Research Article

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Archives in Cancer Research
ISSN 2254-6081

2016
Vol.4 No.4:120

DOI: 10.21767/2254-6081.1000120

Evaluation of Serum Beta-2 Microglobulin as a Diagnostic and Prognostic Marker in Oral Squamous Cell Carcinoma and Leukoplakia

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Received: 12 November 2016; **Accepted:** 19 Dec 2016; **Published:** 23 December 2016

Citation: Diwan NN, Chavan MS, Motgi AA, et al. Evaluation of Serum Beta-2 Microglobulin as a Diagnostic and Prognostic Marker in Oral Squamous Cell Carcinoma and Leukoplakia. Arch Can Res. 2016, 4: 4.

Abstract

Background: The disfiguring defects, functional loss and thus the mortality rate in oral squamous cell carcinoma (OSCC) can be significantly reduced by its early diagnosis. Tumour markers are being studied which could aid in screening, diagnosis, study prognosis and monitor the response to treatment. This research was conducted to study the role of Beta - 2 microglobulin (B2M), a tumour marker in OSCC and leukoplakia.

Method: Serum from 30 cases of OSCC and leukoplakia each and 20 controls was studied Materials and methods: IMMULITE/ IMMULITE 1000 kit was used to assess the B2M levels

Findings: B2M levels in various stages and were higher degree of differentiation of OSCC ($p<0.001$) and as the stages ($p<0.05$) and grades of dysplasia ($p<0.001$) increased in leukoplakia

Conclusion: Serum B2M levels were higher in OSCC and leukoplakia than controls. So B2M can be considered as a diagnostic and prognostic marker.

Keywords: Beta - 2 microglobulin; Tumour marker

Abbreviations: B2M: Beta 2 Microglobulin; OSCC: Oral Squamous Cell Carcinoma; MHC 1: Class I Major Histocompatibility Complex

Introduction

Oral squamous cell carcinoma (OSCC) is an important health problem worldwide not only because of the significant mortality rate associated with the disease, but also because of the disfiguring defects and functional loss of the tissues associated with the treatment. The most important factor that will improve the mortality rate of OSCC is its early detection [1].

Innumerable scientific studies are conducted to find reliable indicators of the biological potential of premalignant diseases and cancerous lesions [2]. These markers have a wide range of potential applications and they have been used as aids to screening, diagnosis, prognosis and monitoring the response to treatment. Estimation of such markers might permit selection of the most appropriate treatment for the individual cancers.

Serum Beta–2 microglobulin (B2M), a tumor marker showed significantly increased concentrations in patients with OSCC and those with keratosis and epithelial dysplasia but with no evidence of invasion [3]. The present study has been carried out to evaluate, compare and establish the role of serum levels of B2M as biological parameter in leukoplakia and OSCC and its different stages.

We considered leukoplakia only because several other premalignant diseases like oral sub-mucous fibrosis which are responsible for OSCC and will require individual consideration.

Materials and Methods

The work had been approved by the appropriate ethical committees related to the institution in which it was performed and the subjects gave informed consent. The study comprised of collection of serum samples from 30 cases of histologically diagnosed OSCC, 30 cases of histologically diagnosed leukoplakia and 20 subjects as control which were grouped into three categories, viz. Group- I, Group- II and Group- III respectively.

Criteria for selection of samples

The patients with a positive history of any renal and liver disorders, allergic conditions, autoimmune diseases, other systemic diseases, and any previous history of major illness were excluded from the study sample [4]. The histopathologically diagnosed patients of OSCC and leukoplakia were identified and selected randomly. The control group patients did not have any history of adverse habits like tobacco use and any obvious oral lesions responsible for the etiology of leukoplakia or OSCC. 4 ml of intravenous blood was collected under aseptic conditions and was immediately transferred into autoclaved test tube and allowed to clot. The serum was separated out by the configuration using an 'Ultracentifuge'. Centrifuged serum samples were immediately processed using IMMULITE/IMMULITE 1000 Beta 2 microglobulin kit in the IMMULITE processor for the estimation of B2M levels.

Comparison of the mean values of B2M between OSCC, leukoplakia and control groups was done using ANOVA and unpaired test. 'Logistic Regression Analysis' was carried out to adjust for age and gender which will nullify the irregularities in age and gender selection.

Results and Observations

The serum B2M values obtained were subjected to statistical analysis.

The TNM stagewise [5] mean B2M levels of group I were noted. Stage I B2M levels were 1840 ± 43.13 ng/ml, stage II were 2870, stage III were 2262 ± 250.50 ng/ml and stage IV were 3062 ± 117.93 ng/ml. Stage II showed an increased level of B2M than stage III because only two patients fell into stage II, so a mean of more cases could not be achieved. It was also noted that the mean levels of B2M in poorly differentiated OSCC were 1840 ± 43.13 ng/ml, moderately differentiated were 2077 ± 97.61 and in well differentiated were 2857 ±285.93 ng/ml.

The mean levels of B2M in group II were found to be increasing as the levels of dysplasia increased. B2M levels with mild dysplasia were 1753 ± 38.08 ng/ml, moderate dysplasia were 1889.6 ± 44.92 and with severe dysplasia were 2052.16 ± 75.91. No cases were graded as *in situ* carcinoma. LSCP [6] classification and their respective B2M levels were – Stage I (1808 ± 65.17), stage II (1995 ± 104.24), stage III (2050 ± 14.14) and none in stage IV. The average B2M levels in group III was 1420 ± 45.08 ng/ml.

It was observed that the B2M levels were increased as the TNM staging of OSCC increased ($p<0.001$) and as the histopathological grading worsened ($p<0.001$), which showed a highly significant co-relation in group I. B2M levels were also raised as the LSCP staging of leukoplakia increased ($p<0.05$) and as the level of dysplasia increased ($p<0.001$). The serum levels of B2M increased in group I as compared to group III ($p<0.05$), in group II as compared to group III ($p<0.05$) and in group I as compared to group II ($p<0.05$). All the above findings were found to be statistically significant.

Discussion

The proverb 'Prevention is better than Cure' and thus the timely diagnosis are a part of successful steps in the prevention of premalignant diseases like leukoplakia and thus OSCC. Early diagnosis will lead to overall control and management of potentially malignant oral mucosal lesions [7]. Several diagnostic modalities, both invasive and non-invasive, have been practiced worldwide for the diagnosis of OSCC and PMD with certain limitations, newer researches have studying tumour markers like B2M for the early detection of such lesions.

A tumor marker can be defined as a molecule that indicates the likely presence of cancer or can also be defined as one that provides information about the likely future behavior of an existing cancer (e.g. ability to metastasize or to respond to therapy) [8]. Crispian Scully was the first to evaluate the role of B2M in premalignancy and malignancy in 1981 [3]. B2M is a low molecular weight protein (11,800 dalton), mainly present on the surface of nucleated cells, abundantly on lymphocytes and tumour cells [9]

Recently, it has been shown that it is equivalent to the light chain of HLA type- I antigen as a subcomponent of HLA antigen [10].

Biological function of Beta- 2 microglobulin

The class I major histocompatibility (MHC 1) antigens are built of two chains, one light (B2M) and one heavy (alpha) which are connected with each other with a non-covalent bond. The light chain presents endogeneous antigens to cytotoxic lymphocytes (CD8+) and thus plays a significant role. It plays a role in the transport of heavy chains through the cytoplasm to the cell surface. Leaving the surface of the cell, the particle of MHC 1 is proteolysed with the use of metabolic energy, and connected with it B2M directly dissociates from the heavy chain. This phenomenon is described as 'shedding'. Then the situation heavy chain of MHC 1 combine with the cellular membrane and stays still with the connection in the cell. The light chain of B2M becomes an independent particle which occurs in the extracellular space as a free monomer. Due to its small size, it can freely transfer from intra to extra cellular space. Appearing on the surface of cells, this B2M can exchange with free B2M from the blood serum which has a high significant role with the heavy chain attachment on the surface of the cells. It is proposed that the high concentration of free B2M can influence the expression of MHC 1 particles on the surface of cells by decreasing the synthesis of cellular B2M. The growth of neoplastic tissues was influenced by the higher production of cytokines: Interleukin- 6, Interleukin- 10 which was influenced by a higher level of B2M [11].

The high levels of B2M in neoplastic diseases may not be fully explained. B2M is known to interact and stabilize the major histocompatibility molecules and also functionally regulate the survival, proliferation, apoptosis, positive and negative growth potentials and even metastasis in cancer cells [12].

Production and releasing of B2M are constant and low in healthy people: 0.13 mg/h/kg b. w. B2M is filtered through the glomerular membrane due to its small size, but then it is nearly completely reabsorbed in the proximal tubules [4].

Concentration of B2M reflects a rate of cellular turnover [4]. Most frequently quoted hypothesis explains this phenomenon with mono or polyclonal activation of lymphocytes, destruction of MHC 1 particles, and increased cellular transformation into neoplastic cells, which could lead to a higher concentration of the protein (B2M).

The concentration of serum B2M was found to be increased in cases of various non-neoplastic disorders like renal diseases, acquired immune deficiency syndrome, psoriasis, non-hodgkin's lymphoma, chronic myelogenous leukemias [10]. Hence the elevated levels of B2M are not specific to OSCC, so all the patients included in this study were screened thoroughly by general clinical examination for these diseases.

Out of the 30 patients with OSCC, 23 of them gave a history of tobacco use with a period ranging from 5 to 40 years. Three patients had a habit of guthka chewing since 7 to 12 years. Three patients had a habit of using mishri made up of tobacco. One patient gave a history of smoking bidi since past 40 years. It is a known fact that guthka and mishri (smokeless tobacco) acts as a synergist in the etiology of OSCC.

In group II with leukoplakia, 22 patients gave a history of tobacco use since past 5 to 30 years, eight gave a history of mava chewing since 3 years and one used mishri since past 30 years. It is a well-known fact that smokeless use of tobacco products may lead to leukoplakia.

The increased levels of B2M may be due to increased production or impaired excretion. However, the patients included in this study did not have any ailments where B2M levels are likely to be raised, the increase in B2M levels appears to be a phenomenon due to the malignant process involving oral carcinoma. The fact that B2M values are elevated in the serum of subjects with oral malignancy is in agreement with reports in the studies conducted by Crispan Scully [3] and Anil S, Beena VT, Raj G, and Nair et al. [13].

The mechanism of increase in B2M levels in malignancies is not known but various possible hypotheses for the increased serum levels have been proposed. The B2M is a cell membrane constituent along with the human leukocyte antigen chain, so an accelerated cell division could increase the shedding of B2M [10]. The ability of carcinoma cells to produce a higher concentration of B2M than the non- neoplastic cells may be due to either active synthesis, increased cell breakdown or both [10].

It was noted in this study that the mean B2M levels of patients with OSCC were increasing as the stage of OSCC and PMD increased and as the stages increased which was noted to be highly significant ($p<0.001$ and $p<0.004$) by Spearmans's rank order correlation test.

Scully [3] conducted research based on B2M changes in the blood serum among patients with keratosis, dysplasia and planoepithelial carcinoma of oral cavity. He observed that the values were found to be higher in patients with oral cancer than in control group ($p<0.05$), whereas with keratosis and different types of epithelial dysplasia the values of B2M were slightly lower than those values in oral cancer but significantly higher than the control group ($p<0.05$). Our study also showed findings similar to this research project.

Silvia and coauthors [10] evaluated the levels of B2M among patients with keratosis and oral cancer. They found that the B2M concentrations in serum were statistically higher than the control group ($p<0.05$), and the values were progressively changing in the course of disease. Among the group with keratosis and oral cancer, the mean values of B2M were higher than in control group. The results of the study suggested that the progression of the neoplasm has an influence on the levels of B2M in the blood serum.

Our study indicates that levels of B2M have been increasing as the staging in OSCC and leukoplakia, in our consideration is increased. This is in correlation with a study conducted by Shabana et al. [14] which studied the B2M levels in the cell surfaces by immunofluorescence in 1991. It was observed that in the carcinoma group, the expression of B2M correlated with tumour differentiation. The results suggested that depletion of B2M levels on the cell surface correlates with the poor prognosis of oral carcinoma.

In another study conducted by C. H. Chen et al. [9], the investigators examined 256 samples from patients with OSCC and studied the expression of B2M at different stages of malignancy. Strong B2M expression was significantly correlated with a relatively advanced stage ($p<0.001$) and TNM stage ($p<0.001$).

The study by Anand Pratap Singh et al. [15] in 2014 on 48 subjects supports that B2M level is a specific and sensitive test for diagnostic and prognostic evaluation of oral squamous cell carcinoma.

Conclusion

In the previous studies the control groups which were included in the studies were of the same age group as were cancer and precancer patients irrespective of their lesions. In our study 'Logistic regression analysis' was carried out to adjust for the age and gender as both the parameters are known to influence the B2M levels. B2M levels were still able to predict the stage of OSCC and PMD when adjusted for age ($p<0.016$) and sex ($p<0.019$).

Thus, we can state that B2M can act as a parameter in the early diagnosis of OSCC and leukoplakia. This can be correlated to a study carried out by N. Vaishali and JV Tupkari [16] to evaluate the role of B2M as a biochemical parameter in leukoplakias and OSCC. The progressively increased serum B2M level has a positive correlation with the histologic grading of OSCC.

Thus we can conclude that:

Serum B2M levels were raised in OSCC and the levels increased as the stages of cancer increased.

Serum B2M levels were raised in leukoplakia and the levels increased as the stages of leukoplakia increased.

The levels of serum B2M in OSCC were significantly higher than oral leukoplakia

Serum B2M can be used as a tumor marker in OSCC and leukoplakia.

It was noted that the serum levels of B2M were raised as dysplasia increased. Thus B2M can be considered as a significant marker to assess the levels of dysplasia. Since the study was undertaken on a small number of patients, it is difficult to draw definite conclusions. Hence further studies with a large sample size are necessary to draw definite conclusions.

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