Beta-2-Microglobulin as a Marker in Patients with Thyroid Cancer

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ABSTRACT:

BACKGROUND:
Thyroid cancer is consider the most common of the endocrine system malignancies, also it represents less than 1% of all tumors in human. Beta-2-microglobulin protein (β2M) is a low molecular weight polypeptide (11800 Dalton), it is found on the surface of cells which contains nucleus except red blood cells. It is a small subunit and the light chain of major histocompatibility complex class I (MHC- 1) also known as human leukocyte antigen (HLA) in human.

Aim of study:
Detection of the possibility of using Beta-2-Microglobulin protein as a marker for thyroid cancer.

METHODS:
A total of 49 patients with thyroid cancer, their mean age 37.77 ± 13.84 years ranged (18-76 years) were included in this study and healthy age-matched control group were 32 healthy donors, their mean age 38.12 ± 12.29 years ranged (19- 69 years) collected from healthy volunteers. Serum creatinine level was determined by automated clinical chemistry analyzer. In addition to Beta-2-Microglobulin protein was determined by Enzyme-linked Immunosorbent Assay technique.

RESULTS:
The results of serum creatinine level showed a normal level of serum creatinine for all subjects (healthy control and patients) except 14 patients with thyroid cancer who had a level of serum creatinine out of normal range were excluded. Also the results showed significant increase (p= 0.0000001) in mean value of serum β2M protein in patients with thyroid cancer (group B) compared to healthy control (group A). Also the results showed no significant correlation between β2M protein and age (r= 0.153 , p-value 0.379). Also no significant correlation between β2M protein and BMI (r= -0.092, p-value 0.598). Moreover, receiver operating characteristics ROC showed β2M level excellent predictor in the diagnosis of thyroid cancer (area under the curve AUC= 0.970 p<0.001).

CONCLUSION:
A level of serum β2M is elevated in thyroid cancer and can be used as a marker for thyroid cancer and an assistant in the diagnosis of this disease.

KEYWORDS: Beta-2-Microglobulin, Creatinine, Thyroid Cancer.

INTRODUCTION:
Thyroid cancer is considered the most common of the endocrine system malignancies, also it represents less than 1% of all tumors in human. The incidence of thyroid cancer differs greatly by gender, age and geographic area [1]. Despite of rise in its rates of thyroid cancer, but the mortality has not risen in equal measure. However, the mortality are higher two times in female [2,3]. Beta-2-microglobulin protein (β2M) is a protein first described by Berggard and Bearn in 1968, it was isolated from the urine of patients with Wilson's disease (4). It is a low molecular weight polypeptide (11800 Dalton) [5] and found on the surface of cells which contains nucleus except red blood cells [6]. It is a small subunit and the light chain of major histocompatibility complex class I (MHC- 1) also known as human leukocyte antigen (HLA) in human and it is noncovalently linked with the alpha chain of MHC-1 and has no direct attachment to the plasma membrane, therefore free soluble β2M circulates in blood after being released from cells surfaces [7]. Under physiologic conditions, β2M protein is released at a constant rate and is cleared from circulation by kidneys, but in case of renal diseases, a level of plasma β2M is elevated [8]. The aim of study is detection of the possibility using β2M protein as a marker for thyroid cancer.

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MATERIALS AND METHODS:
Study population
This study included 49 patients with thyroid cancer (21 males and 28 females) and their mean age 37.77±13.84 years ranged (18-76 years) were selected from Al-Amal National Hospital for Cancer Treatment in Baghdad province/Iraq during the period from October 2017 to January 2018 . The healthy age-matched control group were 32 healthy donors (18 males and 14 females), their mean age 38.12±12.29 years ranged (19- 69 years) collected from healthy volunteers.

Fourteen patients who had a level of serum creatinine out of a normal range (normal range : Adult, Male: 0.72 - 1.25 mg/dL , Adult, Female: 0.57 - 1.11 mg/dL) [9] were excluded, the remaining 35 patients were measured β2M level in their serum. Where β2M is readily filtered through the glomerulus and almost completely reabsorbed and destroyed by proximal tubular cells. Hence, decreased renal function (due to reduced catabolism) results in a proportional rise in serum β2M levels. Therefore, creatinine test was used to evaluate renal function and excluded the subjects to possibility of renal dysfunction [10].

The groups of subjects (patients and healthy control) after excluded 14 patients were divided into two groups:

Group (A): Included 32 healthy donors as control.

Group (B): Included 35 patients with thyroid cancer.

Five milliliters of blood samples were collected in a gel tube free of anticoagulant from each individual, then were separated by centrifuge to obtain the serum, which was transferred into two eppendrof tubes: The first tube was about 2 ml of serum to measure the level of creatinine. The second tube about was 3 ml of serum to measure the level of β2M protein. The sera were stored at (−20 °C) until analysis [11].

Furthermore, body mass index (BMI) equals the weight in kilograms divided by the square of height in meter (BMI = kg/m^2). A persons with BMI (18.5 - 24.9) are a persons of normal weight, a persons with BMI (25 - 29.9) are overweight, while a persons with BMI of ≥ 30 are obese [12].

Determination of serum creatinine
Creatinine was determined by ARCHITECT *c*4000 automated clinical chemistry analyzer using the creatinine kit (Cat. No. 3L81)/Abbott/ Germany to evaluate kidney function and exclude the subjects who had a level of creatinine in their serum out of the normal range.

Enzyme-linked Immunosorbent Assay (ELISA) for determination of serum β2M
Beta-2-Microglobulin (β2M) protein was determined by ELISA technique using the Beta-2-Microglobulin ELISA kit (Cat. No.: DE7610)/ Demeditec Diagnostics GmbH/Germany according to the manufacturer's instructions.

Statistical analysis
Data analysis were performed using SPSS software (statistical package for social sciences) version (20). Data were presented as mean±standard deviation (SD) and range. Differences between means were assessed by Student's t-test. Also r-correlation was used between two variables. Receiver operating characteristics (ROC) curve were used to assess the prediction (diagnostic) accuracy of thyroid cancer by β2M protein. If (p value) <0.05 was considered significant.

RESULTS:
Estimation level of creatinine in serum
A level of serum creatinine was measured for all subjects (healthy control and patients) involved in this study to exclude the subjects to possibility of kidneys dysfunction, where 14 patients with thyroid cancer who had a level of creatinine in their serum out of normal range were excluded, the subjects who had a normal level of creatinine in their serum were included for measuring level of β2M protein in their serum for healthy control (group A: n= 32) and patients with thyroid cancer (group B: n= 35). The results showed that the level of serum creatinine for group A ranged (male: 0.72 – 1.20 mg/dl, female: 0.57 – 0.8 mg/dl) while in group B ranged (male: 0.8 – 1.25 mg/dl, female: 0.62 – 1.07 mg/dl), these results of serum creatinine level within normal range, as shown in Table (1).

Table 1: The level of creatinine in serum as a range for healthy control (group A: n= 32) and patients with thyroid cancer (group B: n= 35).

Groups	Group A Healthy control (n = 32)	Group B Thyroid cancer (n = 35)
Parameter	Range	Range
Creatinine (mg/dl)	Male: 0.72 – 1.20 Female: 0.57 – 0.8	Male: 0.8 – 1.25 Female: 0.62 – 1.07

Estimation level of β2M in serum

The range and Mean±SD values of age for healthy control (group A: n = 32) were (19 – 69 years), 38.12±12.29 years respectively. While in patients with thyroid cancer (group B: n = 35) were (18 – 76 years), 37.77±13.84 years respectively as shown in Table (2).

The range and Mean±SD values of BMI for group A were (20.4 – 30.5 kg/m^2), 23.82±2.06 kg/m^2 respectively. While in group B were (18.6 – 27.7 kg/m^2), 23.75±2.14 kg/m^2 respectively as shown Table (2).

The range and Mean±SD values of level β2M protein in serum for group A were (0.90 - 2.67 µg/ml), 1.91±0.46 µg/ml respectively. While in group B were (2.07 – 11.96 µg/ml), 3.85±2.40 µg/ml respectively as shown in Table (2) and Figure (1).

Table 2: General characteristics for healthy control (group A: n=32) and patients with thyroid cancer (group B: n= 35).

Groups	Group A Healthy control (n = 32)	Group B Thyroid cancer (n = 35)
Parameter	Mean±SD, Range	Mean±SD, Range
β2M (µg/ml)	1.91±0.46 , (0.90 - 2.67)	3.85±2.40 , (2.07 – 11.96)
Age (years)	38.12±12.29 , (19 – 69)	37.77±13.84 , (18 – 76)
BMI (kg/m^2)	23.82±2.06 , (20.4 – 30.5)	23.75±2.14 , (18.6 – 27.7)
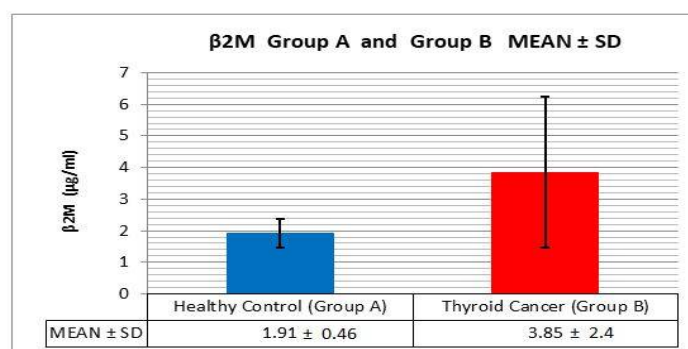
Figure 1: The Mean±SD values of level β2M protein in serum for healthy control (group A: n=32) in blue color and patients with thyroid cancer (group B: n= 35) in red color.

The results in Table (2) and Figure (1) have shown significant increase (p= 0.0000001) in mean value of serum β2M protein in patients with thyroid cancer (group B) as compared with healthy control (group A).

In patients with thyroid cancer, the results showed no significant correlation between β2M protein and age (r= 0.153 , p-value 0.379). Also the results showed no significant correlation between β2M protein and BMI (r= -0.092, p-value 0.598).

The ability of correctly diagnosing patients with thyroid cancer according to β2M protein was assessed using the receiver operating

characteristics (ROC) curve. β2M protein showed a excellent predictor ability for patients with thyroid cancer according to the area under the curve(AUC = 0.970). Where sensitivity= 0.943 and specificity= 0.75 , and the p-value <0.001 as shown in Figure (2).
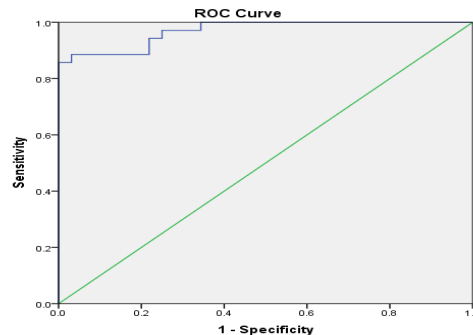

Figure 2: ROC curve analyses for predictive values of β2M protein in detecting patients with thyroid cancer, where AUC = 0.970 and p-value <0.001 .

DISCUSSION:

Creatinine test was used to evaluate the function of kidneys of all subjects (healthy control and patients), this is agreement with the studies [13,14] which showed that using creatinine test is the preferred and more suitable for assessment of renal function. Where the subjects who had a level of serum creatinine out of normal range were excluded, only subjects who had a normal range of serum creatinine level [15] were measured β2M protein in their serum to distinguish the levels of β2M protein in their serum is due to the tumors and not because of kidneys dysfunction, because of serum β2M level elevated in case of renal failure or kidney diseases. This is agreement with study [16] which showed that β2M protein is produced or released at a constant rate in normal subjects and is eliminated from circulation by kidneys, and it is elevated in the serum or plasma in case of renal diseases or renal dysfunction, so it was considered a suitable biomarker to kidneys dysfunction.

Our results showed that level of serum β2M was elevated in patients with thyroid cancer as compared with healthy control, these results are in agreement with study [7] which demonstrated that level of β2M in blood increase in certain malignancies. Also the results are in agreement with study [17] which demonstrated that the higher β2M levels associated with higher tumor burden due to increased β2M protein shedding in the circulation. Also in agreement with study [18] which demonstrated that the level of β2M protein in serum is significant elevated in patients with thyroid cancer.

Also our results showed no significant correlation between β2M protein and age. Also no significant correlation between β2M protein and BMI, these results are in agreement with study [19] which demonstrated no significant correlation between β2M protein and age, also no significant correlation between β2M protein and BMI. Also these the results are in agreement with study [20] which demonstrated no significant correlation between β2M protein and age.

CONCLUSION:

Serum β2M can be used as a marker for thyroid cancer and an assistant in the diagnosis of this disease.

REFERENCES:

1. Aschebrook-Kilfoy B, Ward MH, Sabra MM, Devesa SS. Thyroid cancer incidence patterns in the United States by histologic type, 1992–2006. *Thyroid*. 2011;21:125-34.

2. Edwards BK, Brown ML, Wingo PA, Howe HL, Ward E, Ries LA, *et al*. Annual report to the nation on the status of cancer, 1975-2002, featuring population-based trends in cancer treatment. *J Natl Cancer Inst*. 2005;97:1407-27 .

3. Harris PJ, Bible KC. Emerging therapeutics for advanced thyroid malignancies: rationale and targeted approaches. *Expert Opin Investig Drugs*. 2011;20:1357-75 .

4. Berggård I, Bearn AG. Isolation and properties of a low molecular weight β2-globulin occurring in human biological fluids. *Journal of Biological Chemistry*. 1968;243:4095-103 .

5. Vivian JP, Duncan RC, Berry R, O'connor GM, Reid HH, Beddoe T, *et al*. Killer cell immunoglobulin-like receptor 3DL1-mediated recognition of human leukocyte antigen B. *Nature*. 2011;479: 401-5 .

6. Shi C, Zhu Y, Su Y, Chung LW, Cheng T. β2-Microglobulin: emerging as a promising cancer therapeutic target. *Drug discovery today*. 2009;14:25-30 .

7. Amighi J, Hoke M, Mlekusch W, Schlager O, Exner M, Haumer M, *et al*. Beta 2 microglobulin and the risk for cardiovascular events in patients with asymptomatic carotid atherosclerosis. *Stroke*. 2011;42:1826-33 .

8. Chitra P, Bakthavatsalam B, Palvannan T. Beta-2 microglobulin as an immunological marker to assess the progression of human immunodeficiency virus infected patients on highly active antiretroviral therapy. *Clinica chimica acta*. 2011;412:1151-54 .

9. Fatima Z, Khan A, Tariq U, Sohail MS. Hemophagocytic Lymphohistiocytosis: A Case Series. *Cureus*. 2018;10:2545 .

10. Zeng X, Hossain D, Bostwick DG, Herrera GA, Zhang PL. Urinary β2 microglobulin is a good indicator of proximal tubule injury: a correlative study with renal biopsies. *Journal of biomarkers*. 2014; 2014:7 .

11. Ouda SM, Khairy AM, Sorour AE, Mikhail MN. Serum Beta-2 microglobulin: a possible marker for disease progression in egyptian patients with chronic HCV related liver diseases. *Asian Pacific Journal of Cancer Prevention*. 2015;16:7825-29 .

12. Pasco JA, Holloway KL, Dobbins AG, Kotowicz MA, Williams LJ, Brennan SL. Body mass index and measures of body fat for defining obesity and underweight: a cross-sectional, population-based study. *BMC obesity*. 2014;1:9 .

13. Salazar J H. Overview of urea and creatinine. *Laboratory Medicine*. 2014;45:19-20 .

14. McWilliam A, Macnab R. Laboratory tests of renal function. *Anaesthesia & Intensive Care Medicine*. 2009;10: 296-299.

15. Neves MC, Rocha LA, Cervantes O, Santos RO. Initial surgical results of 500 Parathyroidectomies for Hyperparathyroidism related to chronic kidney disease-mineral and bone disorder. *Brazilian Journal of Nephrology*. 2018 .

16. Shahjahan, Yasmin R, Mahsud MA, Khan M, Subzwari J, Hussain J. Correlation of Beta-2 Microglobulin with serum creatinine and creatinine clearance in patients with different levels of renal function. *Gomal Journal of Medical Sciences*. 2011;9:178-82.

17. Argyropoulos CP, Chen SS, Ng YH, Roumelioti ME, Shaffi K, Singh PP, *et al*. Rediscovering beta-2 microglobulin as a biomarker across the spectrum of kidney diseases. *Frontiers in medicine*. 2017;4:73 .

18. Ma S, Liu Q, Li K. The clinical significance evaluation of serum β 2-microglobulin for thyroid cancer patients. *The Chinese-German Journal of Clinical Oncology*. 2012;11:271-73.

19. Sedighi O, Abediankenari S, Omranifar B. Association between plasma Beta-2 microglobulin level and cardiac performance in patients with chronic kidney disease. *Nephro-urology monthly*. 2015;7: 23563 .

20. Naji NA, Khorsheed SH, Mustafa IF. Study the activity of Tryptase And Beta 2-Microglobulin Levels In the Sera of Patients With Chronic Renal Failure And Rheumatoid Arthritis. *kirkuk university journal for scientific studies*. 2016;11:138-54 .