



Effect of Fasting during Ramadan on Inflammatory Markers in Type II Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. This study is an original research conducted by WSE for master degree in physiology under supervision of Associated Professor author AHE, with assistance of other co-authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Studies have recognized an association between inflammation and T2DM and its complications. Fasting is used as a medical treatment for a variety of ailments. For all healthy adult Muslims, fasting throughout Ramadan is a religious obligation.

Aims: The goal of this study was to back up a recent discovery about the regulatory effects of Ramadan fasting on inflammation in T2D patients.

Study Design: A cross-sectional study was conducted.

Date and Location of Study: The study was conducted in Benghazi Diabetes Center in April 2019, two weeks before the holy Ramadan. Methodology: Blood samples for inflammatory markers (WBC, hs-CRP, Fibrinogen) and glycemic control, fasting blood sugar (FBS), and HbA1C were collected from 72 patients with type 2 diabetes from Benghazi diabetic clinic two weeks before the holy Ramadan fasting.

Following up (at least after the first ten days of Ramadan fasting), another sample of the same Laboratory testing was taken. The difference was tested using a paired T-test, with a P-value of less than 0.05 being considered significant. The trial was completed by 58 individuals, and 14 patients were dropped out.

Results: Our findings revealed that there was no significant difference in FBS and hs-CRP levels before and during Ramadan fasting, but Fibrinogen levels were significantly different ($P = <0.001$)

before and during Ramadan fasting, with Fibrinogen levels being higher during fasting. Interestingly, WBC levels were lower during than before fasting, with the difference being slightly significant ($P = .06$). When it comes to differential counts (neutrophils $P = .009$, lymphocytes $P = .746$), The hemoglobin ($P < 0.001$) and platelets ($P = .028$) were significantly lower during Ramadan fasting compared to pre-Ramadan level.

During Ramadan fasting, the mean value of fasting blood sugar, hs-CRP, and WBC was lower in a group of patients with HbA1C less than 7 compared to a group with HbA1C greater than 7, although the difference was not statistically significant. Fasting blood sugar during Ramadan was strongly connected with hs-CRP (Pearson correlation = 0.289, $P = .032$), but not with HbA1C, even after adjusting for age, waist circumference, and BMI.

The interval of diabetes was not related to any of the inflammatory indicators examined in this investigation.

Conclusion: Except for WBC count, fasting Ramadan (first ten days) had no significant effects on most inflammatory indicators.

Keywords: Ramadan fasting; inflammatory markers; regulatory effect; type2 diabetes mellitus.

1. INTRODUCTION

Diabetes Mellitus (DM) in adults is a global health problem, its rate has generally increased worldwide [1]. According to the IDF, there were 399,200 cases of diabetes in Libya in 2022 [2].

DM is a group of metabolic diseases, characterized by hyperglycemia due to defects in insulin secretion, insulin action or both [3]. There are many types of DM. Type 1 diabetes mellitus (T1DM) or insulin dependent diabetes mellitus (IDDM). T1DM is caused by the absolute deficiency of insulin secretion, while Type 2 diabetes mellitus (T2DM) or non-insulin dependent diabetes mellitus (NIDDM) is caused by the combination of resistance to insulin action and inadequate compensatory insulin secretory response [4].

Several theories have been proposed to explain how hyperglycemia leads to end organ damage. These include the formation of advanced glycosylation end products (AGES), glucose metabolism via sorbitol pathway, activation of protein kinase C, and increased flux through hexosamin pathway [5-7].

Inflammation plays important role in pathogenesis of many diseases like atherosclerosis, obesity, type 2 diabetes, asthma, inflammatory bowel diseases, neurodegenerative diseases, rheumatoid arthritis and cancer.

Inflammatory markers including specific interleukins, tumor necrosis factor-alpha (TNF- α), C- reactive protein (CRP), tissue plasminogen activator (tPA), haptoglobin and fibrinogen levels are found to be increased in type 2 DM (T2DM)

patients probably contributing to the pathogenesis of insulin resistance [8,9].

Fasting can regulate these cytokines, chemokines, and other inflammatory mediators and, reduce the oxidative stress, enhance insulin sensitivity [10].

According to the results of studies, Ramadan fasting not only regulates the biochemical and physiological processes of the body, but it also elicits potent anti-inflammatory responses in both human and animal models, Intermittent fasting during Ramadan down regulates the expression of pro-inflammatory cytokines, chemokines and other pro-inflammatory mediators such as IL-2, IL8 and TNF- α [11,12]. consequently, this study aimed to find the regulatory effects of Ramadan fasting on the level of inflammatory markers in patients with type 2 diabetes mellitus.

2. METHODOLOGY

2.1 Subjects

The study included 72 patients with type 2 diabetes two weeks before Ramadan, however only 58 completed the study and returned for follow-up during Ramadan (as a minimum after the primary 10 days of Ramadan).

The rate of dropout was 14 patient (19.4%). Verbal and written informed consent were obtained, interviewer-administered questionnaire was used to obtain information about phone numbers, duration of diabetes and type of treatment of diabetes, last normal RFT (renal function test), new level of HbA1C The participant's age was (35-84years), twenty-one

were females and thirty-seven were males. The Body mass index was calculated by using Queenlet's Index i.e. Weight (kg) / Height (m²).

We measured waist and hip circumferences, and waist-to-hip circumference ratio (W-HR) by using a tape measure. We exclude the patients who have a history of type 1 diabetes, smoking, acute or chronic inflammation (rheumatoid arthritis-bowel disease), recent infections in the last 3days, taking NSAID (Non-Steroidal Anti-Inflammatory Drug), aspirin intake, fasting Rajab, Shaban lunar months, endocrine and neuroendocrine problem, following special nutritional regimen, consumption of the nutrient supplement, cancer, renal disease.

Also, we excluded obese persons (BMI great than 30 kg/m²), pregnancy, lactation, and the expected date of the menstrual cycle in the first 10 days of Ramadan.

2.2 Estimation of HbA1c

For diabetic patients, HbA1c was estimated by Immunochemical Assay. HbA1c levels were measured according to National Glycohemoglobin Standardization Program (NGSP) guidelines and standardized to Diabetes Control Complication Trial (DCCT) assay, the normal value of HbA1c for adults equal to 4-5.6%, increased risk for diabetes (prediabetes): 5.7-6.4%, Diabetes <7 % good glycemic control, >7% poor glycemic control.

2.3 Estimation of white Blood Cells (Complete Blood Picture)

The white blood cells (WBC) were estimated by Immunochemical Assay. A normal WBC count According to the University of Rochester Medical Center (UMRC). [https:// www.urmc.rochester.edu/encyclopedia/content.aspx?contentid=167&contentid=white_cell_count](https://www.urmc.rochester.edu/encyclopedia/content.aspx?contentid=167&contentid=white_cell_count).

These are the normal ranges of WBCs per microliter of blood (µL):

According to the Leukemia & Lymphoma Society (LLS) the normal percentages of the types of WBCs including Neutrophil, Lymphocyte, Basophil, Eosinophils and Monocytes in healthy subject were illustrated in Table 2 and the link below. <http://www.lls.org/managing-your-cancer/lab-and-imaging-tests/understanding-blood-counts>

Table 1. Normal range of WBC (white cell count)

Age range	WBC count (per mL of blood)
Newborns	9,000 to 30,000
children under 2	6,200 to 17,000
children over 2 and adults	5,000 to 10,000

Table 2. The normal percentages of the types of WBCs:

Neutrophil	55 to 73 percent
Lymphocyte	20 to 40 percent
Eosinophil	1 to 4 percent
Monocyte	2 to 8 percent
Basophil	0.5 to 1 percent

2.4 Estimation of Fasting Blood Sugar (FBS)

For patients in our study fasting blood sugar was estimated by Immunochemical Assay Normal variety of the lab (70-110 mg/dl).

2.5 Estimation of High Sensitive- C Reactive Protein (hs-CRP)

The high sensitive-C reactive protein (hs-CRP) was estimated by Immunochemical Assay. The normal variety of the lab (0.0-5 mg/dl).

2.6 Estimation of Fibrinogen Level

For patients in our study fibrinogen was estimated by Immunochemical assay normal range of the lab (2.5-4 g/L). All investigations before and during Ramadan were done in Alhia lab medical analysis estimated by Immunochemical Assay using COBAS INTEGRA 400 PLUS (Roche).

2.7 Statistical Analyses

The data were collected, organized, tabulated, and then statistically analyzed by SPSS (Statistical Package of Social Sciences, version 20).

Quantitative data were expressed as mean ±SD and analyzed by an independent sample t-test for continuous variables. the mean of biochemical and inflammatory indicators before and during Ramadan fasting in the study subjects, and the anthropometric data of the patients in the study, were estimated. The paired

t-test was used to estimate the degree of significance of the difference between biochemical and inflammatory markers before and during Ramadan fasting.

3. RESULTS

A total of 72 patients with type 2 diabetes were engaged before the holy Ramadan fasting month, of which only 58 accomplished the study and came for follow-up during Ramadan (at least after the first 10 days fast of Ramadan). Fig 1.

Represent the percentage of patients who complete the study.

The mean age of the patients in the study was 56 +/- 10 SD, Twenty one (36%) were females and 37(64%) were males. The mean duration of diabetes was 11+/-8 years, the mean HbA1C for the study sample was 9+/- 2 %, the mean duration of fasting was 12+/- 4 days. Fig 2: show the percentage of patients according to gender.

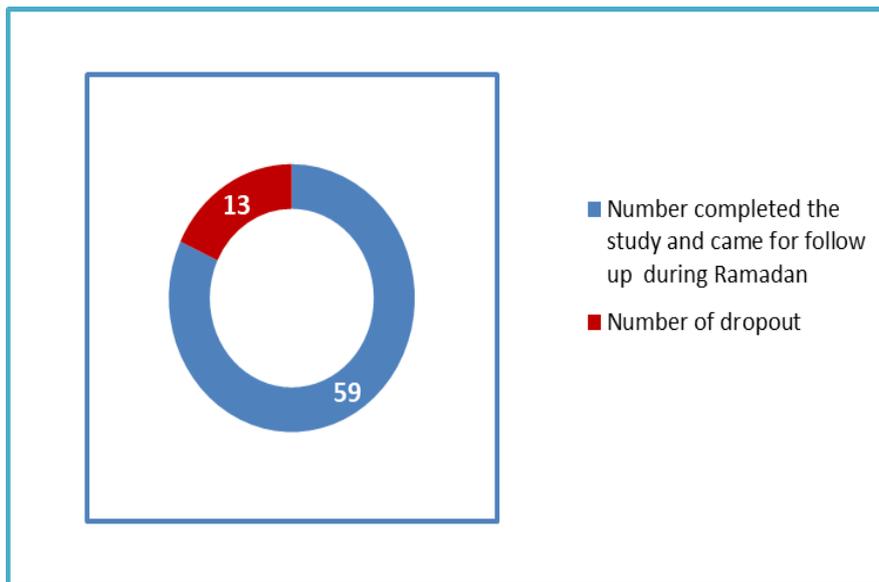


Fig. 1. The percentage of patients who complete the study

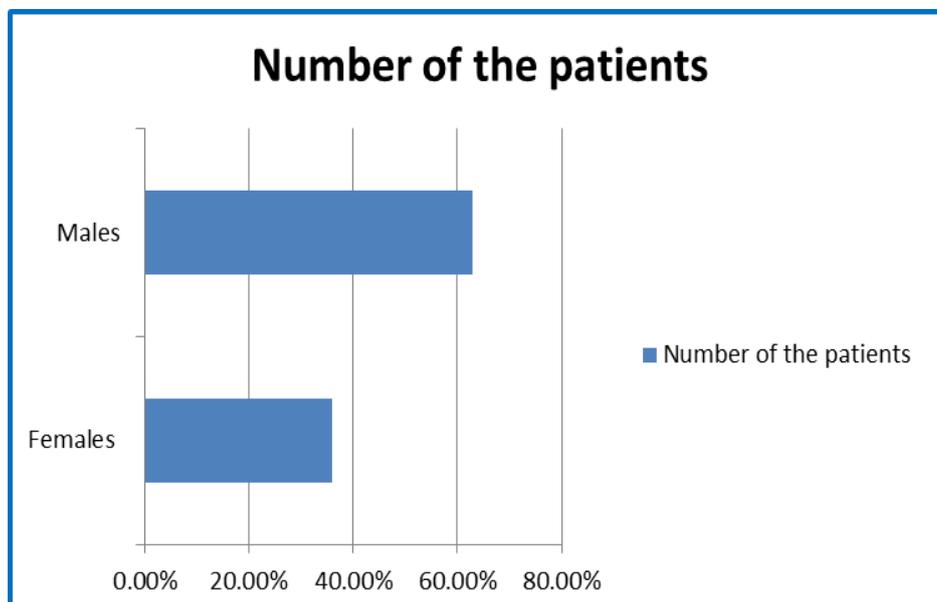


Fig. 2. The percentage of patients according to gender

The anthropometric data of the patients enrolled in the study were illustrated in Table 3.

The mean value of the fasting blood sugar and biochemical and inflammatory markers before Ramadan (FBS= 182+/- 0.82, Fibrinogen level =2.1+/- 0.45, hs-CRP=4+/-4, WBC=8.5+/-2.4) and during Ramadan (FBS= 204+/-72, Fibrinogen level= 3+/-0.65, hs-CRP= 4.3+/-4.1, WBC= 8.1+/-2.14) were illustrated in Fig 3.

Table 3. The anthropometric data of the patients in the study

Anthropometric parameter	Mean+/-SD
weight of the patient	71.2+/- 9.1
Height of the patient	166.3+/-10
waist of the patient	92.03+/- 8
BMI	26+/- 2.1

The paired T-test show no significant difference in the fasting plasma glucose and hs-CRP before and during Ramadan fasting while the Fibrinogen level was strongly statistically different (P = <.001) before and during Ramadan fasting, being higher during the fasting state. Interestingly WBC level was lower during fasting compared to before fasting and the variance was slightly significant (P =.06). Tables 4 and 5 show these findings.

Hemoglobin (13.2+/- 1.4, 14+/- 1.5, P < .001), neutrophils (5+/- 1.5, 5.3+/- 2, P = .009) and platelets (254.4 +/- 78.1, 266+/- 81, P = .03) significantly lower during Ramadan fasting compared to pre-Ramadan level, while no significant change in lymphocyte count (3+/-0.8, 3+/-0 .9, P = .8). Figs 4, 5.

Despite numerically the mean value of fasting blood sugar, hs-CRP, and WBC during Ramadan fasting were lower in a group of patients with HbA1c less than 7 compared to a group whose HbA1c was more than 7 but the difference was statistically not significant Table 6.

When controlling for age, waist circumference and BMI , HbA1c was not correlated with any of inflammatory indicators in the study (Fibrinogen , hSCRp , WBC count) while fasting blood sugar during Ramadan was positively correlated with hs-CRP (Pearson correlation = 0.29 , P = .03).

It's no surprise to see correlation among WBC and other inflammatory markers during Ramadan fasting, as it strongly positively correlated with fibrinogen level (Pearson correlation = 0.37 . P = .005), with hsCRP (Pearson correlation = 0.27 , P = .05) . The duration of diabetes was not correlated with any of the inflammatory markers tested in the study i.e fibrinogen, hs-CRP level, and WBC.

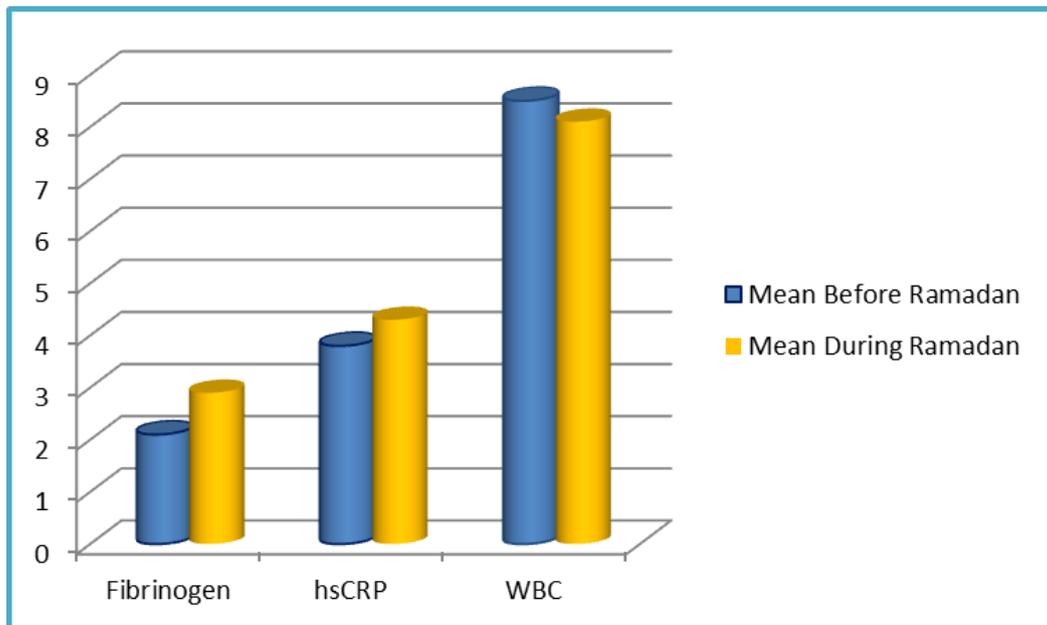


Fig. 3. The mean of inflammatory indicators namely (Fibrinogen level, hs-CRP. WBC) before and during Ramadan fasting

Table 4. Show the paired T test for biochemical and inflammatory markers before and during Ramadan fasting

		Paired Samples Statistics			
		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Fasting blood sugar before fasting Ramadan	182	58	83	11
	Fasting blood sugar during fasting Ramadan	204	58	72	9.39
Pair 2	Fibrinogen level before fasting Ramadan	2.09	58	.45	.058
	Fibrinogen level during Ramadan fasting	3	58	.65	.08
Pair 3	hsCRP before fasting Ramadan	3.82	58	4	.47
	hsCRP during fasting Ramadan	4.32	58	4.17	.54
Pair 4	WBC before fasting Ramadan	8.47	58	2.35	.30
	WBC during fasting Ramadan	8.06	58	2.14	.28

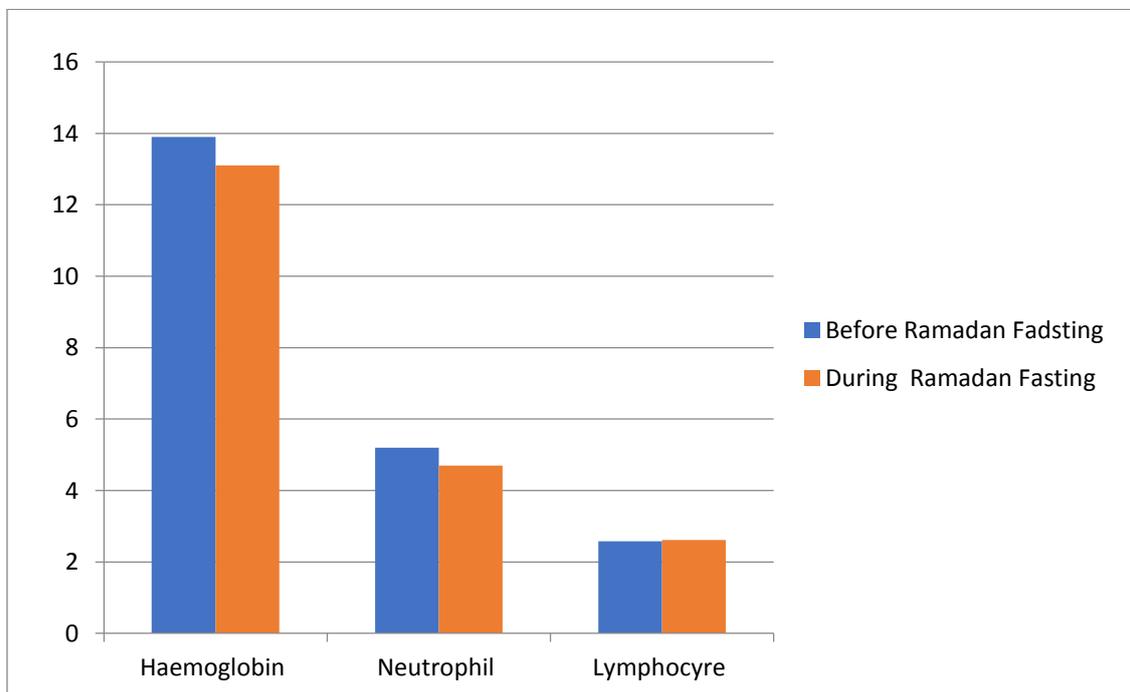


Fig. 4. Demonstrate Compared Mean for Hemoglobin, Neutrophil, Lymphocyte Count Before and During Ramadan Fasting

4. DISCUSSION

Our result suggested Ramadan fasting led to some beneficial changes in inflammatory markers in subject of type 2 diabetes.

The analysis revealed that WBC level was lower during Ramadan fasting compared to pre-

Ramadan level and the difference was marginally significant This mainly driven by decrease in neutrophils significantly, while no significant change in lymphocyte count.

The hemoglobin and platelets were significantly lower during Ramadan fasting compared to pre - Ramadan level. The mean value of fasting blood

Table 5. The Degree of significance of difference between biochemical and inflammatory markers before and during Ramadan fasting

		Paired Differences					T	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	FBS before fasting Ramadan - FBS during fasting Ramadan	-22	101	13.21	-48.07	5	-2	57	.107
Pair 2	Fibrinogen before fasting Ramadan - Fibrinogen during Ramadan fasting	-.86	.78	.10	-1.06	-.65	-8.3	57	.000
Pair 3	hsCRP before fasting Ramadan - hsCRP during fasting Ramadan	-.500	4	.46	-1.42	.42	-1.0	57	.283
Pair 4	WBC before fasting Ramadan – WBC during fasting Ramadan	.40	2	.21	-.02	.83	2	57	.064

Table 6. Illustrate the consequence of glycemic control on the mean of the FBS, hs-CRP, Fibrinogen level, WBC count throughout Ramadan fasting and their degree of significance

	Mean HbA1C	N	Mean	Std. Deviation	Sig. (2-tailed) P value
Fasting blood sugar during fasting Ramadan	>= 7.00	47	208	76.3	.33
	< 7.00	11	184	44	
hsCRP during fasting Ramadan	>= 7.00	47	5	4.31	.4
	< 7.00	11	3.26	3.47	
Fibrinogen level during Ramadan fasting	>= 7.00	47	3	.63	.2
	< 7.00	11	3.21	.70	
WBC during fasting Ramadan	>= 7.00	47	8.22	2.23	.3
	< 7.00	11	7.40	2	

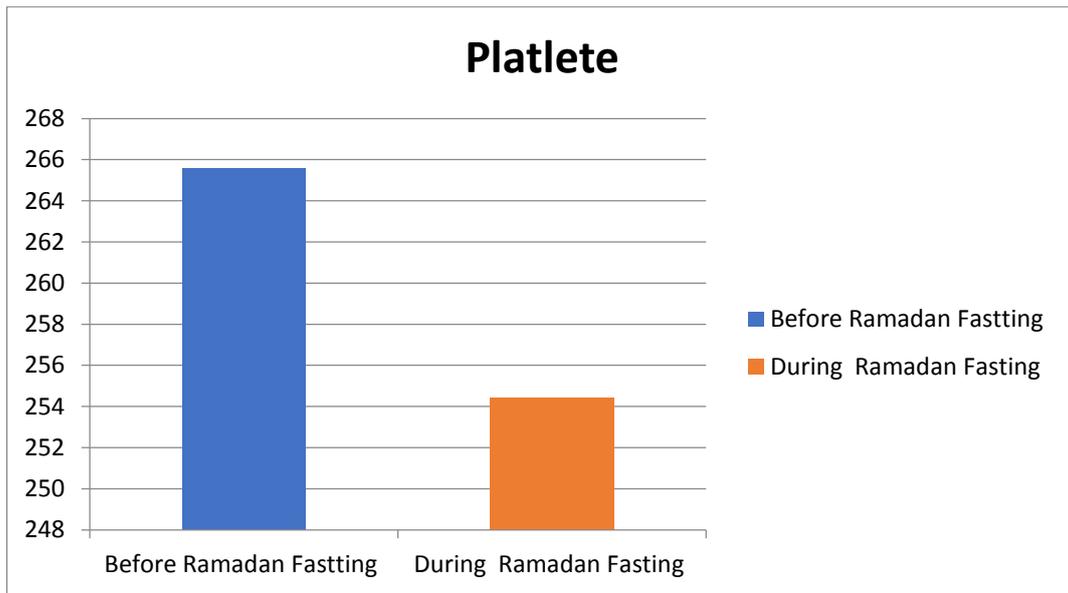


Fig. 5. Demonstrate Compared Mean for Platelet Count Before and during Ramadan Fasting

sugar, hsCRP, and WBC during Ramadan fasting were lower in a group of patients with HbA1c less than 7 compared to a group whose HbA1c more than 7 but the difference was statistically not significant. Our observations are in agreement with [13,14].

Also Abdel-Fattah (1994) in his study in Saudi Arabia reported a significant decrease in WBC number [15].

While Hosseini, et al. Sarraf-Zadegan, et al. showed no changes in WBC count or any other hematological parameters throughout Ramadan [16,17].

In contrast, a study by Bouhlel, et al. (2006) shows a significant increase in Hb and HCT. And Nematy, et al. [18,19] reported a significant increase in WBC, RBC and platelet (PLT) counts after fasting in Ramadan.

Controversial results obtained from different studies could be attributed to several factors, Ramadan can occur at any time of the year marking the duration of fasting differ between 11-18 hours which effect on the individuals' lifestyle such as sleep duration and their physical activities as important factors influence on the inflammatory biomarker [11,20].

Another potential hypothesis may explain variation on finding would be considerable variation in habitual dietary lifestyle that might involve in onset of inflammation.

It should be considered that humidity and temperature in different seasons in which Ramadan fall are potential confounders in fasting study.

While our observation about the fibrinogen level was strongly statistically different before and during Ramadan fasting being higher during fasting state may be because of hyperglycemia. Hyperglycemia has pro inflammatory consequences, including increases in levels of factor VIII and Von Willebrand factor and impaired fibrinolysis and increase pro inflammatory cytokines. This result was in agreement with Ebrahimi, et al. [21].

There is no significant difference in the fasting plasma glucose and hsCRP before and during Ramadan fasting, this in accordance with result of Khafaji et al. [22]. while was not in agreement with other study done by Askaria V.R and their colleagues (2016) which demonstrate reduction of hsCRP level during Ramadan fasting [23].

The duration of diabetes was not correlated with any of the inflammatory markers which tested in the study i.e fibrinogen, hsCRP level and WBC.

5. CONCLUSION

Fasting Ramadan (first 10 days) doesn't show significant improvement in most inflammatory markers except WBC count.

5. LIMITATION

The limitation of the study was inadequate sample size of 58 and the males number more than females for subset analyses of the differential effects of Ramadan on gender, age group, ethnicity and other population differences

ETHICAL APPROVAL

"All authors hereby declare that the study have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki." Benghazi university and The Libyan Commission for Scientific Research. www.aonsrt.ly. Approval number 1860/21

CONSENT

written informed consent were obtained, interviewer-administered questionnaire was used to obtain information about phone numbers, duration of diabetes and type of treatment of diabetes, last normal RFT (renal function test), new level of HbA1C

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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