metastasis (4 cases); this, too, was not statistically significant (p=0.370) (**Table 7**)

Discussion

The age distribution among the 21 case group subjects showed most of them to be within the 60 to 69 age range, which is consistent with Malaysian oral cancer statistics. Malaysian statistics also show an almost equal gender incidence; however, gender distribution in the study sample revealed more male subjects within the case group (61.9%). This could be a reflection of the localized distribution seen in Kelantan.

The racial distribution of case subjects was mostly Malay, reflecting the racial composition of Kelantan state where Malays account for about 95% of the state population. Other minority races include those of immigrant Chinese and Indian descent [26].

Of the three most important risk factors associated with OSCC (smoking, betel quid chewing and alcohol consumption), smoking was found to be more prevalent in the case group (71.4%) as compared to betel quid chewing (23.8%) and alcohol consumption (0%). This is probably due to Malay Muslim religious beliefs that prohibit alcohol consumption among our predominantly Malay case subjects. Consistent with global data on common OSCC subsites, we found that the most common site of presentation of primary tumour was the tongue (47.6%) followed by buccal mucosa (33.3%) [27].

All the OSCC patients presenting to us were in stage IV of the disease, and moderately differentiated squamous cell carcinoma (52.4%) was the most common histopathological presentation, followed by well-differentiated histology (47.6%), with no cases of poorly differentiated disease. While it is well established that patients with OSCC tend to present late, the overwhelming presence of stage IV cases in our study has not been previously reported. We posit that the status of HUSM as a tertiary referral centre, and a centre for oncological management in the East Coast of Peninsular Malaysia, to be the main reason for this unusual finding. Patients amenable to surgery would have been treated at their respective state hospitals.

The local cultural preference for traditional and alternative therapy during the early stage of disease could be a further contributory factor.

Serum B2M level in the OSCC group was shown to have a statistically significant ($p<0.001$) increase, and is in line with findings of previous studies, although the mechanism of altered B2M level is not yet clearly understood [21, 22, 28, 29]. Various postulates have been put forth, which include increased cellular activity, cell membrane turnover and cell division in malignancy [30]. Our study showed 90.5% sensitivity and 90.5% specificity by ROC analysis, consistent with other studies [23, 31]. B2M was also found to have good diagnostic test accuracy measurements, with a Positive Likelihood Ratio of 9.53, Negative Likelihood Ratio of 0.10, Diagnostic Odds Ratio of 95.3 and a Youden Index value of 0.81. This is in agreement with a recent meta-analysis which concluded that B2M was one of four (prolactin, glutathione, and catalase) biomarkers with the highest diagnostic test accuracy [18].

Serum B2M levels were seen to correlate with a worsening degree of histological differentiation, although this was not statistically significant. This finding is incongruent with other studies; the possible reasons include a small sample size and the absence of poorly differentiated histology among our patient pool [32]. Median serum B2M levels were also not significantly increased for variables of nodal status and distant metastasis, unlike in tissue studies, where the association of B2M expression with progression and metastasis of OSCC lesions was statistically significant [24, 25]. Other serum based studies, however, did not analyse those variables [21-23, 28, 29, 31], thus ruling out comparisons.

It is therefore apparent that B2M is a sensitive and specific serum tumour marker at a cut off value of 1.57 mg/l to differentiate cases from controls, for a case group consisting of primarily Malay subjects. This can serve as an initial reference value for countries in the region with large Malay populations such as Malaysia and neighbouring Indonesia, since currently none exists.

The utility of B2M serum tumour marker as a singular screening tool is diminished because an elevated level has also been reported in cases of haematological malignancies, tumours of the breast, lung, and gastrointestinal and genitourinary tracts [29]. In addition, its role as a serum prognostic marker has yet to be verified conclusively in literature.

However, it can serve well an adjunct diagnostic tool for the early diagnosis of OSCC, and to determine if a biopsy is warranted. Its utility lies in the fact that it is cost effective, minimally invasive, and yields results within a minimal timespan, as compared to more expensive alternatives such as an oral brush biopsy. Furthermore, it avoids inter-observer variation associated with other protocols such as fluorescence spectroscopy and toluidine blue staining [33]. Most importantly, raised B2M levels serve to increase the index of suspicion regarding the presence of OSCC, in the case of a negative biopsy.

Conclusion

The early diagnosis of oral squamous cell carcinoma (OSCC) is of paramount importance and the first step to achieving this is the further development of rapid, less invasive and accurate methods of screening and diagnosis. B2M holds great promise, when used as an adjunct with other recently developed techniques because of its low cost, minimally-invasive nature and quick availability of results.

Limitations

Our study has a few limitations: first, the sample size was small; second, our findings may have limited external validity due to it being localised to a single centre and was confined to a largely Malay sample. Finally, all of our patients presented an advanced stage of disease.

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