

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

ABSTRACT:

Background: Studies have recognized an association between inflammation and T2DM and its complications. Fasting is recommended as a medical treatment for various conditions. Fasting in the month of Ramadan is the religious duty of all healthy adult Muslims.

Aims: This study aimed to confirm a recent finding regarding the regulatory effects of Ramadan fasting on inflammation in patients with T2D.

Study Design: Across sectional study.

Study Date and Place: study was conducted April 2019 two weeks before holey Ramadan in Benghazi diabetes center.

Methodology: We Recruited 72 patients with type 2 diabetes from Benghazi diabetic center two weeks before holey Ramadan fasting month and blood sample for inflammatory markers (WBC, hs- CRP, Fibrinogen) and glycemic control, fasting Blood sugar (FBS)and HbA1C were collected. Another sample of same Laboratory tests was retaken on follow up (at least after first10 days fast of Ramadan) . Paired T test was used to test the difference and P value <0.05 considered significant.

Results: There were 59 patients completed the study and came for follow up and one patient was dropped because Urinary tract infection.

Our results showed that no significant difference in the FBS and hs-CRP before and during Ramadan fasting, while the Fibrinogen level was strongly statistically different ($P = 0.000$) before and during Ramadan fasting being higher during fasting state, Interestingly WBC level was lower during fasting compared to before fasting and the difference was marginally significant ($P = 0.06$). As regard to differential count (neutrophils $P=0.009$, lymphocytes $P =0.746$) .

The hemoglobin ($P=0.000$) and platelets ($P=0.028$) were significantly lower during Ramadan fasting compared to pre-Ramadan level.

Despite Numerically the mean value of Fasting blood 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. When controlling for age, waist circumference and BMI. fasting blood sugar during Ramadan was positively correlated with hsCRP (Pearson correlation = 0.289, $P = .032$) while HbA1C was not. The duration of diabetes was not correlated with any of the inflammatory markers which tested i study.

Conclusion: Fasting Ramadan (first 10 days) doesn't show significant effects in most inflammatory markers except WBC count

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 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 recruited 72 patients with type 2 diabetes before holly Ramadan fasting by two weeks, of which only 59 completed the study and came for follow up during Ramadan (at least after first 10 days fast of Ramadan). One of these 59 participants his data was cancelled because he develops urinary tract infection during Ramadan.

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, waist-to- hip circumference ratio (W-HR) by using tape measure.

We exclude the patients who have history of type 1 diabetes, smoking, acute or chronic inflammation (rheumatoid arthritis-bowel disease), recent infections since last 3days, taking NSAID (Non-Steroidal Anti-Inflammatory Drug), aspirin intake, fasting rajjab, shaban lunar months, endocrine and neuroendocrine problem, following special nutritional regimen, consumption of nutrient supplement, cancer, renal disease. Also, we excluded obese

persons (BMI more than 30 kg/m²), pregnancy, lactation and the expected date of menstrual cycle in the first 10 days of Ramadan,

2.2 Estimation of HB A1c:

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, normal value of HBA1c for adults equal 4-5.6%, increased risk for diabetes (prediabetes): 5.7-6.4%, Diabetes <7% good glycaemic control, >7% poor glycaemic control.

2.3 Estimation of white blood cell (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 \(UMRC\)](#), these are the normal ranges of WBCs per microliter of blood (µL):

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

Age range	WBC count (per µL 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

According to the [Leukemia & Lymphoma Society \(LLS\)](#):

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

Types of WBCs	Normal percentage
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 range of the lab (70-110).

2.5 Estimation of high sensitive- C Reactive protein(hs-CRP):

The high sensitive-C reactive protein (hs-CRP) was estimated by Immunochemical Assay. Normal range of the lab (0.0-5)

2.6 Estimation of fibrinogen level:

For patients in our study fibrinogen was estimated by Immunochemical assay normal range of the lab (2.5-4g/L).

All investigation before and during Ramadan done in Alhia lab medical analysis estimated by Immunochemical Assay using COBAS INTEGRA 400 PLUS (Roche).

2.7 Statistical analyses:

The data was collected, organized, tabulated and then statistically analyzed by SPSS (Statistical Package of Social Sciences, version 20). Quantitative data was expressed as mean \pm SD and analyzed by independent sample t-test for continuous variables. the mean of biochemical and inflammatory markers before and during Ramadan fasting in the study subjects, the anthropometric data of the patients in the study , were estimated . The paired t test was used to estimate the Degree of significance of difference between biochemical and inflammatory markers before and during Ramadan fasting.

3. RESULTS AND DISCUSSION

A total of 72 patients with type 2 diabetes were recruited before holey Ramadan fasting month, of which only 59 completed the study and came for follow up during Ramadan (at least after first 10 days fast of Ramadan) .

One of these 59 his data was cancelled because he develops urinary tract infection during Ramadan. **Figure1.**

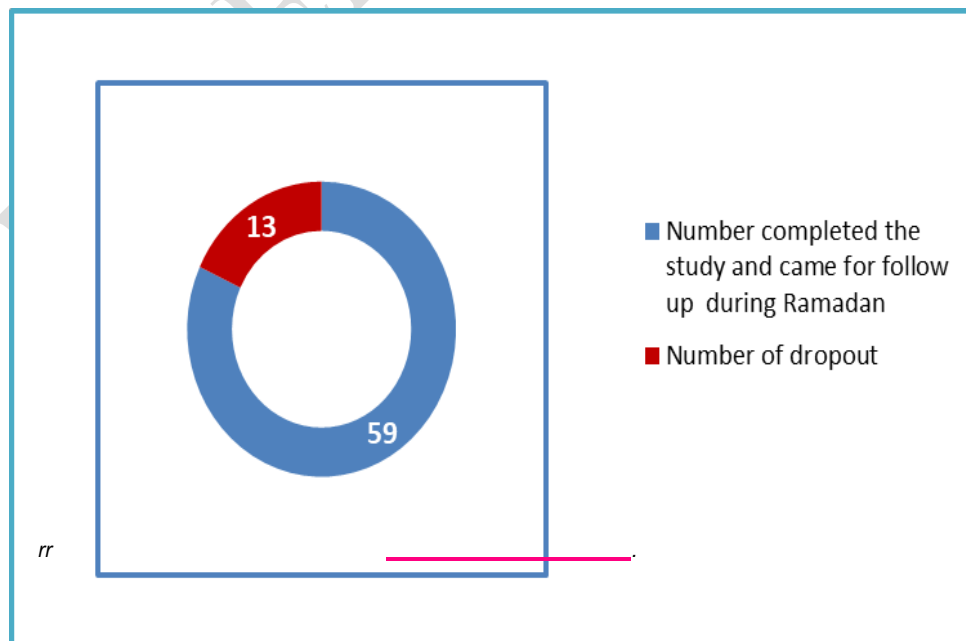


Fig 1: The percentage of patients who complete the study

The mean age of the patients in the study were 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 and the mean duration of fasting was 12+/- 4. **Figure 2 .**

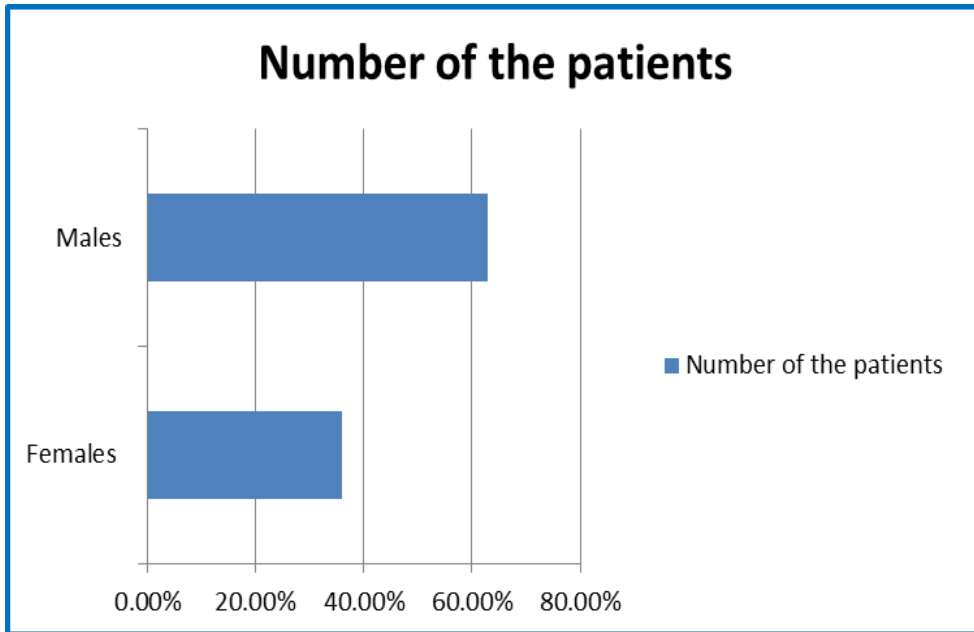


Fig 2: The percentage of patients according to gender.

The anthropometric data of the patients enrolled in the study were illustrated in the following table. **Table 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 mean value of the fasting blood sugar and biochemical and inflammatory markers before Ramadan (FBS= 182+/- 0.82, Fibrinogen level =2.1+/- 0.45 , HsCRP=4+/-4,WBC=9+/-2.4)and during Ramadan(FBS= 204+/-72, Fibrinogen level= 3+/-0.65, HsCRP= 4.3+/-4.1,WBC= 8.1+/-2.14)were illustrated in **Figure3**

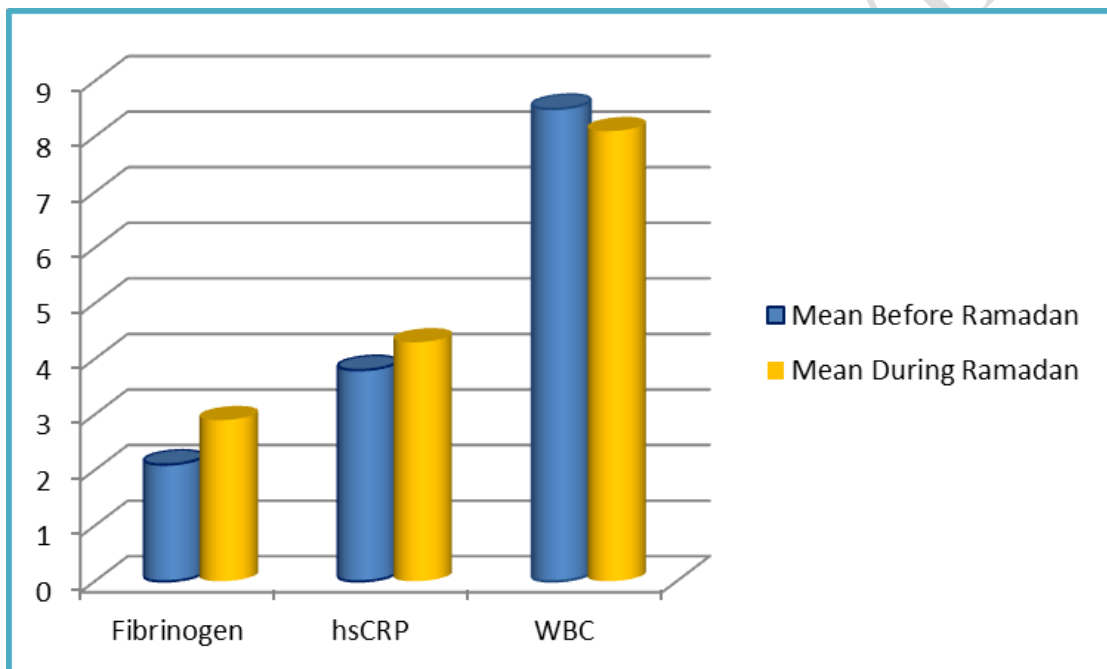


Fig 3 : The mean of inflammatory markers namely (Fibrinogen level, hs-CRP. WBC) before and during Ramadan fasting .

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 fasting state Interestingly WBC level was lower during fasting compared to before fasting and the difference was marginally significant ($P = .06$) . **Table 4** show these finding.

Table 4: The Degree of significance of difference between biochemical and inflammatory markers before and during Ramadan fasting

Biochemical and inflammatory markers		Mean	N	Std. Deviation	Sig. (2-tailed)
Pair 1	FBS before fasting Ramadan	182	58	83	.107
	FBS during fasting Ramadan	204	58	72	
Pair 2	Fibrinogen level before fasting Ramadan	2.09	58	.45	.000
	Fibrinogen level during Ramadan fasting	3	58	.65	
Pair 3	hsCRP before fasting Ramadan	3.82	58	4	.283
	hsCRP during fasting Ramadan	4.32	58	4.17	
Pair 4	WBC before fasting Ramadan	8.47	58	2.35	.283
	WBC during fasting Ramadan	8.06	58	2.14	

Hemoglobin (13.2+/- 1.369, 14+/- 1.48 , $P < .001$), neutrophils(5+/- 1.49, 5.25+/- 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+/-76, 3+/- 85 , $P = .8$) . **Figure 4, 5**

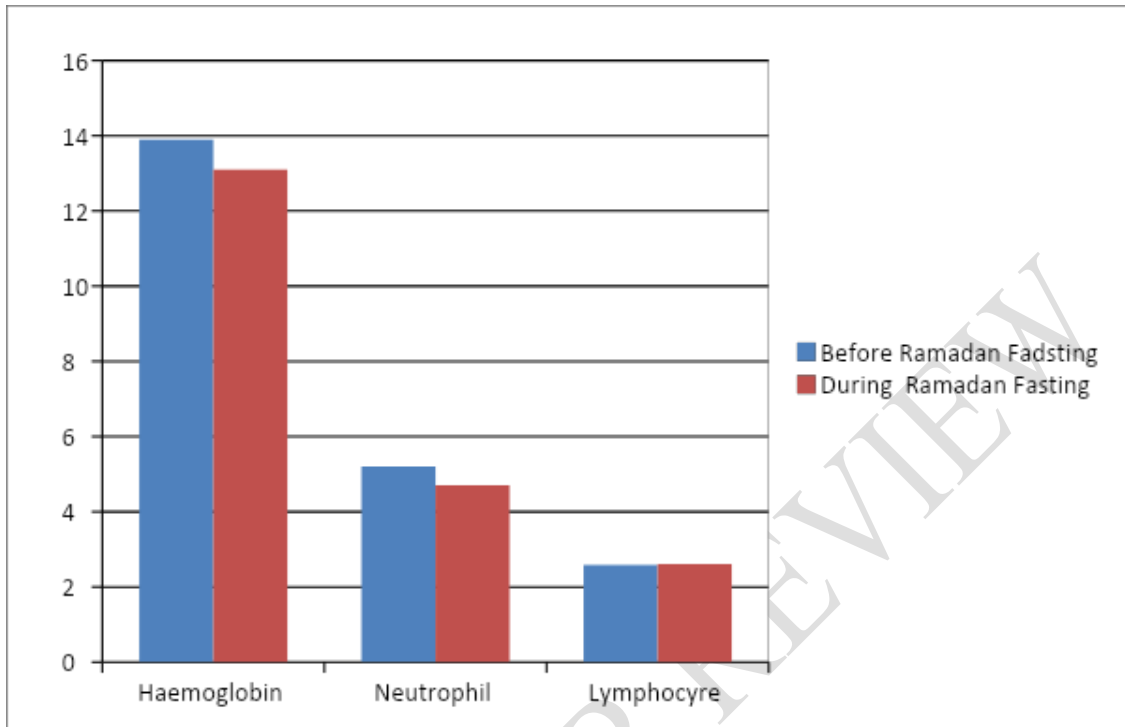


Fig 4 : Demonstrate Compared Mean For Hemoglobin, Neutrophil, Lymphocyte Count Before And During Ramadan Fasting.

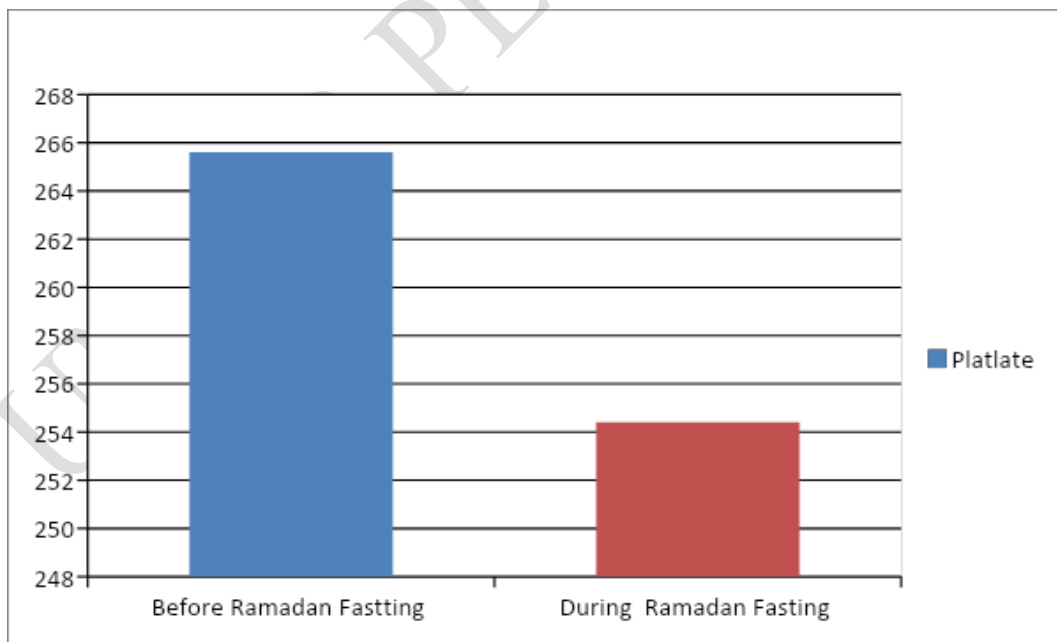


Fig 5 : Demonstrate Compared Mean For Platelet Count Before And During Ramadan Fasting.

Despite numerically the mean value of fasting blood 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 **Table 5.**

Table 5 : illustrate the effect of glycemic control on the mean of the FBS, hsCRP, Fibrinogen level , WBC count during Ramadan fasting and their degree of significance .

	Mean HbA1C	N	Mean	Std. Deviation	Sig. (2-tailed) P value
FBS during fasting Ramadan	>= 7.00	47	208	76.27	.33
	< 7.00	11	184	44	
hsCRP during fasting Ramadan	>= 7.00	47	5	4.31	.35
	< 7.00	11	3.26	3.47	
Fibrinogen level during Ramadan fasting	>= 7.00	47	3	.63	.15
	< 7.00	11	3.21	.70	
WBC during fasting Ramadan	>= 7.00	47	8.22	2.23	.26
	< 7.00	11	7.40	2	

When controlling for age, waist circumference and BMI , HbA1c was not correlated with any of inflammatory markers in the study (Fibrinogen , hSCRp , WBC count) while fasting blood sugar during Ramadan was positively correlated with hsCRP (Pearson correlation = 0.29 , $P = .03$) . It's not surprise to see correlation between WBC and other inflammatory markers during Ramadan fasting, as it strongly positively correlated with fibrinogen level (Pearson correlation = 0.37 . $P = 0.005$) , with hsCRP (Pearson correlation = 0.27 , $P = .05$) . The duration of diabetes was not correlated with any of the inflammatory markers which tested in the study i.e fibrinogen, hs-CRP level and WBC.

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 ($P = .06$), This mainly driven by decrease in neutrophils ($P = .009$) significantly, while no significant change in lymphocyte count ($P = 0.8$),

The hemoglobin ($P = .000$) and platelets ($P = 0.03$) were significantly lower during Ramadan fasting compared to pre -Ramadan level. The mean value of fasting blood 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 Ramadan, et, al (1994) and farshidfar, et al . (2006) [13,14].

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

While hosseini, et al ,(2013) , sarraf-zadegan, et al,(2000) showed no changes in WBC count or any other hematological parameters throughout Ramadan [16,17].

In contrast study did by bouhleb, et al (2006) show a significant increase in Hb and HCT And nematy, et al (2012) reported a significant increase in WBC, RBC and platelet (PLT) counts after fasting in Ramadan [18,19].

controversial results obtained from different studied could be attributed to several facts, 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 ($P = .000$) 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 2016 [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 .etal 2012 [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 .

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.

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*

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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UNDER PEER REVIEW