

Original Article

Significant relationship between salivary and serum β 2-microglobulin in prostate cancer and benign prostatic hyperplasia patients

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Received: 2 April, 2017, Accepted: 14 May, 2017

Abstract

Background: The presence of beta-2microglobulin (β 2M) in biological fluids due to lack of binding to the membrane, increases at the time of cell turnover such as cancer and chronic infectious. In recent years, study on the standardization of saliva for clinical diagnosis is increased. The objective of this study was to determine β 2M levels and the relationship between salivary and serum β 2M in Prostate cancer (PCa) and Benign prostatic hyperplasia (BPH) patients.

Materials and Methods: In a case-control study, forty Subjects including 20 PCa and 20 BPH were enrolled. The concentration of β 2M was measured by an enzyme-linked immunosorbent assay. The comparison between β 2M levels in the PCa and BPH groups as well as the correlation between salivary and serum β 2M were tested using Mann-Whitney U test and spearman correlation coefficient, respectively.

Results: Statistically significant difference was observed between salivary and serum β 2M in the PCa and BPH groups ($P < 0.05$). Spearman correlation analysis showed that salivary β 2M is correlated positive and significantly with serum β 2M in the PCa ($r = 0.747$, $P < 0.05$) and BPH ($r = 0.513$, $P < 0.05$) groups.

Conclusion: β 2M can be a suitable biomarker for the diagnosis of prostate diseases, as well as salivary β 2M can be used as an alternative approach to serum β 2M for monitoring and diagnosis of prostate diseases.

Keywords: Beta-2 microglobulin , Saliva, Prostate cancer , Benign prostatic hyperplasia

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Please cite this article as: Amri J, Farahani H, Baghinia M R. Significant Relationship between Salivary and Serum β 2-Microglobulin in Prostate Cancer and Benign Prostatic Hyperplasia Patients. Arch Med Lab Sci. 2016;2(4):129-34.

Introduction

The most common prostate diseases are prostate cancer (PCa) and benign prostatic hyperplasia (BPH). PCa is the second leading cause of cancer related death among men, while BPH is non-cancerous enlargement of the prostate gland that often occurs with aging (1, 2). Early detection is the important way for cancer treatment. such that,

delayed diagnosis contributes to cancer progression and lead to loss of the initiative to treat it (3). The incidence of prostate cancer has decreased due to early detection and identify factors specific diagnosis (3, 4). Despite the advanced diagnostic methods, such as magnetic resonance imaging (MRI), computed tomography (CT) and biopsy, these methods cause suffering and impose heavy costs on patients (5). As a result, studies have great interest in tumor markers due

to they are simple, low cost, and relatively sensitive screening tool for detecting malignancies. These biomarker also increased in the presence of cancer and found in blood, urine and body tissues, and are used in cancer care including diagnosis, screening, monitoring, staging and prognosis (6). Beta-2 microglobulin (β 2M) constitutes a (11.8 kDa) light chain of the major histocompatibility complex class I (MHC I). β 2M is produced in all nucleated cells. Studies have shown increased β 2M in tissue/serum of breast, lung, gastrointestinal, gastrointestinal and prostate cancer cells (7). Based on studies, saliva can show the tumor markers such as PSA in prostate cancer (8), CA15-3 in ovarian cancer (9), CA125 in ovarian cancer(10), etc. Therefore, the use of saliva as a biological fluid could be beneficial due to non-invasive, inexpensive, no need for special equipment and special techniques and repeated sampling without suffering of patients

The aim of this study was to assess the salivary and serum β 2M levels and the relationship between salivary and serum β 2M in patients with prostate cancer and benign prostatic hyperplasia.

Methods

Study design. This study was designed as a case-control study in Arak University of Medical Sciences, Arak, IR Iran and governmental referral hospital Ayatollah Khan Sari in Arak, IR Iran for 1 year's duration on 40 men aged between 50 to 55 years. An ethical clearance was obtained from the ethical committee of the Arak University of Medical Sciences. The study groups included 20 prostate cancer and 20 benign prostatic hyperplasia subjects. Sample size was calculated based on a pilot study (8). The inclusions and exclusion criteria for the PCa and BPH groups were as below:

Inclusions criteria

PCa group. Subjects with prostate cancer and without metastasis, chemotherapy and surgery, capable of giving informed consent, lack of specific diseases and no oral and dental disease.

BPH group. Subjects without a history of prostate cancer that has been diagnosed by a specialist, capable of giving informed consent, lack of specific diseases and no oral and dental disease.

Exclusion criteria

PCa group. Subjects with benign tumors or already treated and having oral and dental disease.

BPH group. Subjects with prostate cancer that has been diagnosed by a specialist and having oral and dental disease.

The inclusions and exclusion criteria for this study was achieved by accurate laboratory analysis and under the supervision of consultant physician in urology medicine. The study was received ethical clearance and approval from Arak University of Medical Sciences, which was consistent with the Helsinki Declaration. All of the subjects in the study were asked to completed a consent form of information questionnaire and demographic before sample collection.

Blood and Saliva sample collection. Five milliliter of blood samples were taken from the participants by venipuncture, and centrifuged at 3000rpm for 5 minutes. Unstimulated saliva was collected as follows: each participant were asked to and refrained from drinking, eating, and smoking or oral hygiene procedures for 2 hr before saliva collection. They rinsed her mouth with plain water several times and seat before collecting for ~15 min before collection. In general, each participant donated ~5–10 ml of saliva. All saliva samples were centrifuged for 10 min at 3000 rpm to obtain clear supernatant. All sample were aliquot in tubes then coded for each test and was stored at -70°C for later determination of salivary and serum β 2M.

Analysis of salivary and serum β 2M. Salivary and serum β 2M levels were determined using sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol. The kit was purchased from Bioassay technology laboratory (Shanghai, China), and is designed to quantify the β 2M levels in biological fluids (serum, saliva, cell lysate etc.). The minimum β 2M detection level reported by the manufacturer is 0.5 mg/dl. Intra- and inter-assay coefficients of variation were <8 and <10%, respectively. All instruments were calibrated before use by second author. Sample absorbance at 450 nm was measured with an ELISA reader (ELX 800 TM ELISA reader Bio Tek, Winooski, VT, U.S.A.).

Statistical analysis. Mann Whitney U test (non-

Table 1: Baseline characteristics of participants

Characteristics	PCa group n=20	BPH group n=20	P value
Age (Year)	50.95 $\pm$ 2.76	50.45 $\pm$ 2.61	0.8
BMI (Kg/m2)	22. 3 $\pm$ 0.5	22.7 $\pm$ 0.5	0.7
Tobacco usage	35%	20%	
Alcohol usage	0	0	

Data are expressed as mean $\pm$ SEM and P < 0.05 is statistically significant

PCa: Prostate cancer; BPH: Benign prostatic hyperplasia; BMI: Body Mass Index

Table 2: Mann–Whitney U test comparing salivary and serum β 2M between the PCa and BPH groups.

Characteristics	PCa group n=20	BPH group n=20	P value
β 2M (Serum)	26.5 $\pm$ 8.5 mg/dl	7.0 $\pm$ 0.5 mg/dl	0.02
β 2M (Salivary)	11.4 $\pm$ 1.3 mg/dl	6.1 $\pm$ 0.5 mg/dl	0.0005

Data are expressed as mean (ng/ml) $\pm$ SEM and *P < 0.05 is statistically significant

PCa: Prostate cancer; BPH: Benign prostatic hyperplasia; β 2M: Beta-2 microglobulin

parametric test) were used for comparison between values of salivary and serum β 2M in two groups. Correlations between salivary and serum β 2M was assessed by applying Spearman's correlation test. The p-value<0.05 was considered statistically significant. All Statistical analysis was done by software Graph Pad Prism software (Version 6.00).

Results

Demographic information. Demographic characteristics of the study groups are summarized in Table 1. The mean $\pm$ SEM age of PCa and BPH subjects were 50.95 $\pm$ 2.76 and 50.45 $\pm$ 2.61 years, respectively which were not significantly different between PCa and BPH groups (P=0.8). The body mass index for the PCa group was 22. 3 $\pm$ 0.6 and for the BPH group was 22.7 $\pm$ 0.5, that was no significant between two groups (P=0.7). All the 40 participants were married. As well as, the two groups in terms of alcohol consumption and smoking were

compared: Thirty-five percent of the PCa and twenty percent of the BPH cases were smoker but no subjects consumed alcohol.

β 2M levels in saliva and serum. The results showed that there was a significant difference between PCa and BPH in terms of β 2M levels in saliva and serum (Table 2). The β 2M level in serum of the PCa group (26.5 $\pm$ 8.5) were higher than serum of the BPH group (7.0 $\pm$ 0.5), which was statistically significant (P=0.02). As well as, the β 2M levels in saliva of the PCa group (11.4 $\pm$ 1.3) were higher than saliva of the BPH group (6.1 $\pm$ 0.5), that was statistically significant (P=0.0005). In general, it was found that the values of salivary β 2M in all subjects were less than its serum.

Correlation between salivary and serum β 2M. There was a significant positive correlation between salivary and serum β 2M showed (Figure 1). In the PCa group (Figure1-A), saliva β 2M level was correlated significantly and positive with serum β 2M (r=0.747,

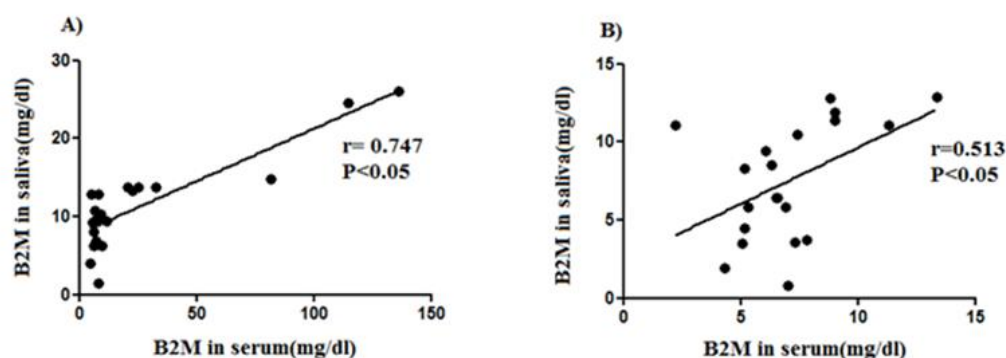


Figure 1. Correlation curves of salivary and serum β 2M in PCa and BPH groups. The salivary β 2M showed significantly and positive correlated with the serum β 2M in two groups. This correlation coefficient was ($r = 0.747$, $P < 0.05$; Figure 1-A) and ($r = 0.513$, $P < 0.05$; Figure 1-B) for PCa and BPH groups, respectively. Spearman's correlation coefficient (r) was used to analysis of the correlation between salivary and serum β 2M. *P-value is for spearman correlation, $P < 0.05$ is statistically significant.

$P < 0.05$). As well as, in the BPH group, salivary β 2M levels were correlated significantly and positive with serum β 2M ($r = 0.513$, $P < 0.05$; Figure 1-B).

Discussion

There are many biological samples, but some of them are very important and acceptable for patients and clinical laboratory (11). On the other hand, since prostate diseases often occurs in adult men, the use of saliva due to its benefits as an inexpensive, non-invasive, simple, and no painful method has been derived interest for diagnosis and monitoring (12, 13). Additionally, the use of specific biomarkers owing to its advantages such as simple, low cost, relatively sensitive screening tool for monitoring prostate cancer and benign prostatic hyperplasia can be useful. Recently, interest in saliva as a biological sample for measure tumor markers has increased exponentially (14). For this purpose, in our study β 2M were measured in saliva and serum to investigate its changes in prostate disease and the estimate the relationship between salivary and serum in this subjects.

The results of our study showed that there was a significant difference between PCa and BPH in terms of serum and saliva β 2M. These results suggest that the β 2M can be effectively used for the diagnosis and differentiation of PCa and BPH. In addition, our results showed that there is a significant and positive

correlation between salivary and serum β 2M in both groups, as well as in all participants the salivary β 2M was less than serum β 2M. These results indicate the potential use of saliva to measure the β 2M as an alternate to serum β 2M in the future.

Studies have indicated that when β 2M is released from the complex MHC-I acts as a ligand that bind to growth factors receptors and causes angiogenesis, growth and differentiation of the cells. The presence of β 2M in biological fluids is due to lack of binding to the membrane, which increases at the time of cell turnover such as cancer and chronic infectious (15). In this regard, our results showed that level of β 2M in serum of the PCa was significantly increased compared with BPH group. Consistent with this result, Gross et al. reported that the β 2M significantly increased in prostate cancer patients compared to healthy subjects (16). Abdul et al. also showed that the value of β 2M has been higher in urine of patients with androgen-independent prostate cancer compared to androgen-dependent prostate cancer (12). In addition Abdul et al. in another study, reported that the β 2M on cells derived from patients with prostate cancer metastasis and in the urine of patients with bone and visceral metastases is higher than normal cells and healthy group, respectively (17), which these results are in agreement with our results. As well as, our data revealed that the β 2M significantly increased in saliva of the PCa group compared to the BPH group. As well as, our findings indicted that serum of

all participants had β 2M higher than of their salivary and the significant positive correlation were observed between salivary and serum β 2M in both groups. These results suggest that saliva as a non-invasive sample can reflect the serum value of β 2M. To our knowledge, this is the first study to evaluate saliva β 2M in prostate patients and also determination of the correlation between salivary and serum β 2M in these patients. However, Vahedi et al and Assareh et al in various studies showed that salivary β 2M levels cannot be an indicator for diagnosis of patients with renal failure, but, in line with our results they reported the salivary levels of β 2M are less than the serum β 2M (18, 19). For further confirmation of our results in order to use saliva as a non-invasive samples, previous studies showed that the correlation is positive and significant between the saliva and serum levels of other biomarkers in various diseases, such as cancer antigen (CA) 15-3 and CA125 in breast cancer (20, 21), PSA in prostate cancer(8) and creatine phosphokinase (CPK) in patients with acute myocardial infarction (22). In general, considering the benefits of tumor markers and also advantages of saliva, future studies are needed to investigate alterations of the other tumor markers in the prostate diseases and indicate whether the saliva can be used to evaluate tumor markers or not.

Conclusion

Our data underline the potential of β 2M as a tumor marker for diagnosis and differentiation of prostate cancer and benign prostatic hyperplasia and confirm the possibility of using saliva as a possible diagnostic fluid. In conclusion, the positive and significant correlation between salivary and serum β 2M found in our study suggests that saliva could be an alternative approach to blood for diagnosis and monitoring prostate diseases. However, further studies in other diseases, different parameters and a larger sample size are needed to confirm this decision.

Conflicts of Interest

The authors have no conflict of interest in this study.

Acknowledgment

The authors are grateful all the patients who took part in this study. This research was supported by a grant of deputy in research and technology, Arak Medical Science University, with ethic permission number IR.ARAKMU.REC.1394.2.

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