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Journal of Bioscience and Applied Research
www.jbaar.org

Exploring the Biophysical Mechanisms of Taurine's Effect on Myeloperoxidase Enzymatic Kinetics in Pre-Diabetic and Type 2 Diabetic Patients

Suha A. Muneam[1*], Nada.A. Muneam [2], Mihad Shakir N[3].

Al-Iraqia University/ College of Medicine – Iraq

[1*] Corresponding email[*]: Suha. A.Muneam.
Email: Suha.a.muneem@aliraqia.edu.iq , https://orcid.org/0009-0004-3027-731
Department of Chemistry and Biochemistry, Al-Iraqia University/ College of Medicine -Baghdad, Iraq.

[2]Nada.A. Muneam.
Email: nadalaheeb@gmail.com or nada.a.muneam@aliraqia.edu.iq
https://orcid.org/0009-0007-8397-9187
Department of Physiology and Medical Physics, Al-Iraqia University/ College of Medicine -Baghdad, Iraq.
[3] Mihad Shakir N.
Email: mehadshakr@gmail.com https://orcid.org/0009-0001-0575-4165
Department of Physiology, Al-Iraqia University/ College of Medicine -Baghdad, Iraq.

DOI: 10.21608/jbaar.2023.331983

Abstract

Background: investigate the enzymatic activity of Myeloperoxidase (MPO) in pre-diabetic and diabetic individuals and explore the modulation of this activity by taurine supplementation, considering its potential anti-oxidative properties and the emerging evidence of its role in glucose metabolism. **Methods:** This case-control study was done at the Iraqi University College of Medicine. It used advanced spectroscopic techniques and kinetic modeling to measure the amount of MPO activity in the sera of people who were healthy, pre-diabetic, and diabetic. The Lineweaver-Burk plot derived from the Michaelis-Menten equation was used to ascertain the Km and Vmax of MPO. Taurine inhibition assays were also performed to understand its effect on MPO kinetics. **Results:** The data showed that MPO activity increased significantly from the control group to the diabetic group. This was in line with rising HbA1c levels and BMI, suggesting a link between MPO activity, glycemic control, and obesity. The gender distribution showed no significant deviation, suggesting that the observed enzymatic and metabolic alterations are not gender biased.

Conclusion: The pronounced elevation in MPO activity in diabetic individuals underscores the enzyme's potential significance in glycemic diseases. The results mean that more research needs to be done on how taurine, which is known to have anti-inflammatory and antioxidant properties, could improve MPO activity and possibly restore metabolic homeostasis, opening a new way to treat type 2 diabetes.

Keywords: Myeloperoxidase, Taurine, Type 2 Diabetes, Enzyme Kinetics, Glycemic Control.

Received: October 3, 2023. Accepted: December 10, 2023. Published: December 25, 2023

Introduction

Myeloperoxidase (MPO) stands out as an integral member of the peroxidase enzyme family [1]. Its presence is predominantly noted within specific immune cells, namely neutrophils, monocytes, and macrophages. Beyond these immune cells, other cellular structures in our body also partake in MPO production, indicating its broader physiological significance. Diving deeper into cellular mechanisms, it's fascinating to note that MPO resides in unique compartments called azurophilic granules within these cells [2]. For this purpose, the enzyme uses H_2O_2 in conjunction with halides or other compounds. The whole working mechanism may be thought of as a circular process, with MPO as its starting point [3]. MPO undergoes a transition to an intermediate state, a designated molecule when interacting with H_2O_2. This is where the cycle diverges, with MPO primarily producing HOCl with the help of halides. In contrast, when H_2O_2 is abundant, MPO takes a different route [4, 5]. This alternative pathway involves numerous steps and different chemicals to achieve its result. When MPO takes in an additional electron, it becomes inactive, adding another degree of complication to the situation. However, there are redemptive routes provided by nature; inactive MPO may be converted back into its active form via interactions with oxygen and other mechanisms. In conclusion, MPO's capacity to adapt to surrounding molecules is crucial to its function in guarding the body against potential hazards [6, 7, 8]. The amino acid taurine, or 2-aminomethane-sulfonic acid, plays a crucial role in biology. The Latin word "Taurus," from which the name is derived, gives some idea of the historical background of its discovery. It's a sulfur-rich amino acid found in high concentrations in marine organisms and other animal tissues. On the other hand, its concentration in plants and fungal sources is still rather low. Muscles, the brain, the liver, and the kidneys all contain significant amounts of taurine. Taurine serves a wide variety of vital functions. It mostly contributes to the development of the functions of skeletal muscles, cardiovascular systems, central nervous systems, and the retina. Studies have shown that not getting enough taurine might lead to subpar performance and trigger certain physiological abnormalities. Nutritionally speaking, taurine is a must-have for humans, and it becomes even more so in situations when the liver is unable to produce enough of it on its own [9, 10, 11, 8].

Taurine may interact with proteins on a molecular level, mostly because of its amine ($NH3^+$) group. Methionine metabolism, bile acid production, intracellular transport systems, and general Sulphur metabolism are just a few of the many metabolic pathways in which it plays a role. Methionine, a source of Sulphur, is the first step in the production of homocysteine, from which taurine may be synthesized in many species. This molecule binds to serine and triggers the production of cysteine. The production of taurine involves a series of oxidation reactions: cysteine is converted to cysteine sulfonic acid by the enzyme cysteine dioxygenase (CDO). In the next enzymatic processes, guided by cysteine sulfonate decarboxylase (CSD) and hypotaurine dehydrogenase, hypotaurine, and then taurine are formed [12, 13, and 14]. Understanding the pace and type of enzyme-catalyzed processes is made possible via the study of enzyme kinetics. In most cases, a clear rectangular hyperbola is seen when graphing the starting velocity of an enzyme versus its substrate concentration. The increase in enzyme activity is attenuated and eventually reaches a plateau as the substrate concentration rises. In contrast, the reaction rate in uncatalyzed reactions continues increasing with increasing reactant concentration [15]. An increase in substrate concentration causes a linear or first-order increase in the rate of an enzyme-catalyzed reaction at low substrate concentrations. In contrast, increasing the concentration of the substrate does not significantly increase the rate of the enzyme-catalyzed reaction at extremely high concentrations. Zero-order kinetics describes this situation. It seems that the substrate concentration has little effect on the rate of this

reaction. The Michaelis-Menten model, named after the eminent German scientist Leonor Michalis [16, 17, 18] is one of the most respected frameworks in the study of enzyme kinetics. An equation detailing the relationship between reaction velocity (V) and substrate concentration (S) is illustrative of this concept. Two key elements are highlighted in the well-known Michaelis-Menten equation: the Michaelis constant (Km), which represents the enzyme's affinity towards its substrate, and the maximum rate (V_{max}) obtained by the system at maximal substrate saturation. The substrate concentration at which the response velocity (V_{max}) is 50% of its highest value (Km) is particularly relevant. It is interesting to note that Km is invariant concerning enzyme concentration. In this framework, the rate of conversion of substrate to product is denoted by the velocity (v), which is often expressed as a mole per minute (mM) of product. The Lineweaver-Burk plot (also known as the double reciprocal plot) is a graphical representation of the Lineweaver-Burk equation for enzyme kinetics used in biochemistry. This graph was developed in 1934 by Hans Lineweaver and Dean Burk to compare the starting reaction rate (VO) to the substrate concentration [S]. The hyperbolic curve has a very little slope at large substrate concentrations, making it difficult to identify the Vmax accomplishment. The Lineweaver-Burk plot, however, is a linear graph that results from contrasting 1/V with 1/[S]. Extracting data like Km and Vmax and understanding the mechanism of enzyme inhibitors is a breeze with the help of this visual aid [19]. Type 2 diabetes mellitus is becoming increasingly common, and it is a chronic, debilitating condition that has serious consequences [20]. A combination of decreased pancreatic insulin production and the development of resistance to the activities of insulin on its target tissues leads to the onset of type 2 diabetes (T2D), a complicated metabolic condition. The accumulation of ectopic fat in vital organs such as the liver, pancreas, and skeletal muscles is central to the pathogenesis of T2D. Insulin resistance in these organs and the dysfunction of pancreatic beta cells are both exacerbated by the buildup of fat. Hyperglycemia (high blood sugar) is a complication that occurs in people with T2D [21, 22, and 23]. The gradual development of T2D is a defining feature of the disease. Oftentimes, the typical signs of diabetes are ignored because the steady increase of hyperglycemia in the early stages is not severe enough. Therefore, many people go undetected for a long time. Undiagnosed people have an increased risk for both macrovascular and microvascular problems, even if they do not have any noticeable symptoms. In addition, some people with T2D may have normal or even increased insulin levels, but their raised blood glucose concentrations imply that their insulin values would be much higher if their beta cells were functioning normally. The failure of insulin production to adequately overcome insulin resistance is shown by this difference. While it is possible to improve insulin sensitivity with measures like weight reduction and medication, this improvement is usually only temporary [24, 25, 26]. The possibility of developing T2D is increased by several risk factors. Age, excessive weight, and lack of physical activity are just a few examples. T2D is more common in women who have had gestational diabetes mellitus (GDM). High blood pressure, abnormal lipid profiles, and a strong hereditary propensity, particularly in close relatives, are additional risk factors. It's worth noting that, unlike type 1 diabetes, the genetics underlying T2D are still poorly understood. It is critical to do antibody testing to rule out a diagnosis of type 1 diabetes in people who appear with abnormal risk profiles for T2D or who develop the illness at a younger age [27, 28]. Myeloperoxidase (MPO) enzymatic activity in the sera of pre-diabetic and type 2 diabetic persons will be measured using state-of-the-art spectroscopic methods. On top of that, we use cutting-edge kinetic modeling to figure out what these two factors do to MPO's Michaelis constant (Km) and maximal rate of enzymatic reaction (V_{max}). Focusing on Km and

Vmax, this study aims to determine how the addition of taurine affects these kinetic parameters of MPO from a molecular dynamics standpoint. As new data suggests that taurine has a function in glucose metabolism, it seemed a natural choice.

Materials and Methods

This case-control study was conducted in the Department of Chemistry, Biochemistry, and Physiology, Iraqi University College of Medicine. The study was executed during the term from December 2022 to March 2023. The Ethics Committee of the College of Medicine at Iraqi University duly approved the research protocol for this study, in compliance with the stipulations outlined in the 2013 Helsinki Declaration. All study participants provided informed consent before their involvement.

Quantification of MPO Activity:

Formula:

$$MPO\ activity\ (U/L) = \frac{\Delta A}{11.3 \times b} \times \frac{V_{Total}}{\frac{V_{Sample}}{V_1} \times V_2} \times 1000 \times f$$

- MPO Activity is in units per liter (U/L).
- ΔA represents the change in absorbance.
- V_{Total} Is the total volume.
- V_{Sample} Is the sample volume.
- f Is a factor.
- b, V_1, V_2 are constants.[29]

Preparation of Substrate Diluted Concentrations:

Substrate concentrations are prepared by diluting Reagent 7 (200mM) with distilled water. As indicated in Table

Inhibitor Preparation:

A 103.5 mM inhibitor solution is prepared by dissolving 64.7mg of Taurine powder in 3 mL of distilled water. Add 771µl of sodium hypochlorite (5%) and adjust the total volume to 5 ml, this concentration was chosen based on preliminary assays demonstrating its efficacy.

Assay Procedure:

1. Combine 0.45 mL of sample with 0.45 mL of Reagent 2 solution and mix thoroughly.
2. Add 0.1 mL of Reagent 3 solution.
3. Incubate at 37°C for 15 minutes.
4. Follow the steps detailed in Table 1.

Table 1 Assay Procedure

	Control tube	Sample tube
Double distilled water (mL)	3	
Sample (mL)	0.2	0.2
Reagent 5 (mL)	0.2	0.2
Chromogenic agent (mL)		3
Mix thoroughly,	incubateat37℃ water bath for	30 min
Reagent 8 (mL)	0.05	0.05

Determination of Km and V_{max}:

To ascertain the Km and Vmax of MPO in patient sera, we utilize five varying substrate concentrations of H_2O_2. We aim to ascertain the Km and V_{max} of MPO in patient sera. The Lineweaver-Burk plot, derived from the Michaelis-Menten equation, is used. It's acknowledged that the Lineweaver-Burk plot can distort data due to transformation; thus, data integrity will be ensured by repeated measurements.

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times 1/\{S\} + \frac{1}{V_{max}}$$

V: is the reaction velocity.

V_{max}: is the maximum reaction velocity.

Km: is the Michaelis constant (substrate concentration at half-maximal reaction velocity) [S]: is the substrate concentration [16].

Km and Vmax Calculation:

Utilize the Lineweaver-Burk plot. The X-axis intercept indicates -1/Km, and the Y-axis intercept represents $1/V_{max}$.

Km = $-\frac{1}{point\ of\ intercept\ straight\ line\ with\ Xaxis}$

And

Vmax = $\frac{1}{point\ of\ intercept\ straight\ line\ with\ Yaxis}$

Assay Procedure with Taurine Inhibition:

The assay procedure mirrors the earlier steps with the addition of a 0.3 mM concentration of the taurine inhibitor. This concentration was chosen based on its efficacy as observed in preliminary results. For detailed steps, refer to the assay procedure section above.

Validation & Controls:

1- Negative controls (without substrate) were used to ensure specificity.
2- Positive controls from known MPO activity samples were used for assay accuracy.
3- Triplicates were performed for each sample to ensure reproducibility.
4- Inter-day and intra-day variations were measured for assay precision.

Results and Discussion:

Sample period: During the examination, 90 patients were studied. These samples included 30 people with type 2 diabetes, 30 were pre-diabetic subjects, and 30 were age- and gender-matched healthy controls (Figure 1).

Activity, MPO, HbA1c, BMI, age, and gender are compared between control, pre-diabetic, and diabetic groups in the table. The study analyses data using mean ± SD for continuous variables and frequencies and percentages for categorical variables (gender). The P-values show the statistical significance of the three groups' differences. Activity There is a steady rise in mean activity MPO from the control group to the diabetes group The P-value (p <0.001) shows a significant difference between them and the activity there is a steady rise in mean activity MPO from the control group to the prediabetes group The P-value (p = 0.0002*) of shows a significant difference between them. The mean HbA1c level was found to grow gradually from the control group to the diabetes group (%). Since HbA1c is a marker of long-term blood glucose management, the P-value of <0.001suggests a very significant difference between the three groups. The mean BMI rose significantly from the control to the diabetes group, with a P-value of <0.001. A higher BMI may lead to pre-diabetes and diabetes. The mean age rises somewhat from the control group to the diabetes group, although the change is not statistically significant (P-value = 0.2NS).

The asterisk (*) adjacent to P-values shows significant differences (p<0.05) across the three groups for activity MPO, HbA1c, and BMI, but not for age or gender.

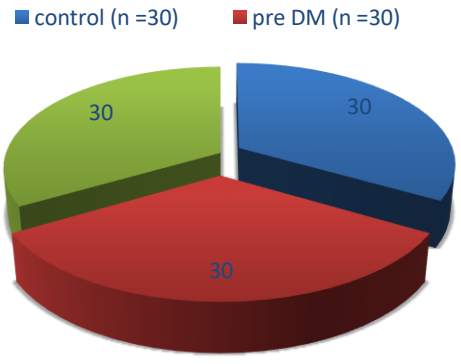

Figure1 : Pie- charts showing the distribution of the studied groups.

Table 2: Demographic distribution of the three studied groups:

Tested value	Control(n=30)	Pre-Diabetic (n=30)	Diabetic (n=30)	P
	Mean ±SD	Mean ±SD	Mean ±SD	
HbA1c (%)	4.84±0.29	5.93±0.23	8.87±1.9	<0.001
BMI (kg/m²)	24.79±1.62	31.94±6.04	32.66±5.92	<0.001
Age (years)	45.63±8.81	46.23±11.11	49.90±9.71	0.2 NS
Gender Male female	 M=13(43.4%) F = 17 (56.6%)	 M=16 (53.4%) F=14 (46.6%)	 M=14 (46.6%) F=16 (53.4%)	0.21 NS
*: (p<0.05)				

Table (3) Comparison activity of serum MPO between study groups:

Activity MPO (IU/l))	Control(n=30)	Pre-Diabetic (n=30)	Diabetic (n=30)	P
Mean ±SD	137.52±29.03	185.99±41.95	----------	0.0002*
Mean ±SD	137.52±29.03	----------	252.04±59.77	<0.001
Mean ±SD	----------	185.99±41.95	252.04±59.77	<0.001
*: (p < 0.05)				

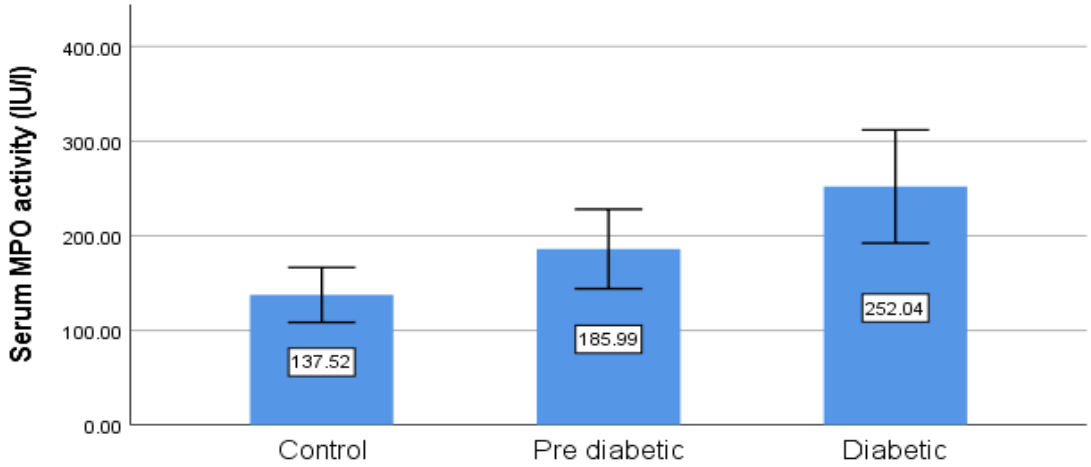

Figure 2: mean and standard deviation of activity MPO in control, P-DM, and DM.

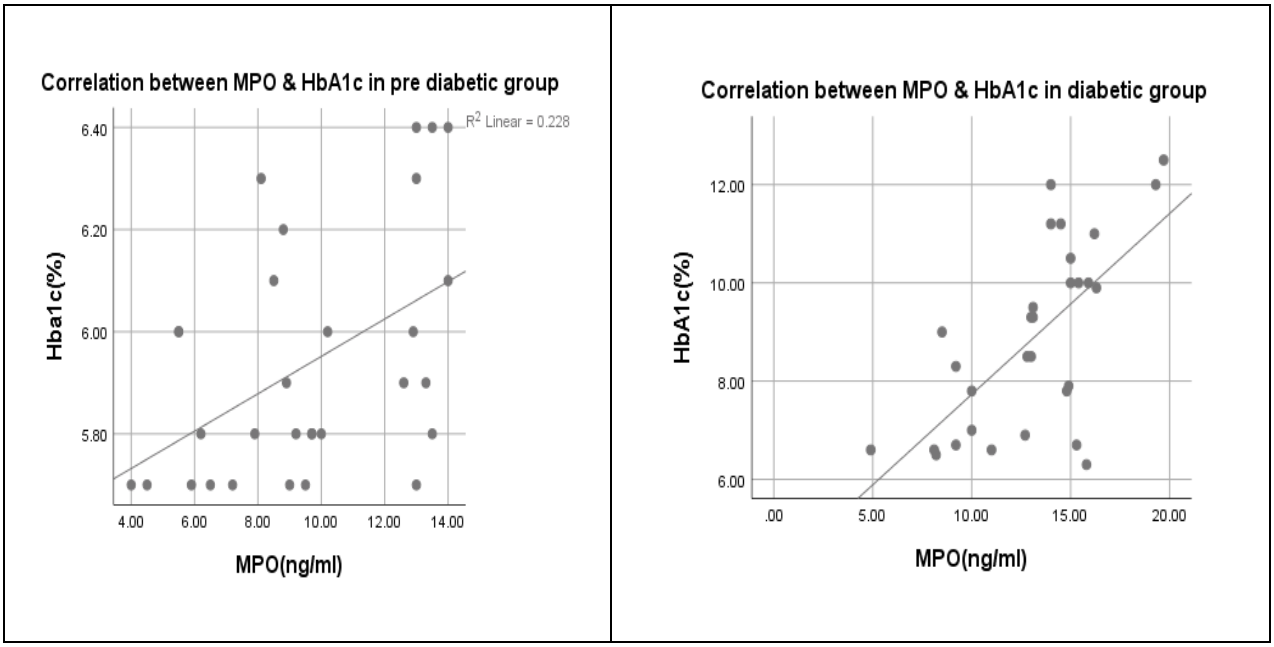

Figure 3: Correlation between MPO &HbA1c in P-DM, and DM

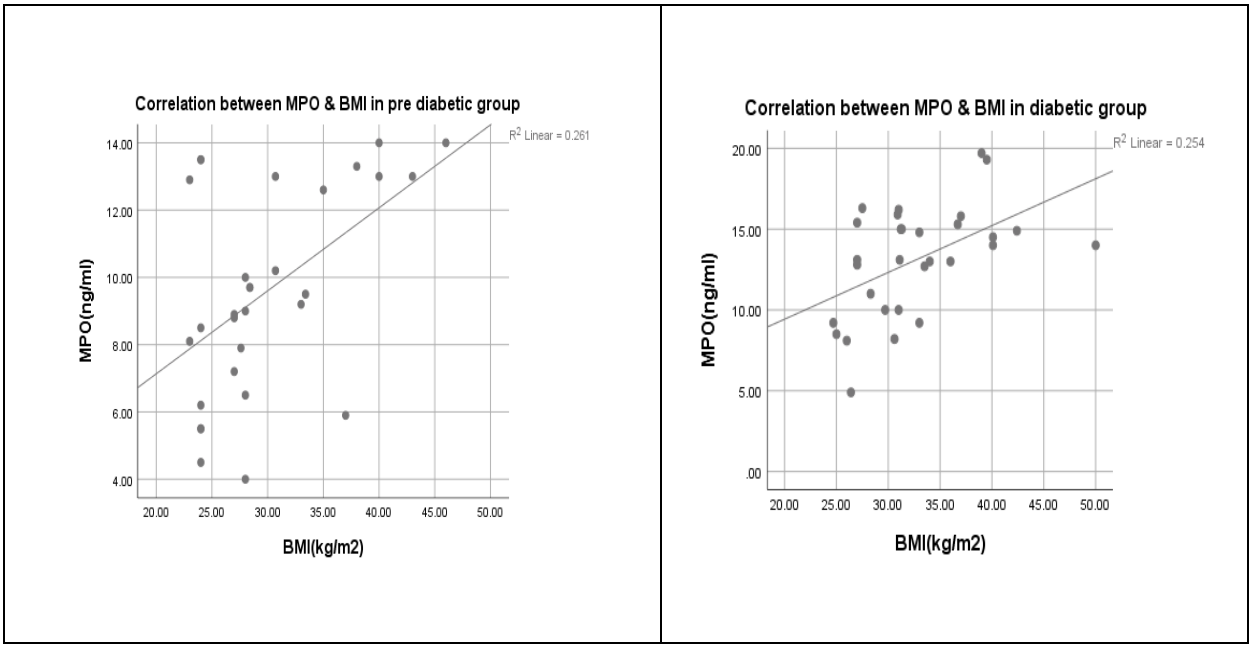

Figure 4: Correlation between MPO &BMI in P-DM, and DM

Our study proved the rising activity of MPO in both healthy individuals and those with diabetes, highlighting the enzyme's possible significance in the development and evolution of glycemic diseases. Table 2 shows the demographic distribution of the three studied groups. High levels of oxidative stress and inflammation are commonly linked with type 2 diabetes, which may explain the dramatic increase in MPO activity in diabetics. As shown in Figure 2 [30], the progressive increase in MPO activity across the groups, with the most pronounced elevation in the diabetic group, suggests a potential correlation between impaired glycemic control and enhanced MPO activity [31]. This is consistent with the literature linking MPO to inflammatory and oxidative processes, which are hallmark features of diabetes. The HbA1c levels, an established indicator of long-term glycemic control, validate the categorization of individuals into control, pre-diabetic, and diabetic groups [32]. The exponential rise in HbA1c levels from control to diabetic individuals is emblematic of deteriorating glycemic control, warranting further exploration of how taurine supplementation might mitigate this rise and possibly restore the enzymatic balances shown in Figure 3 [33]. The data portrays a discernible trend of increasing BMI from control to diabetic individuals. This trajectory, coupled with escalating MPO activity, accentuates the possible interplay between obesity, inflammation, and diabetes. Investigating the potential modulatory effects of taurine on BMI and its subsequent impact on MPO activity could unveil novel therapeutic avenues. As shown in Figure 3 [34], although not statistically significant, the slight age increment across the groups may hint at the accruing risk of glycemic disorders with advancing age [35]. The gender distribution does not exhibit a significant deviation, suggesting that the observed enzymatic and metabolic alterations are not gender-biased [36]. The core of this research pivots around understanding how taurine supplementation modulates MPO activity in pre-diabetic and diabetic states. The conspicuous escalation in MPO activity coupled with adverse glycemic indices necessitates a thorough investigation into how taurine, known for its antioxidant and anti-inflammatory properties, could ameliorate MPO activity, potentially reinstating metabolic homeostasis.

Conclusion

The observed increase in myeloperoxidase (MPO) activity among persons with diabetes highlights the potential importance of this enzyme in the context of glycemic disorders. The results of this study indicate a need for more investigation into the possible effects of taurine, a compound recognized for its antioxidant and anti-inflammatory characteristics, on MPO activity and its ability to restore metabolic balance. These findings suggest a new and promising approach to treating type 2 diabetes.

Acknowledgment

The Iraqi University College of Medicine for their essential support in this research.

Disclaimer: None
Conflict of Interest: None
Funding Disclosure: None

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