Journal of Umm Al-Qura University for Medical Sciences

journal homepage: https://uqu.edu.sa/en/mj

The Association between oxidative stress marker Beta-2 microglobulin and type 2 diabetes mellitus in context of glycaemic index

Riyad A Almaimani [a].

[a] *Department of Biochemistry, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.*

ARTICLE INFO

Article History:
Submission date: 20/12/2020
Accepted date: 8/3/2021

Keywords:
Beta2-microglobulin, Oxidative stress, Type 2 Diabetes Mellitus, Haemoglobin A1c, Glycaemic index.

Conflict of Interest:
None to declare.

ABSTRACT

Beta-2 microglobulin (B2M) protein has been shown to be elevated in response to oxidative stress and metabolic disorders and it modulates human immune and inflammatory responses. However, little is known about the association between B2M and glycaemic index in diabetic patients. Hence, this case-control study was conducted to measure the effects of type 2 diabetes mellitus (T2DM) on the plasma concentrations of B2M. A total of 50 T2DM male patients alongside another 50 age-matched and healthy men were enrolled. The plasma concentrations of B2M and haemoglobin A1c (HbA1c) were measured. Data related to smoking, daily consumption of multivitamins and regular performance of exercise were obtained through a questionnaire. The participants body mass index (BMI) was calculated after the body weight (Kg) and height (cm) of each participant was recorded. The results showed comparable age, BMI and rate of active smoking between the study groups. However, the levels of HbA1c were significantly higher, whereas the frequencies of consuming multivitamins and regularly performing exercise were significantly lower, in the T2DM group when compared with healthy controls. The plasma B2M levels also showed moderate significant positive correlations with age, BMI, and HbA1c plasma levels. In conclusion, the plasma Concentration of the oxidative stress marker B2M, was associated with T2DM and a significant elevation in its levels was positively correlated with age and the levels of HbA1c.

1. Introduction

Beta2-microglobulin (B2M) is a plasma membrane protein that is a component of major histocompatibility complex class I molecules (MHC-I) and is expressed in all nucleated cells. It has been reported that B2M is highly secreted in the blood in response to immune and inflammatory reactions [1]. For example, B2M can interact with mediators of inflammation such as interleukin 6 (IL-6) and interleukin 8 (IL-8) in many cells. Moreover, it can modulate cellular functions, and hormone secretion, and regulate the binding between ligands and their receptors [2, 3]. Previously, it had been reported that B2M acts as a growth promoter factor supporting the tumour progression [4]. Zhu et al, 2009 reported that the injection of B2M promotes stem cell accumulation through expression of endothelial growth factors, and it induces osteosarcoma cell proliferation and metastasis [5]. In another context, B2M induces cell death in many leukemic tumour cells [6, 7]. For patients under long term haemodialysis, an increase in the B2M level results in its accumulation in joints, leading to the build up of amyloid plaques, which is considered to be a major part of amyloid fibrils. In addition, it has been reported that point mutation in the B2M gene increases febrile development [8].

B2M has been used in biological samples as a marker for different disorders [9-18]. While there are many studies that note its role in different diseases related to aging and cancer. It has been observed in several solid tumours including human lung, breast, renal, and prostate cancers, it is less common in liquid tumours, such as lymphomas and leukaemias [19, 20]. In amyloidosis cases that result from long term haemodialysis, B2M abnormally increases in the blood . In rare cases, B2M in cerebrospinal fluid(CSF)can also be utilized to assess the nervous system [21]. Many studies have reported the increase of B2M in the blood of people with progressive prostate cancer [22]. This may be a result of the regulatory role of the androgen hormone that may stimulate B2M shedding from prostate tumour cells[23]. In addition, higher B2M levels in the blood could be the result of inflammatory bowel disorders [24]. It also increases in the blood as a result of viral infections, such as cytomegalovirus and HIV(human immunodeficiency virus) [25]. In the case of bacterial infections, such as Helicobacter pylori, gastric biopsies reveal high levels of B2M expression that underscore the presence of immune cell infiltration and possible regional inflammation in the stomach [26]. Moreover, a previous study recommended B2M to be used as a potential blood biomarker for neurological diseases during infection with T-cell leukemic virus [27]. In lupus nephritis disease, B2M/ cystatin C ratio is recommended to be used as a biomarker for renal function test [28, 29]. In renal transplantation, B2M level is used for long-term monitoring of the recipient after kidney grafting [30].

Lifestyle habits are believed to be the main risk factors of chronic disease development [31, 32]. Smoking, a Western diet, and a sedentary lifestyle are considered to trigger the low-grade inflammation that is subsequently responsible for chronic diseases in developed countries [33, 34]. Different biomarkers have been studied to assess the role of lifestyle on inflammation such as C-reactive protein. A systematic review has reported that different levels of physical activity and lifestyle had a different inflammatory response [35]. As far as we know, no studies reported to check the direct relationship between B2M and HbA1c in non-complicated diabetic patients. Therefore, this study aims to investigate the possible association between these two biomarkers.

* **Corresponding Author**
Department of Biochemistry, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia.
E-mail address: ramaimani@uqu.edu.sa (*Riyad A Almaimani*)

2. Materials and methods

2.1. Subjects and blood collection

This case-control study was approved by the Medical Ethics Committee of the Faculty of Medicine, Umm Al-Qura University, and a written consent was obtained from all participants prior to enrolment. The study participants included 50 male patients with T2DM and another group of 50 age-matched men who were apparently healthy with no history of chronic diseases (e.g., hypertension, cardiovascular diseases, metabolic disorders, respiratory diseases, etc.) according to the clinical history and investigations. The patients' group (T2DM) diagnosed with diabetes mellites for at least five years, were treated with oral hypoglycaemic or insulin injections, and had no symptoms or signs of other co-morbid illnesses. According to the American Diabetes Association (ADA), the participants were categorized into four groups based on HbA1c levels; normal (HbA1c level below 5.7%), prediabetics (HbA1c level between 5.7% and 6.4%), controlled group of diabetic patients (HbA1c level between 6.5% and 7%) and uncontrolled group of diabetic patients (HbA1c level more than 7%) [36].

Information related to smoking, physical exercise and consumption of multivitamin supplements were obtained through a questionnaire. For each participant, a non-fasting blood sample was collected in an EDTA tube and the samples were immediately stored in ice. The plasma was separated by centrifugation at 3000 rpm for 10 minutes and was then stored in 1.5 Eppendorf tubes at −80°C until further analysis.

2.2. HbA1c assay

HbA1c was measured in the collected plasma samples by a human HbA1c kit (CATALOG# 80099, Crystal Chem) according to the manufacturer's instructions. In brief, the whole blood samples were added to a mixture of protease buffer A and of buffer B, then 25 µL sample lysate was incubated for 5 min at 37°C. The released amino acid including glycated valine residues from the haemoglobin beta chains (substrate) was incubated with 70 µL enzyme solution (fructosyl valine oxidase (FVO) enzyme) for 3 minutes at 37°C. Finally, an automated microplate reader was used to measure the absorbance at 700 nm (SpectraMAX M2; USA).

2.3. B2M measurement

B2M was analysed using a commercially available kit (PARS BIOCHEM beta 2 microglobulin ELISA kit, PRS-01661hu). The wells in the plate were pre-coated with B2M-specific antibody. Standards or plasma samples were added to the pre-coated wells with specific antibody. After that, the plates were washed before adding secondary labelled antibody that can bind to B2M. The pre-coated plates were then washed to remove unbound secondary antibody before adding Streptavidin-Peroxidase conjugate that binds to the secondary antibody. Addition of 3,3′,5,5′-Tetramethylbenzidine (TMB) developed the color of the enzymatic reaction and the reaction was then stopped by the addition of acid. The levels of B2M in the samples were then measured on a fully automated plate reader (SpectraMAX M2; USA).

2.4. Statistical analysis

Data were analysed by SPSS software version 26. Normality and homogeneity of continuous data were assessed by the Kolmogorov-Smirnov test and the Levene test, respectively. Ordinal and discontinuous data were presented as numbers and percentages, and cross-tabulation followed by Chi-square (χ^2) test were used to test for significant differences among studied groups. Student t-test was applied to test for the hypothesis of numerical data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of study participants

This study included 50 males with type 2 diabetes mellitus and another 50 age-matched apparently healthy participants. As shown in Table (1), there was no significant difference between the cases and controls in the mean ±SD of age, body weight, height and body mass index as well as the rate of active smokers. In contrast, the levels of HbA1c were significantly higher, whereas the frequencies of consuming multivitamins and regularly performing regular physical exercise were significantly lower, in the diabetic group compared with the controls (Table 1).

Table 1: The characteristics of controls (n = 50) and T2DM patients (n=50) according to diabetic status. The data expressed as Mean ± SD of numerical data and as a percentage for nominal data. Chi-square (χ^2) test and Student t-test was applied to test of the hypothesis. $P < 0.05$ was considered statistically significant.

	Controls (n = 50)	Type 2 DM (n = 50)	P value
Mean ± SD of Age (year)	54 ± 10.5	57.1 ± 11.1	0.1
Mean ± SD of HbA1c (%)	5.6 ± 0.7	8.1 ± 2.1	**< 0.0001**
Mean ± SD of Weight (kg)	85.2 ± 17.6	85.6 ± 16.1	0.8
Mean ± SD of Height (cm)	170.8 ± 7.6	166.8 ± 7.1	0.08
Mean ± SD of BMI (kg/m^2)	29.2 ± 5.9	30.8 ± 5.7	0.2
BMI Classes			
Underweight	4 (8%)	3 (6%)	
Normal	15 (30%)	9 (18%)	
Overweight	12 (24%)	14 (28%)	0.2
Obese	13 (26%)	15 (30%)	
Morbid obesity	6 (12%)	9 (18%)	
Active smoking			
Yes	14 (28%)	15 (30%)	0.8
No	36 (72%)	35 (70%)	
Use of multivitamins			
Yes	29 (58%)	15 (30%)	**0.001**
No	21 (42%)	35 (70%)	
Regular physical exercise			
Yes	33 (66%)	22 (44%)	**0.02**
No	17 (34%)	28 (56%)	

3.2. B2M plasma concentrations

Overall, the plasma concentrations of B2M were significantly elevated in the diabetic group than the in control group (Figure 1A). Moreover, prediabetic control (HbA1c level between 5.7% and 6.4%) individuals demonstrated markedly higher levels of B2M compared with normoglycemic participants (Figure 1B). The diabetic patients with uncontrolled glycaemic index (HbA1c level equal 6.5% or higher on two separate tests) similarly had significantly increased concentrations of plasma B2M than those with controlled glycaemic index. Moreover, the highest B2M concentrations were detected in diabetic patients with uncontrolled elevated HbA1c compared with other groups (Figure 1B). Also, the results showed that regular performance of physical exercise (Figure 1C) as well as daily consumption of multivitamins (Figure 1D) showed comparable plasma B2M levels within the non-diabetic and diabetic groups.

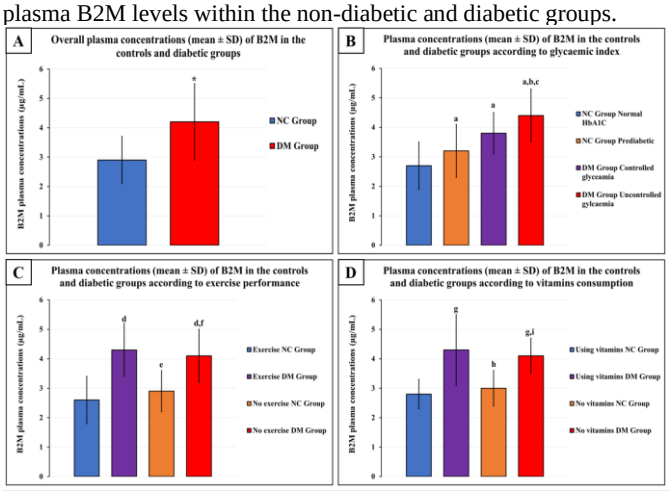

Figure 1: The overall plasma concentration of B2M in diabetic patients. A) The plasma concentrations (mean ±SD) of B2M in the controls and diabetic groups. B) Plasma concentrations (mean ±SD) of B2M in the controls and diabetic groups according to glycaemic index. C) Plasma concentrations (mean ±SD) of B2M in the controls and diabetic groups according to exercise performance. D) Plasma concentrations (mean ±SD) of B2M in the controls and diabetic groups according to vitamins consumption. (Data expressed as mean ± SD, (* = $P < 0.05$ compared with overall NC group; a = $P < 0.05$ NC group with normal HbA1c; b = NC group; c = $P < 0.05$ compared with DM group with controlled glycaemia, d = $P < 0.05$ compared with NC group performing exercise; e = $P < 0.05$ compared with DM group performing exercise; f = $P < 0.05$ compared with NC group not performing exercise; g = $P < 0.05$ compared with NC group using vitamins; h = $P < 0.05$ compared with DM group using vitamins and I = $P < 0.05$ compared with NC group not using vitamins).

Furthermore, significant moderate positive correlations were observed observed in the plasma concentrations of B2M with the participants' age, BMI, and the levels of HbA1c (Table 2).

Table 2: Results of correlation analysis using Pearson's test between the plasma concentrations of B2M with age, BMI and HbA1c of the controls (n = 50) and T2DM patients (n=50).

		Plasma B2M (µg/ml)
Age (Years)	*r value*	0.90
	P value	p <0.001
BMI (kg/m^2)	*r value*	0.21
	P value	0.01
Plasma HbA1c (%)	*r value*	0.46
	P value	p <0.001

4. Discussion

B2M has been reported to be highly elevated in blood in response to immune and inflammatory reactions [1]. It has been documented to be increased in in various types of solid tumours and in multiple myeloma[19, 20]. Our results showed an increase in B2M plasma levels in diabetic subjects compared to healthy controls. In addition, diabetic patients with uncontrolled glycaemia had higher B2M levels compared to diabetic patients with controlled glycaemia.

A recent study showed that a reduction in the inflammatory markers was seen remarkably in subjects with low BMI [37]. It has been shown that a short period of exercise have beneficial effects on endothelial function, but no effects were seen in reducing low grade inflammation. In contrast, long-term exercise was associated with anti-inflammatory effects [38]. Consistent with these observations, a recent systematic review concluded that long-term and intense exercise increased inflammatory biomarkers, however, mild exercise with adequate resting time showed lower inflammatory response [39]. Our results showed no significant difference in B2M levels between the exercise group of DM patients and healthy controls. This might be due to the fact that different types of exercise have different impact on B2M levels.

Our findings showed that B2M levels were increased in the plasma of DM patients compared to controls. It has been reported that higher B2M levels were associated with a poor prognosis of complications in diabetic patients [40]. Previous studies reported an association between glucose concentration and B2M in the plasma of diabetes patients who were under haemodialysis [41]. However, to the best of our knowledge, no study has investigated the direct relationship between B2M level and diabetes aetiology. Therefore, future studies should include in vivo model to explore a possible link between B2M and insulin resistance, pancreatic function and diabetes.

In this study, we showed that B2M levels were higher in diabetic patients compared to healthy controls, and HbA1c was positively correlated with B2M level. Therefore, B2M can be used to assess the prognosis of diabetic patients. However, a larger sample size should be used to validate the association between B2M and HbA1c in diabetic patients. A possible explanatory mechanism is that B2M could be involved in the inflammatory network of insulin resistance and hence hyperglycaemia. This may explain the elevated level of B2M in diabetic patients.

In conclusion, the study showed that B2M levels increased in patients with type 2 diabetes compared to healthy controls. In addition, the mean value of B2M is higher in prediabetic subjects compared to healthy controls. A slight increase in the mean value of B2M in diabetic patients with uncontrolled glycemia compared to the diabetic patients with controlled glycemia. In addition, B2M correlated positively with HbA1c levels in the patients studied. Cumulative evidence from our finding support the association between B2M levels and DM. However, A large sample size with the addition of female participants with diabetes should be considered in future studies to strengthen these findings. The study had some limitation that should be highlighted, firstly, the sample size of the study was limited. In addition, the study included people from one city, adding more samples from different cities could strengthen the study. Moreover, the amount and frequency of multivitamins consumption has not been reported. In addition, in diabetic patients, the study has not recorded the anti-diabetic drug that has been used.

5. Acknowledgment

The author would like to thank Dr. Mohammad Althubiti and Dr Mahmoud El-Readi for their help and support in the collection of samples, supervision of all experiments and ethical approval as well as Dr. Bassem Refaat for his advice and comments. The author would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by grant code:19-MED-1-01-0022.

6. References

[1] Shi, C., et al., Beta2-*microglobulin: emerging as a promising cancer therapeutic target.* Drug Discov Today, 2009. **14**(1-2): p. 25-30.

[2] Balint, E., *Role of beta(2)-microglobulin in the immune response in renal osteodystrophy.* Semin Dial, 2001. **14**(2): p. 113-6.

[3] Yang, J., et al., *Anti beta2-microglobulin monoclonal antibodies induce apoptosis in myeloma cells by recruiting MHC class I to and excluding growth and survival cytokine receptors from lipid rafts.* Blood, 2007. **110**(8): p. 3028-35.

[4] Huang, W.C., et al., *beta2-microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis.* Cancer Res, 2006. **66**(18): p. 9108-16.

[5] Zhu, Y., et al., *Beta2-microglobulin as a potential factor for the expansion of mesenchymal stem cells.* Biotechnol Lett, 2009. **31**(9): p. 1361-5.

[6] Mori, M., et al., *Antitumor effect of beta2-microglobulin in leukemic cell-bearing mice via apoptosis-inducing activity: activation of caspase-3 and nuclear factor-kappaB.* Cancer Res, 2001. **61**(11): p. 4414-7.

[7] Gordon, J., et al., *Beta2-microglobulin induces caspase-dependent apoptosis in the CCRF-HSB-2 human leukemia cell line independently of the caspase-3, -8 and -9 pathways but through increased reactive oxygen species.* Int J Cancer, 2003. **103**(3): p. 316-27.

[8] Leney, A.C., et al., *Insights into the role of the beta-2 microglobulin D-strand in amyloid propensity revealed by mass spectrometry.* Mol Biosyst, 2014. **10**(3): p. 412-20.

[9] Banner, B.F., et al., *Immunophenotypic markers in renal cell carcinoma.* Mod Pathol, 1990. **3**(2): p. 129-34.

[10] Mogi, M., et al., *Brain beta 2-microglobulin levels are elevated in the striatum in Parkinson's disease.* J Neural Transm Park Dis Dement Sect, 1995. **9**(1): p. 87-92.

[11] Mogi, M., et al., *Increased beta 2-microglobulin in both parotid and submandibular/sublingual saliva from patients with Sjogren's syndrome.* Arch Oral Biol, 1994. **39**(10): p. 913-5.

[12] Gao, Y.M., Y. Gao, and G.Z. Lu, *[The changes of urine protein and serum beta 2-microglobulin in autoimmune thyroid disease].* Zhonghua Nei Ke Za Zhi, 1994. **33**(9): p. 605-7.

[13] Kim, M.K., et al., *Clinical utility of serum beta-2-microglobulin as a predictor of diabetic complications in patients with type 2 diabetes without renal impairment.* Diabetes & Metabolism, 2014. **40**(6): p. 459-465.

[14] You, L., et al., *High levels of serum β2-microglobulin predict severity of coronary artery disease.* BMC Cardiovasc Disord, 2017. **17**(1): p. 71.

[15] Argyropoulos, C.P., et al., *Rediscovering Beta-2 Microglobulin As a Biomarker across the Spectrum of Kidney Diseases.* Front Med (Lausanne), 2017. **4**: p. 73.

[16] Wang, H.J., et al., *The prognostic values of beta-2 microglobulin for risks of cardiovascular events and mortality in the elderly patients with isolated systolic hypertension.* J Res Med Sci, 2018. **23**: p. 82.

[17] Saddiwal, R., et al., *Estimation of Serum Beta 2-Microglobulin Levels in Individuals Exposed to Carcinogens: Clinical Study in Indian Population.* J Maxillofac Oral Surg, 2017. **16**(1): p. 53-57.

[18] Behairy, O.G., et al., *Role of serum cystatin-C and beta-2 microglobulin as early markers of renal dysfunction in children with beta thalassemia major.* Int J Nephrol Renovasc Dis, 2017. **10**: p. 261-268.

[19] D'Anastasi, M., et al., *Tumor load in patients with multiple myeloma: beta2-microglobulin levels versus whole-body MRI.* AJR Am J Roentgenol, 2014. **203**(4): p. 854-62.

[20] Molica, S., et al., *Clinico-prognostic implications of simultaneous increased serum levels of soluble CD23 and beta2-microglobulin in B-cell chronic lymphocytic leukemia.* Eur J Haematol, 1999. **62**(2): p. 117-22.

[21] Jeffery, G.M., et al., *Cerebrospinal fluid B2-microglobulin levels in meningeal involvement by malignancy.* Pathology, 1990. **22**(1): p. 20-3.
[22] Zhang, Y.X., et al., *Correlation of serum beta2-microglobulin levels with prostate-specific antigen, Gleason score, clinical stage, tumor metastasis and therapy efficacy in prostate cancer.* Arch Med Res, 2013. **44**(4): p. 259-65.
[23] Mink, S.R., et al., *Beta-2-microglobulin expression correlates with high-grade prostate cancer and specific defects in androgen signaling.* Prostate, 2010. **70**(11): p. 1201-10.
[24] Yilmaz, B., et al., *Serum beta 2-microglobulin as a biomarker in inflammatory bowel disease.* World J Gastroenterol, 2014. **20**(31): p. 10916-20.
[25] Chitra, P., B. Bakthavatsalam, and T. Palvannan, *Beta-2 microglobulin as an immunological marker to assess the progression of human immunodeficiency virus infected patients on highly active antiretroviral therapy.* Clin Chim Acta, 2011. **412**(11-12): p. 1151-4.
[26] Conz, P.A., et al., *Beta-2-microglobulin and Helicobacter pylori infection in uraemic dialysed patients.* J Gastroenterol Hepatol, 1992. **7**(2): p. 191-3.
[27] Toledo-Cornell, C., et al., *Soluble IL-2 receptor and beta-2 microglobulin as possible serologic markers of neurologic disease in HTLV-1 infection.* J Med Virol, 2014. **86**(2): p. 315-21.
[28] Madureira Silva, M.V., et al., *Serum Beta 2-microglobulin/cystatin C index: a useful biomarker in lupus nephritis?* Nephron Extra, 2012. **2**(1): p. 169-76.
[29] El-Frargy, M.S., et al., *Serum cystatin-C and BETA 2-microglobulin as accurate markers in the early diagnosis of kidney injury in neonates: A single center study.* Saudi J Kidney Dis Transpl, 2015. **26**(4): p. 712-7.
[30] Astor, B.C., S. Shaikh, and M. Chaudhry, *Associations of endogenous markers of kidney function with outcomes: more and less than glomerular filtration rate.* Curr Opin Nephrol Hypertens, 2013. **22**(3): p. 331-5.
[31] Abete, I., et al., *Association of lifestyle, inflammatory factors, and dietary patterns with the risk of suffering a stroke: A case-control study.* Nutr Neurosci, 2018. **21**(1): p. 70-78.
[32] Margină, D., et al., *Chronic Inflammation in the Context of Everyday Life: Dietary Changes as Mitigating Factors.* Int J Environ Res Public Health, 2020. **17**(11).
[33] Henson, J., et al., *Sedentary time and markers of chronic low-grade inflammation in a high risk population.* PLoS One, 2013. **8**(10): p. e78350.
[34] Ruiz-Núñez, B., et al., *Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context.* The Journal of Nutritional Biochemistry, 2013. **24**(7): p. 1183-1201.
[35] Kasapis, C. and P.D. Thompson, *The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review.* J Am Coll Cardiol, 2005. **45**(10): p. 1563-9.
[36] *Standards of medical care in diabetes--2013.* Diabetes Care, 2013. **36 Suppl 1**(Suppl 1): p. S11-66.
[37] Fedewa, M.V., E.D. Hathaway, and C.L. Ward-Ritacco, *Effect of exercise training on C reactive protein: a systematic review and meta-analysis of randomised and non-randomised controlled trials.* Br J Sports Med, 2017. **51**(8): p. 670-676.
[38] Sallam, N., M. Khazaei, and I. Laher, *Effect of Moderate-Intensity Exercise on Plasma C-Reactive Protein and Aortic Endothelial Function in Type 2 Diabetic Mice.* Mediators of Inflammation, 2010. **2010**: p. 149678.
[39] Cerqueira, É., et al., *Inflammatory Effects of High and Moderate Intensity Exercise—A Systematic Review.* Frontiers in Physiology, 2020. **10**(1550).
[40] Kim, M.K., et al., *Clinical utility of serum beta-2-microglobulin as a predictor of diabetic complications in patients with type 2 diabetes without renal impairment.* Diabetes Metab, 2014. **40**(6): p. 459-65.
[41] Raikou, V.D. and D. Kyriaki, *The relationship between glycemic control, beta2-microglobulin and inflammation in patients on maintenance dialysis treatment.* J Diabetes Metab Disord, 2015. **14**: p. 34.