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Ramadan Fasting Improves Kidney Functions and Ameliorates Oxidative Stress in Diabetic Patients

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Abstract: The current investigation was carried out to evaluate and characterize the effects of Ramadan fasting and the possible influence of anti-oxidants supplementation with vitamin E on parameters of renal function and oxidative stress in patients with *diabetes mellitus*. Two study groups of 20 subjects each (n = 20) having an average age of 50 ± 5 years were investigated. One group included normal control subjects recruited from the general population. The second group had patients with *diabetes mellitus*. Each of the two groups was subdivided into 2 subgroups (n = 10) during the month of Ramadan. One subgroup fasted during the month of Ramadan and the other subgroup fasted and ingested 800 IU of vitamin E per day during Ramadan. Parameters of kidney function namely: blood urea, serum creatinine, creatinine clearance, serum electrolytes and urinary albumin to creatinine ratio (ACR) and parameters of oxidative stress namely: serum malondialdehyde (MDA) and blood reduce glutathione (GSH) were all measured during the 4th week of the month of Shabaan (i.e. before fasting), during the 4th week of Ramadan and 6 weeks post-Ramadan fasting. We reported that Ramadan fasting resulted in significant $P<0.05$ reductions in: blood urea from its pre-fasting baseline values by 16.6and25.6 and 5.6and0.4 serum creatinine by 1.7and16.4 and 12.5and17.8 ACR by 3.9and26.2 and 5.6and6.3 and serum MDA by 52.2and57.6 and 39.6and44.0 during the 4th week of fasting alone and fasting with vitamin E supplementation in the control and diabetic groups respectively. Conversely, creatinine clearance was significantly $P<0.05$ elevated from its baseline values by 0.1and16.1 and 8.5and12.8 and GSH by 139.4and141.1 and 241.4and251.4 during the 4th week of fasting alone and fasting with vitamin E supplementation in the control and diabetic groups respectively. There were insignificant changes in serum electrolytes $P>0.05$ during the 4th week of fasting with or without vitamin E supplementation in the two groups. The values of blood urea, serum creatinine, creatinine clearance and ACR returned to levels statistically indifferent from their corresponding pre-fasting baseline values, whereas serum MDA were maintained significantly $P<0.05$ lower by 19.7 and 20.9 and GSH significantly $P<0.05$ higher than the pre-fasting baseline values by 54.7 and 70.3, 6-weeks post-Ramadan fasting in the control and diabetic groups respectively. In conclusion, results of the present study confirmed that Ramadan fasting doses not have adverse effects; rather it has favorable effects on renal function in the diabetic patients. Furthermore, Ramadan fasting significantly lowers oxidative stress in the body.

Key words: Ramadan Fasting · Kidney Functions · Oxidative Stress · Diabetes

INTRODUCTION

Diabetes mellitus (DM) has been long recognized as the most common metabolic disorder worldwide. Recent estimate showed that there were 171 million people in the world with DM in the year 2000 and this is projected to increase to 366 million by 2030 [1,2].

Numerous animal, clinical and epidemiological studies have shown that cardiovascular disease (CVD) and diabetic nephropathy (DN) to be the primary cause of morbidity and mortality among diabetic [3-5]. DN, defined clinically as the presence of microalbuminuria or overt nephropathy in patients with diabetes who lack indicators of other renal diseases, is the major cause of end stage

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renal disease (ESRD) throughout the world [6,7]. Additionally, the reduced glomerular filtration rate (GFR) and albuminuria caused by DN are independent risk factors for CVD and death [8,9],. Screening for albuminuria and reduced GFR could thus help in slowing disease progression by allowing earlier implementation of risk reduction measures and the selection of aggressive treatment [10].

Patients with chronic renal dysfunction and ESRD have been shown there oxidative stress markers to have raised levels of such as MDA [11,12]. Several clinical studies have demonstrated an inverse correlation between MDA and GFR and showed that graded increases in oxidative stress are associated with longer duration of dialysis therapy [13]. The increased oxidative stress in patients with renal dysfunction is probably due to increased production of reactive oxygen species (ROS) or a deficiency in the antioxidant defense system [14].

The effects of Ramadan fasting on public health are of importance to physicians and health care personnel wherever there is a sizeable Muslim population. Up to 400 million Muslims observe the fast during that holy Islamic month of Ramadan world-wide. There is a growing public belief that Ramadan fasting deteriorates kidney function in diabetic patients. Several studies have shown no deleterious effects of Ramadan fasting on renal functions in renal transplant patients [15]. This comprehensive study was carried out to investigate the effects Ramadan fasting on parameters of renal function and oxidative stress and the possible influence of vitamin E supplementation with fasting on these parameters in normal control and diabetic subjects. Additionally, the influences of Ramadan fasting on body mass index (BMI), fasting blood glucose (FBG) and glycosylated hemoglobin (hemoglobin A1c; HbA1c) were studied.

MATERIALS AND METHODS

Volunteers: The study was conducted in Benha Teaching Hospital, Faculty of Medicine, Benha University, Benha, Egypt, during the period from 5 August 2010 to 25 November 2010. This corresponds to the period from 24 Shaaban 1431 to 17 Dhul Al-Qi'dah 1431 in the Islamic Calendar. Subjects were recruited from the general population. Potential volunteers were first requested to complete a screening questionnaire. Subjects excluded form the study included those who are current smokers or ex-smokers who had stopped smoking within the past six months; and those with any history of a major illness. All participants gave written informed consent. Eligible volunteers were then finally screened prior to participation and the study design and requirements were thoroughly explained to them. Ethical approval of the study was obtained from the relevant Research and Ethics Committee of the University.

Forty subjects having an average age of 50 ± 5 years were included in the study. Participants were arranged into two study groups of 20 subjects each (n = 20); one group included normal control subjects and the second one comprised known patients with type II DM who used to come to the Medical Outpatient Clinic for regular monthly checkup. Both the control and the diabetic groups had 30% males and 70% females. Thus, subjects were carefully matched for age and sex. All subjects had their body weight, BMI, FBG, HBA1c, blood urea, serum creatinine, creatinine clearance, serum electrolytes, ACR, serum MDA and blood GSH measured during the 4th week of the month of Shaaban which immediately precedes the month of Ramadan (i.e. shortly before fasting), in order to make sure that they are in a good health and can sustain fasting and to obtain pre-fasting baseline data for these parameters.

Experimental Design: The study lasted for a total of 12 weeks, each of the two study groups was subdivided into 2 subgroups (n = 10). Participants in one subgroup from each of the two main study groups were instructed to fast during the month of Ramadan and to take their regular medications as usual, as advised by their physicians. In addition to fasting and taking the regular medications, the remainder two subgroups were provided with vitamin E capsules and instructed to ingest 800 IU of the vitamin per day during Ramadan. All participants came to the Medical Outpatient Clinic during the 4th week of the month of Ramadan for their regular monthly checkup. They had been able to fast for the four weeks of Ramadan and the above-mentioned parameters of BMI, glycemic control, kidney function and oxidative stress were re-measured. All participants were instructed to continue taking their regular medications and not to have additional fasting for 6 weeks post-Ramadan. Those who had been taking vitamin E were told to stop taking the vitamin by the end of Ramadan. They all came 6 weeks post-Ramadan for their regular checkup and the same parameters were again re-measured.

Whole Blood, Serum and Urine Samples: Early morning fasting urine and blood samples were collected from each participant at each visit. Urine samples were collected in serialized urine containers and used to detect albuminuria. A total of 10 ml sample of venous blood was obtained from each participant at each visit; 4 ml was taken in two tubes with heparin and the rest in a biochemical tube. Samples were immediately transferred to the laboratory in a cool box at +4°C. Serum was obtained from samples collected in biochemical tubes by centrifuging blood samples at 3,000 rpm for 15 min at 4°C. Serum samples were then stored at -20°C until analyzed.

FBG and HbA1c Analyses: FBG was measured with a glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA). Whole blood samples were hemolyzed in 4 times its volume of ice and then centrifuged. HbA1c was then separated from non-glycosylated forms of hemoglobin, based on differences in charge and using gel electrophoresis [16].

Urea and Creatinine Analyses: Urea was measured in the serum samples by the Modified Berthelot enzymatic method, using BIODIAGNOSTIC kits (BIODIAGNOSTIC, Egypt). [17] Serum creatinine was measured based on enzymatic colorimetric test [18] using BIODIAGNOSTIC kits (BIODIAGNOSTIC, Egypt). Creatinine clearance was calculated by Cockcroft and Gault relationship [19].

Serum Electrolytes Analyses: Serum electrolytes measured included sodium, potassium, calcium and phosphorus. Electrolytes were measured colorimetrically [20, 21] using Biodiagnostic Kits (Biodiagnostic, Egypt).

Albumin to Creatinine Ratio (ACR): Albuminuria was detected using a reagent stick with tetrabromophenol. Albumin combines with the blue divalent anionic form of the indicator and this changes color from yellow to green to blue. Albuminuria was then quantified colorimetrically [22,23] using Biodiagnostic Kits (Biodiagnostic, Egypt). Then, ACR was estimated by dividing urinary albumin in milligrams (mg) by serum creatinine in grams (g).

Oxidant/Antioxidant Status Analyses

MDA Determination: MDA was measured in the serum samples by the thiobarbituric acid (TBA) method [24], using BIODIAGNOSTIC kits (BIODIAGNOSTIC, Egypt).

GSH Determination: Whole blood samples were hemolyzed in 4 times its volume of ice and then centrifuged. The clear upper supernatant (erythrocyte lysate) was then collected and the levels of GSH were measured based on the reduction of 5,5` dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound [25], using BIODIAGNOSTIC kits (BIODIAGNOSTIC, Egypt).

Statistics: Statistical analyses were performed using SPSS software version 13 (SPSS, Chicago, IL, U.S.A.). Results are presented as means ± standard errors of the means (S.E.M) and $P<0.05$ was chosen as the level of significance. The independent-samples t-test was used to compare baseline values between the control and the diabetic groups and levels of the control and diabetic subgroups during Ramadan and 6 weeks post-Ramadan; a value of $P < 0.05$ was considered significant from the diabetic group/subgroup. One-way analysis of variance (one-way ANOVA) followed by Tukey's test were used to analyze within-group changes in the measured parameters.

RESULTS

Effects of Ramadan Fasting on Body Mass Index (BMI): Table 1 and Figure 1A show the baseline pre-fasting BMI (kg/m^2) of the control and diabetic groups and demonstrate no statistically significant differences between the 2 groups in their pre-fasting baseline ($P>0.05$) . The effects of Ramadan fasting on BMI of the two groups are shown in Figure 1A, which reveals that Ramadan fasting for 4 weeks without vitamin E supplementation resulted in insignificant reductions in the BMI 1.1 and 5.1% in the control and diabetic groups. When Ramadan fasting was supplemented with vitamin E, the BMI was lowered by 3.0 and 3.9% respectively. These reductions in BMI were however statistically insignificant. Furthermore, reductions in BMI induced by Ramadan fasting alone were statistically insignificant from the reductions produced by Ramadan fasting supplemented with vitamin E in the two study groups. At 6 weeks post-Ramadan fasting, although the BMI remained lower than its baseline pre-fasting value by 1.4 and 1.0% in control and diabetic groups, these post-fasting BMI values were statistically indistinguishable from the corresponding pre-fating levels. Furthermore, there were no statistically significant differences in BMI during the 4^{th} week of Ramadan fasting with or without vitamin E supplementation ($P>0.05$) and the 6-weeks post-fasting levels in the two study groups. Interestingly, there were no statistically differences in BMI between the two groups during the 4^{th} week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

Effects of Ramadan Fasting on Glycemic Control FBG: Table 1 and Figure 1B demonstrate that the baseline pre-fasting fasting blood glucose levels FBG; mg/dl of the diabetic group was significantly higher than the corresponding levels in control group ($P < 0.05$). Figure 1B also shows the effects of Ramadan fasting on FBG of the two study groups. Ramadan fasting for 4 weeks with no vitamin E supplementation resulted in insignificant reductions in the FBG by 5.8% in control group and significant reductions by 23.0% in diabetic group. Supplementation of fasting with vitamin E lowered FBG by 13.0 and 25.9% in control and diabetic groups. These reductions were also statistically insignificant in control and significant in diabetic group. The reductions

Table 1: Pre-fasting baseline parameters of BMI, glycemic control, kidney function and oxidative stress

Parameter	Control Group © (n = 20)	Diabetic Group (d) (n = 20)	P-value
Body mass index; BMI (kg/m^2)	31.9±0.8	30.2±1.1	> 0.05
Fasting blood glucose; FBG (mg/dl)	122±10.0	252.6±24.5	<0.05
Glycosylated hemoglobin; HbA_1C (%Hb)	5.5±0.3	6.3±0.2	<0.05
Blood urea; (mg/dl)	34.4±2.8	26.9±4.9	> 0.05
Serum cereatinine; (mg/dl)	0.86±0.01	0.90±0.02	> 0.05
Creatinin clearance; (ml/min)	123.8±9.0	107.4±7.0	> 0.05
Albumin to creatinine ratio; ACR (mg/g creatinine)	13.0±1.1	36.0±5.5	<0.001
Calcium; (mg/dl)	9.7±0.2	9.2±0.3	> 0.05
Phosphorus; (mg/dl)	4.1±0.3	4.2±0.2	> 0.05
Sodium; (mEq/l)	138.6±2.2	137.5±1.9	> 0.05
Potassium; (mEq /l)	4.8±0.1	4.4±0.3	<0.01
Malondialdehyde (MDA); (nmol/ml)	0.94±0.02	0.91±0.09	> 0.05
Glutathione; (mg/dl)	3.6±0.9	3.7±0.7	> 0.05

Data are presented as means and standard errors of the means (SEM); the independent-samples t-test was used to compare baseline values between groups. A value of $P < 0.05$ was considered significant.

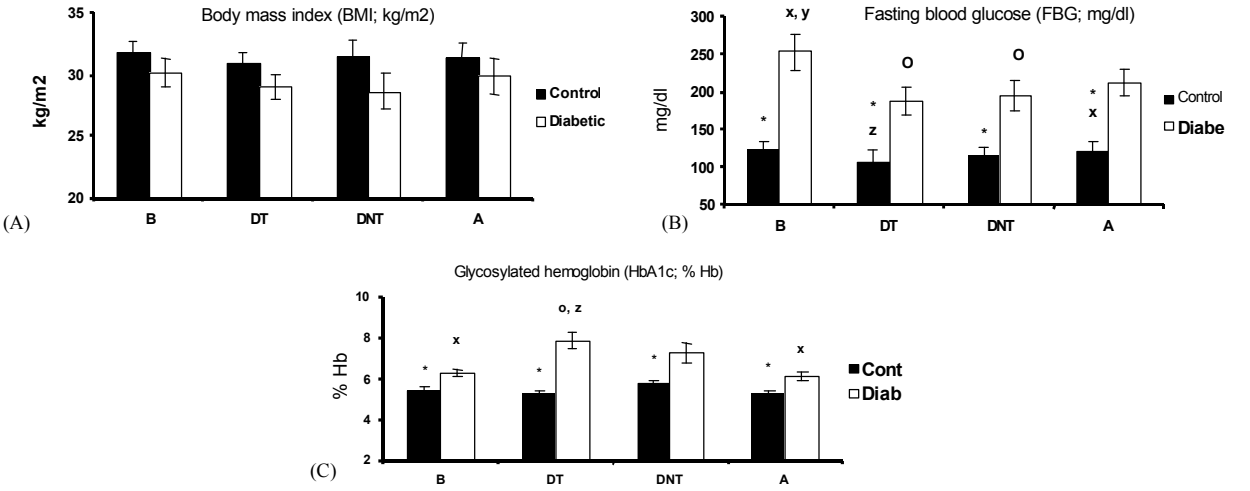

Fig. 1: Effects of Ramadan fasting on BMI, FBG and HbA1cChanges in BMI (A), FBG (B) and HbA1c (C) for the control group *(black bars)* and the diabetic group *(white bars)* one week before fasting Ramadan (B on x axis), 4th week of Ramadan fasting with vitamin E supplementation (DT), 4th week of Ramadan fasting without vitamin E supplementation (DNT) and 6 weeks after Ramadan fasting (A). Data are presented as means ± SEM. One-way ANOVA followed by Tukey's test were used to compare the changes during DT; the changes during DNT and the changes at A from the baseline pre-fasting level of the measured parameter. A value of $P < 0.05$ was considered significant from ^{o}B, ^{x}DT, ^{y}DNT and ^{z}A respectively. The independent-samples t-test was used to compare baseline values between the control and the diabetic groups and levels of the control and diabetic subgroups during Ramadan and 6 weeks post-Ramadan; a value of $^{*}P < 0.05$ was considered significant from the diabetic group/subgroup.

produced by Ramadan fasting alone were statistically indistinguishable from the corresponding reductions induced by Ramadan fasting and supplementation of vitamin E in the two groups. Six weeks post-Ramadan, the FBG remained lower than its baseline pre-fasting value by 1.7 and 15.91% in control and diabetic groups. However, these post-fasting FBG levels were statistically indifferent from the corresponding pre-fasting baseline values in the two study groups. Furthermore, there were no statistically significant differences in FBG during the 4th week of Ramadan fasting without vitamin E supplementation and the post-fasting values in the two study groups. However, the FBG during the 4th week of Ramadan fasting with vitamin E supplementation was significantly lower than the 6-week post-fasting value in control group. The FBG levels of diabetic group were significantly higher than the corresponding values of control group during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

HbA1c: Table 1 and Figure 1C show that diabetic group had significantly higher baseline pre-fasting baseline glycosylated hemoglobin levels (HbA1c; % Hb) than control group (P < 0.05). Figure 1C also demonstrates that

the levels of HbA1c during the 4th week of Ramadan fasting with and without vitamin E supplementation and also the corresponding levels 6-weeks post-Ramadan fasting in the two study groups. Interestingly, the levels of HbA1c during the 4th week of Ramadan fasting alone were higher than their corresponding baseline pre-fasting levels by 5.5 and 15.9% in control and diabetic groups. These changes in HbA1c were however statistically insignificant in the two groups. The levels of HbA1c during the 4th week of Ramadan fasting supplemented by vitamin E were significantly higher than the corresponding baseline pre-fasting levels by 25.4% in diabetic group, but insignificantly lower than the corresponding baseline pre-fasting levels by 3.6% in control group. Furthermore, the levels of HbA1c during the 4th week of Ramadan fasting without vitamin E supplementation were statistically indistinguishable from the corresponding levels during the 4th week of Ramadan fasting supplemented by vitamin E in two groups. Six weeks post-Ramadan fasting, the HbA1c levels were lower than their corresponding baseline pre-fasting values by 3.7 and 1.6% in control and diabetic groups, but these changes were statistically insignificant. Furthermore, these post-fasting HbA1c levels were statistically indifferent from the values during the 4th week of Ramadan fasting with or without vitamin E supplementation in the two study groups. In parallel with the FBG levels, the Hba1c levels of diabetic group were significantly higher than the corresponding values of control group during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

Effects of Ramadan Fasting on Kidney Function Urea: Table 1 and Figure 2A showed that the baseline pre-fasting blood urea (mg/dl) levels of control group were insignificantly higher than the corresponding levels in diabetic group (P>0.05). Figure 2A demonstrated that Ramadan fasting without vitamin E supplementation reduced the serum urea levels insignificantly by 16.8 and 0.70% in control and diabetic groups (P>0.05). When Ramadan fasting was supplemented with vitamin E, the serum urea levels were also lowered insignificantly by 25.6 and 0.4% in control and diabetic groups (P>0.05). The reductions in serum urea induced by Ramadan fasting alone were statistically indistinguishable from the corresponding reductions produced by Ramadan fasting supplemented by vitamin E in the two study groups (P>0.05). Six weeks following Ramadan, serum urea increased insignificantly by 0.9 and 1.1% in control and diabetic groups (P>0.05). Moreover, there were no statistically significant differences in serum urea during the 4th week of Ramadan fasting with or without supplementation of vitamin E and the post-fasting levels in the two study groups. There were no statistically differences in serum urea between the two groups during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

Serum Creatinine: Table 1 and Figure 2B demonstrate that the baseline pre-fasting serum creatinine levels of control group were slightly and insignificantly lower than the corresponding levels in diabetic group (P>0.05). The effects of Ramadan fasting on serum creatinine of the two groups were shown in Figure 2B, which reveals that Ramadan fasting without vitamin E supplementation reduced the serum creatinine levels insignificantly by 1.2% in control group and significantly by 12.50% in diabetic group. Ramadan fasting supplemented by vitamin E resulted in significant reductions in serum creatinine by 16.4 and 14.8% in control and diabetic groups. Additionally, reductions in serum creatinine produced by Ramadan fasting supplemented by vitamin E were statistically greater than the corresponding reductions during the 4th week of Ramadan fasting without vitamin E supplementation in control group only. Six weeks post-Ramadan, the serum creatinine levels were still lower than the corresponding baseline pre-fasting values by 0.6 and 1.1% in the control and diabetic groups. However, these post-fasting serum creatinine reductions were statistically insignificant in the two study groups. Furthermore, the 6-weeks post-Ramadan fasting serum creatinine levels were significantly higher than the corresponding values during the 4th week of Ramadan fasting supplemented by vitamin E, but indistinguishable from the values during Ramadan fasting alone in control group. On the other hand, the 6-weeks post-fasting serum creatinine values were statistically higher than the levels during the 4th week of Ramadan fasting with or without vitamin E supplementation in the d group. Nevertheless, there were no statistically differences in serum creatinine between the two groups during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

Creatinine Clearance: Table 1 and Figure 2C demonstrate that control group had insignificantly higher baseline pre-fasting creatinine clearance levels (ml/min) than diabetic group (P>0.05). The effects of Ramadan fasting on creatinine clearance levels are shown in Figure 2C, which demonstrates that Ramadan fasting for 4 weeks with no vitamin E supplementation raised creatinine clearance insignificantly by 0.1 and 8.5% in control and diabetic groups. On the other hand, Ramadan fasting supplemented by vitamin E elevated creatinine clearance significantly by 16.1% in control group (P<0.05) and insignificantly by 12.8% in diabetic group (P>0.05). Additionally, elevations in creatinine clearance produced by Ramadan fasting alone were statistically indistinguishable from the elevations induced by

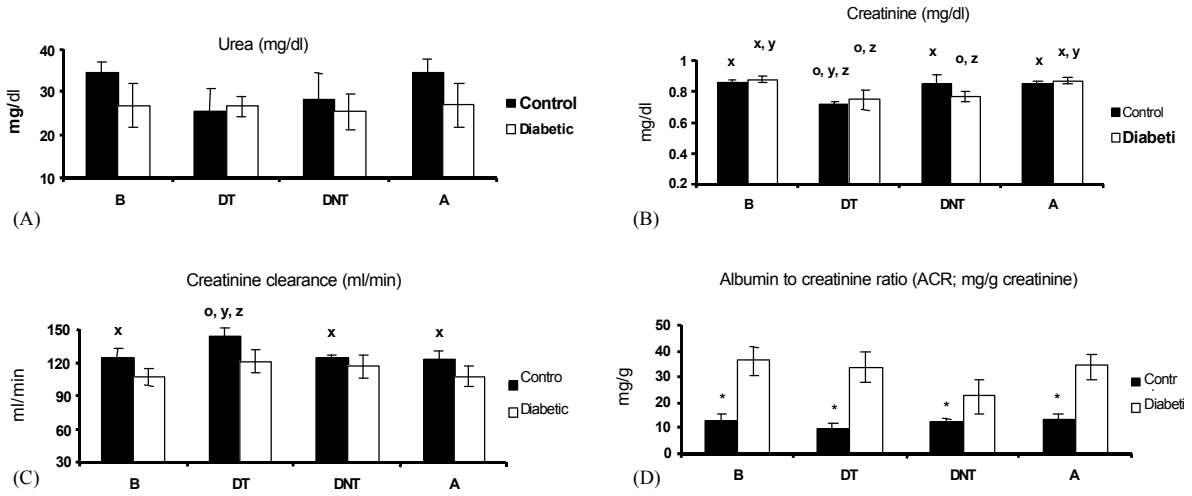

Fig. 2: Effects of Ramadan fasting on blood urea, serum creatinine, creatinine clearance and albumin to creatinine ratio (ACR)Changes in blood urea (A), serum creatinine (B), creatinine clearance (C) and ACR (D) for the control group (black bars) and the diabetic group (white bars) one week before fasting Ramadan (B on x axis), 4th week of Ramadan fasting with vitamin E supplementation (DT), 4th week of Ramadan fasting without vitamin E supplementation (DNT) and 6 weeks after Ramadan fasting (A). Data are presented as means ± SEM. One-way ANOVA followed by Tukey's test were used to compare the changes during DT; the changes during DNT and the changes at A from the baseline pre-fasting level of the measured parameter. A value of $P < 0.05$ was considered significant from oB, xDT, yDNT and zA respectively. The independent-samples t-test was used to compare baseline values between the control and the diabetic groups and levels of the control and diabetic subgroups during Ramadan and 6 weeks post-Ramadan; a value of $*P < 0.05$ was considered significant from the diabetic group/subgroup.

Albumin to Creatinine Ratio (ACR): Table 1 and Figure 2D show that diabetic group had significantly higher baseline pre-fasting albumin to creatinine ratios (ACR; mg/g creatinine) than control group ($P < 0.05$). Figure 2D demonstrates the effects of Ramadan fasting on ACR of the two groups and reveals that Ramadan fasting for 4 weeks without vitamin E supplementation lowered ACR insignificantly by 3.9 and 5.6% in control and diabetic groups. Interestingly, Ramadan fasting supplemented with vitamin E resulted in insignificant reductions in ACR by 26.2 and 6.3% in control and diabetic groups. Additionally, the reductions in ACR produced by Ramadan fasting alone were statistically insignificant from the corresponding reductions induced by Ramadan fasting supplemented by vitamin E in the two study groups. Six weeks after Ramadan, ACR ratios remained lower than their corresponding baseline pre-fasting values by 5.4% in diabetic group but increased above their baseline pre-fasting levels by 3.1% in control group. However, these post-fasting ARC ratios were statistically indifferent from their pre-fasting baseline values in the two groups. Furthermore, ACR ratios during the 4th week of Ramadan fasting with or without vitamin E supplementation were statistically indistinguishable from the 6-weeks post-fasting values in the four study groups. There were no statistically differences in ACR between the two groups during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

Serum Electrolytes: Table 1 and Figure 3A-D show the baseline pre-fasting levels of the main serum electrolytes for the two study groups; calcium (Ca; mg/dl; Figure 3A), phosphorus (P; mg/dl; Figure 3B), sodium (Na; mEq/l; Figure 3C) and potassium (K; mEq/l; Figure 3D) and demonstrate that there were no statistically significant differences between the two groups in their baseline pre-fasting serum ($P>0.05$) for all studied electrolytes. Figure 3A-D reveals the effects of Ramadan fasting on serum electrolytes levels of the study groups and show that Ramadan fasting for 4 weeks without vitamin E supplementation resulted in insignificant reductions in serum Ca levels by 6.7 and 8.7%, Na by 0.6 and 1.1%, K by 5.5 and 4.6% and insignificant elevations in serum P levels by4.6 and 4.8% in control and diabetic groups ($P>0.05$). On the other hand, Ramadan fasting supplemented by vitamin E lowered serum Ca levels by 3.1 and 7.6%, Na by 0.1 and 0.5%, K by 7.6 and 6.8% and elevated serum P levels by (1.2 and 7.1% in control and diabetic groups. These changes in serum electrolytes were however statistically insignificant ($P>0.05$). Additionally, changes induced by Ramadan fasting alone were statistically indifferent f rom changes produced by Ramadan fasting

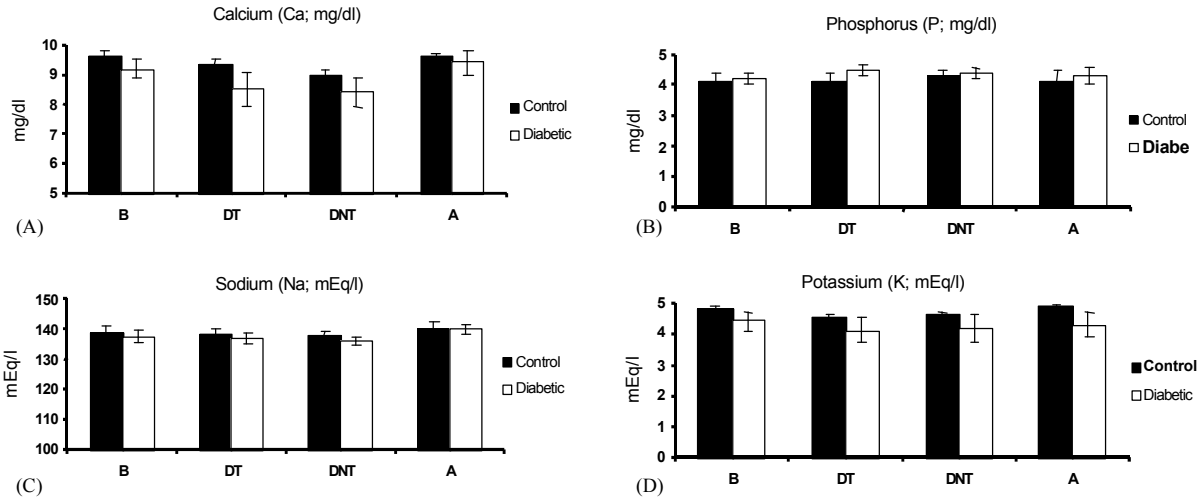

Fig. 3:

supplemented by vitamin E in the two groups. Six weeks following Ramadan, the serum Ca levels remained lower than their corresponding baseline pre-fasting values by 0.6% in control group, while in diabetic group, the serum Ca levels increased by 2.2%. However, these post-fasting changes in serum Ca levels were statistically insignificant. Furthermore, at six weeks post-Ramadan, the serum Phosphate and sodium levels were higher than the corresponding baseline pre-fasting values by 1.2%and1.0% and 2.4and1.7% for Phosphate and sodium in control and diabetic groups. However, again these post-fasting serum P and Na levels were statistically indifferent from their corresponding baseline pre-fasting values in the two groups. The serum K levels were reduced from their baseline pre-fasting values by 2.3% in diabetic group but increased by 1.0% in control group at 6 weeks post fasting. However, the post-fasting serum K levels were statistically indifferent from their pre-fasting baseline values in the two study groups. Moreover, there were no statistically significant differences in serum electrolytes levels during the 4th week of Ramadan fasting with or without supplementation of vitamin E and the post-fasting levels in the two groups. Interestingly, there were no statistically differences in serum electrolytes between the two groups during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at six-weeks post-fasting.

Effects of Ramadan Fasting on Oxidative Stress

Serum Malondialdehyde (MDA): Table 1 and Figure 4B demonstrate that the baseline pre-fasting serum malondialdehyde (MDA; nmol/ml) levels of the (c) were statistically indistinguishable from the corresponding levels in diabetic group; (P>0.05). The effects of Ramadan fasting on serum MDA levels are shown in Figure 4B, which reveals that Ramadan fasting for 4 weeks without vitamin E supplementation resulted in significant reductions in serum MDA by 52.2 and 39.6% in control and diabetic groups (P<0.05). Supplementation of fasting with vitamin E lowered serum MDA even more significantly by 57.6 and 44.0% in control and diabetic groups the reductions produced by Ramadan fasting alone were statistically indistinguishable from the corresponding reductions induced by Ramadan fasting and supplementation of vitamin E in the two groups (P>0.05). Six weeks post-Ramadan, serum MDA levels remained significantly lower than the corresponding baseline pre-fasting values by 19.7 and 20.9% in control and diabetic groups. These post-fasting MDA levels

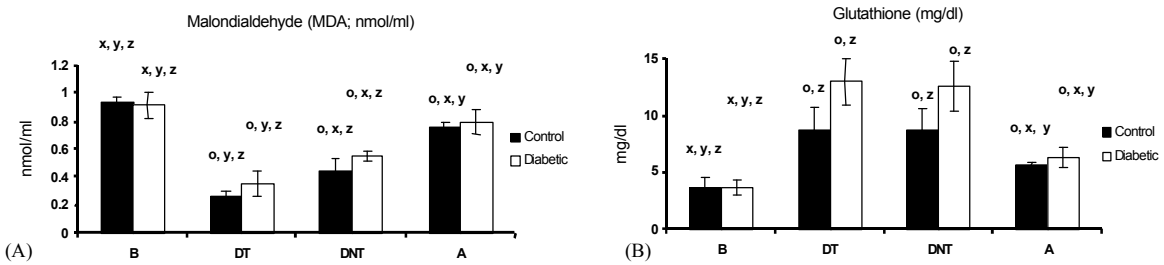

Fig. 4: Effects of Ramadan fasting on glutathione (GSH) and MDAChanges in GSH (A) and MDA (B) for the control group (black bars) and the diabetic group (white bars) one week before fasting Ramadan (B on x axis), 4th week of Ramadan fasting with vitamin E supplementation (DT), 4th week of Ramadan fasting without vitamin E supplementation (DNT) and 6 weeks after Ramadan fasting (A). Data are presented as means ± SEM. One-way ANOVA followed by Tukey's test were used to compare the changes during DT; the changes during DNT and the changes at A from the baseline pre-fasting level of the measured parameter. A value of $P < 0.05$ was considered significant from ^{o}B, ^{x}DT, ^{y}DNT and ^{z}A respectively. The independent-samples t-test was used to compare baseline values between the control and the diabetic groups and levels of the control and diabetic subgroups during Ramadan and 6 weeks post-Ramadan; a value of $*P < 0.05$ was considered significant from the diabetic group/subgroup.

were however significantly higher than the corresponding levels during the 4th week of Ramadan fasting with or without vitamin E supplementation in the two study groups ($P<0.05$). There were no statistically differences in serum MDA between the two groups during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

Blood Reduced Glutathione (GSH): Table 1 and Figure 4A show that the baseline pre-fasting blood glutathione (GSH; mg/dl) levels of the (c) group were statistically indifferent from the corresponding levels in the (d) group; ($P > 0.05$). Figure 4A demonstrate the effects of Ramadan fasting on blood GSH in the two groups. Interestingly, Ramadan fasting for 4 weeks with no vitamin E supplementation raised blood GSH levels very significantly by 139.4 and 241.4% in control and diabetic groups($P<0.05$). Similarly, Ramadan fasting supplemented by vitamin E elevated blood GSH levels much significantly by 141.1 and 251.4% in control and diabetic groups ($P<0.05$). Elevations in blood GSH produced by Ramadan fasting alone were statistically indistinguishable from the elevations induced by Ramadan fasting supplemented by vitamin E in the two study groups. Six weeks post-Ramadan, the blood GSH levels were still significantly higher than their corresponding baseline pre-fasting values by 54.7 and 70.3% in control and diabetic groups ($P<0.05$). Furthermore, the blood GSH levels during the 4th week of Ramadan fasting with or without vitamin E supplementation were significantly higher than the corresponding 6-weeks post-Ramadan fasting values in the two study groups. However, there were no statistically differences in blood GSH between the two groups during the 4th week of Ramadan fasting with or without vitamin E supplementation and also at 6-weeks post-fasting.

DISCUSSION

In our study, both diabetic patients and control group showed insignificant reduction in body weight during Ramadan as compared to before Ramadan with or without vitamin E supplementation This finding agree with Aksungar *et al*. [26]. and Adlouni *et al.* [27]. This could be attributed to the lower energy intake during Ramadan. Although other studies showed that weight reduction has been shown among controls but not in diabetics. A review of literature showed controversy about weight changes in diabetics during Ramadan. Some studies show weight gain instead of loss[28],while others show no change [15]. It has been attributed to reduced daily activities by diabetics in fear of hypoglycemia. It has also been reported that overweight persons lose more weight than normal or underweight subjects [29].

Fasting blood sugar showed significant reduction in diabetic group but not in control group. This was reconfirmed by HbAIc which showed significant improvement among diabetic group and there was more reduction in FBG after vitamin E intake. This can be explained by decreased carbohydrate intake and decreased number of meals during Ramadan and among diabetics increased gluconeogenesis to keep blood sugar in normal rang. [30, 31]. These findings agree with Khaled *et al*. [32], who found significant decreases in HbA1c during Ramadan. However other studies have however reported no change in the mean HbA1c during Ramadan fasting [33].

In our study both diabetic patients and control group showed insignificant reductions in blood urea, serum creatinine and ACR and parallel insignificant elevations in creatinine clearance. The mechanism by which fasting results in these changes needs to be explained, in spite of the expectation of elevation of blood urea, serum

creatinine and the reduction of GFR and creatinine clearance due to dehydration and starvation associated with fasting but fasting Ramadan is only from dawn to sunset (12-14 hour), Furthermore, the blood and urine samples were collected in the early morning and it seems that participants were relatively well hydrated in the early morning and it could be also that participants had reduced their dietary intake of proteins during Ramadan. Interestingly, after supplementation with vitamin E there were more reductions in serum creatinine, ACR and elevations in creatinine clearance in the two studied groups but statistically insignificant.

In this study, it was cleared that fasting Ramadan with or without vitamin E supplementation resulted in minor insignificant changes in the serum electrolytes Na, K, Ca and P and their values stayed within the proper physiological range in the two study groups.

These findings are generally consistent with the results of a recent study reporting no changes in serum electrolytes during fasting [30]. And with the results of several studies conducted on kidney transplant patients and showed no significant changes in the serum values of creatinine, calcium, protein, sodium, potassium, urinary protein excretion or estimated GFR during fasting [34]. These studies thus suggest that fasting during the month of Ramadan is safe and has no significant harmful effects on kidney transplant recipients with normal renal function.

Fasting Ramadan has also been shown to reduce oxidative stress in the body. Our results reported that Ramadan fasting resulted in significant reductions in the mean levels of MDA and parallel significant elevations in the mean values of blood GSH in the two groups. Supplementation of vitamin E resulted in slightly more reductions in the MDA and more elevations in the GSH levels in the two groups during fasting but statistically insignificant. This confirms that fasting ameliorates oxidative stress and that this effect was primarily due to fasting with additional effects of vitamin E.

How fasting Ramadan reduces oxidative stress needs to be elucidated; the high intake of fresh vegetables and fruits rich in anti-oxidant during Ramadan could be a possibility. The maintained low levels of MDA and high values of GSH at 6-weeks post-fasting could be attributed to prolonged effects of vitamin E.

Our results are generally consistent with the results of recent studies reported significantly lower MDA levels during fasting in healthy males and females [26] and healthy pregnant women [35] during Ramadan fasting.

CONCLUSION

Based on these findings, it was concluded that fasting during the month of Ramadan is relatively safe and devoid of any serious complications among stable diabetic patients provided they are properly educated about drug regimen adjustment, diet control, daily activities and possible complications and how to deal with them. It is beneficial as it leads to body weight reduction and improvement of glycaemic control. Rather it has favorable effects on renal function and significantly lowers oxidative stress in the body Benefits of fasting can be explained to them so that they maintain dietary control in future.

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