APPENDICES
# APPENDIX I

## METABOLIC PERTURBATIONS IN TYPE 2 DIABETIC PATIENTS DURING RAMADAN FASTING

<table>
<thead>
<tr>
<th><strong>PROFORMA</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAEMATOLOGICAL TESTS</strong></td>
</tr>
<tr>
<td>RBC</td>
</tr>
<tr>
<td>WBC</td>
</tr>
<tr>
<td>Hb</td>
</tr>
<tr>
<td>HCT</td>
</tr>
<tr>
<td>PLT</td>
</tr>
<tr>
<td><strong>BIOCHEMICAL TESTS</strong></td>
</tr>
<tr>
<td>FBG</td>
</tr>
<tr>
<td>PPBG</td>
</tr>
<tr>
<td>HbA1c</td>
</tr>
<tr>
<td>MBG</td>
</tr>
<tr>
<td>TC</td>
</tr>
<tr>
<td>TG</td>
</tr>
<tr>
<td>HDL- cholesterol</td>
</tr>
<tr>
<td>LDL- cholesterol</td>
</tr>
<tr>
<td>Blood urea</td>
</tr>
<tr>
<td>Serum creatinine</td>
</tr>
<tr>
<td>Uric acid</td>
</tr>
<tr>
<td>Bil. D</td>
</tr>
<tr>
<td>Bil. T</td>
</tr>
<tr>
<td>Total protein</td>
</tr>
<tr>
<td>Albumin</td>
</tr>
<tr>
<td>Globulin</td>
</tr>
<tr>
<td>A/G ratio</td>
</tr>
<tr>
<td>SGOT</td>
</tr>
<tr>
<td>SGPT</td>
</tr>
<tr>
<td>ALP</td>
</tr>
<tr>
<td>CBMN Assay</td>
</tr>
<tr>
<td>Urine Microalbumin</td>
</tr>
</tbody>
</table>
APPENDIX II

REQUEST FOR PARTICIPATION

I, Ms. Sandhya A M, request you to be kind enough to volunteer as a subject in the research project entitled "METABOLIC PERTURBATIONS IN TYPE 2 DIABETIC PATIENTS DURING RAMADAN FASTING" under the supervision of Dr. J.K Mukkadan, Research Director, Little Flower Medical Research Centre. No invasive procedure will be undertaken and no drugs will be given as a part of the study. We will be collecting only Urine and Blood samples for the investigation and the results will be given to you or to your clinician for treatment purpose. Your identity will not be disclosed to anyone without your permission and the data collected will be utilized only for research for the benefit of patients. You have the right to withdraw from the study at any moment and the data so far collected will be utilized only with your permission.

Note: The request letter will be printed in English as well as in the language of the subjects. If the subject is illiterate, it will be read by one of the close relatives of the subject in his presence.

Ms. Sandhya A M
Investigator.

169
APPENDIX III
CONSENT LETTER OF SUBJECTS

I have no objection in participating in the study entitled "METABOLIC PERTURBATIONS IN TYPE 2 DIABETIC PATIENTS DURING RAMADAN FASTING". I understand that my identity will not be disclosed to anyone without my permission and the data collected will be utilized only for research for the benefit of the patients. I have the right to withdraw from the study at any moment and the data so far collected will be utilized only with my permission.

Note :- The consent letter will be printed in English as well as in the language of the subjects. If the subject is illiterate, it will be read by one of the close relatives of the subject and will be signed and or thumb impressed by both of them.

Signature / thumb impression of the subjects

Name

Signature of the relative

Name and relation

170
APPENDIX IV

ETHICAL CLEARANCE CERTIFICATE

INSTITUTIONAL ETHICS COMMITTEE,
Dr. SURESH’S DIABCARE INDIA.

[Prof] Dr. P.V Narayanan [Chairman],
Prof & HOD, Pharmacology & Vice
Principal, Govt. Medical College,
Calicut. Ph: 09946316580

[Prof] Dr. T. Vijayakumar [Member],
CChem FRSC [London] FIMSA FABNIS,
FICS, HOD, Biochemistry and
Physiology, Chief Basic Medical
studies, Educare Institute of Dental
Science, Chottirampuram, Malappuram. 
Ph: 9447141307

[Prof] Dr. Ram Manohar [Member],
Prof., Oral Pathology, Educare Institute
of Dental Science, Chottirampuram,
Malappuram. Ph: 9895055663

Adv. P. Madhavan Kutty Menon
[Member] Vigilance Tribunal [Ltd],
Calicut, Ph: 9349111526

Mrs. C K Renuka Devi [Member]
Councillor, Calicut Corporation

Dr. E Sreekumaran [Member] Reader
in Physiology, Dept of Life Sciences,
University of Calicut, Ph: 9539254721

Dr. P. Suresh Kumar [Member]
Secretary, Chief Consultant
Diabetologist, Dr. Suresh’s Diabcare
India, Calicut. Ph: 9744056000

Ms. Reshmi J [Member],
Administrator, Dr. Suresh’s Diabcare
India, Calicut. Ph: 9447267433

ETICAL CLEARANCE

IEC/DIABCARE/1/2014
20/6/2014

To,
Ms. Sandhya A M

The PhD thesis titled "Metabolic Perturbations in Type 2 Diabetic
Patients during Ramadan Fasting" by Sandhya A M on scrutiny by the
Institutional Ethics Committee of Dr. Suresh’s Diabcare India, has been given
ethical clearance to conduct the study for the stipulated period.

please inform the ethic committee when the study is complete.

Member Secretary

Institutional Ethics Committee

Dr. SURESH KUMAR MD, PhD

A mission with a vision to care & cure diabetics
APPENDIX V
List of Publications


Awards

1. Best oral presentation award at the first international conference on “Neutraceuticals and Chronic Diseases” INCD 2016, jointly organized by Society for Translational Cancer Research and Indian Institute of Technology, Guwahati.
APPENDIX VI

PAPER PRESENTATION CERTIFICATE I

National Conference cum Workshop on Advances in Clinical Research

CERTIFICATE

This is to certify that Mr. Ms./Dr. Sandhya A.M. has participated/presented Scientific Paper in the National Conference cum Workshop on Advances in Clinical Research held at MES Medical College, Perinthalmanna on 9th and 10th of August 2014.

Dr. George Abraham
Secretary, S.O.K.

Dr. T. Vijayakumar
Organizing Secretary

Dr. D.M. Vasudevan
Chairman

Dr. Jithesh T.K.
Convenor
APPENDIX VI
PAPER PRESENTATION CERTIFICATE II

SOUTHERN REGIONAL CONFERENCE OF ASSOCIATION OF CLINICAL BIOCHEMIST OF INDIA
NATIONAL CONFERENCE ON ADVANCES IN LABORATORY PRACTICE

CERTIFICATE OF SCIENTIFIC PRESENTATION

This is to certify that Mr./Ms./Dr. Sandhya AM has made a Scientific Presentation (Oral / Poster) in the National Conference on Advances in Laboratory Practice held at MES Medical College, Perinthalmanna, Malappuram District, Kerala, on 13th and 14th of June 2015.

Dr. T. Vijayakumar
Organizing Chairman

Dr. Jithesh TK
Organizing Secretary

Dr. Antony George
Convener

Council Observer
APPENDIX VIII

PAPER PRESENTATION CERTIFICATE III

First International Conference On
“Nutraceuticals and Chronic Diseases-2016”
(INCD 2016)

Certificate of Participation

This is to certify that Mrs. Sandhya A. M. has delivered a talk at the First International conference on “Nutraceuticals and Chronic Diseases” (INCD-2016) jointly organized by Society for Translational Cancer Research and Indian Institute of Technology Guwahati in Cochin, Kerala, India, from 9th – 11th September, 2016.

Organizing Secretary
Ajaikumar B. Kunnumakkara

Organizing Secretary
Bharat B. Aggarwal

Convenor
Oommen V. Oommen

Chairman
Perumana R. Sudhakaran
APPENDIX IX
AWARD CERTIFICATE

First International Conference On

“Nutraceuticals and Chronic Diseases-2016”
(INCD 2016)

Certificate

This is to certify that Ms. Sandhya A. M. has received the Best Oral Presentation Award at the First International Conference on “Nutraceuticals and Chronic Diseases” (INCD-2016) jointly organized by Society for Translational Cancer Research and Indian Institute of Technology Guwahati in Cochin, Kerala, India, from 9th – 11th September, 2016.

Organizing Secretary
Ajaikumar B. Kunnumakkara
APPENDIX X
PUBLICATION 1

ANTHROPOMETRIC AND BIOCHEMICAL PROFILE OF DIABETES PATIENTS AND ITS RISK FOR CARDIOVASCULAR DISEASE

Sandra A M, Mukkadan J K and Suresh Kumar P

1. Research scholar, Little Flower Medical Research Centre, Kerala.
2. Research Director, Little Flower Medical Research Centre, Angamaly, Kerala.
3. Diabetic India Ltd. Calicut, Kerala.

Manuscript Info

Abstract

Diabetes mellitus (DM) is a major risk factor for the development of cardiovascular disease (CVD). Due to the ever increasing incidence of both DM and CVD were coexistence of these disorders, numerous agents have been developed over the years to target complications. The present study focuses on the anthropometric and biochemical profile of diabetes patients and its risk for cardiovascular disease. Seventy two study subjects were diagnosed as diabetes mellitus by American Diabetes Association's (ADA) criteria and sixty healthy subjects without any chronic illness were selected as control for the present study. Levels of fasting blood glucose, cholesterol, LDL, triglycerides were found to be significantly high compared to controls and the levels of HDL were lower than that of the controls, thus constituting a risk factor for CAD. The correlation between anthropometric and biochemical profile shows the importance of CVD in subjects with diabetes. Early screening for DM and aggressive management of risk factors is important to optimizing the outcome of patients with DM. These results indicate the risk of developing CVD was higher in diabetic subjects than non-diabetic subjects.

Introduction:-

Diabetes Mellitus (DM) is a chronic disorder resulting from a number of factors in which an absolute or relative deficiency of insulin or its function. It is projected that by the year 2025, India alone would have 57 million diabetics mainly of type 2 diabetes constituting 90% of the diabetic population (Ramachandran et al., 2002). The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Type 2 diabetes is commonly associated with obesity, hypertension, CVD and lipid abnormalities. Glycated Hemoglobin (HbA1c) is a well known marker for long term glycemic control. It indicates mean blood glucose level and predicts the risk for developing complications in diabetic population (Mahato et al., 2011).

Cardiovascular disease (CVD) is the most threatening complication of diabetes. While the leading cause of mortality worldwide, it is three to four times more common in diabetics than non-diabetics individuals (Barr et al., 2007). The most common disorder that besets type 2 diabetic subjects is coronary heart disease (CHD). Irrespective of the ethnic background the risk for CHD among diabetic subjects is greater by a factor of 2 to 4 compared to non-diabetic subjects (Deepa et al., 2002).

Approximately 58% of diabetes and 21% of ischemic heart disease globally are attributable to a body mass index (BMI) above 21 kg/m² (WHO, 2002). An increase in body fat is generally associated with an increase in risk of metabolic diseases such as type 2 diabetes mellitus, hypertension and dyslipidaemia (WHO, 2007). In addition, many patients with these metabolic diseases are either overweight or obese (Bays et al., 2007). In 2008, more than 200 million men and approximately 300 million women were obese. Overweight and obesity is one of the leading
risk factors for mortality, estimated to account for 23% of the ischaemic heart disease burden (WHO, 2012). It results in the deterioration of the entire cardiovascular risk profile (NCEB, 2002).

General and central obesity are associated with CVD risk (Ryan et al., 2008; Sato et al., 2010; Ying et al., 2010). Currently used general and central obesity anthropometric measures for assessing adiposity related risk include BMI, waist circumference (WC), hip circumference (HC), waist-to-hip ratio (WHR) and body adiposity index. BMI or WC is most commonly used to measure body fatness (Park and Kim, 2012).

Anthropometric parameters are commonly used as research tools to assess the noncommunicable disease risk factors in the populations as they are inexpensive and easy to monitor at the community level (Jaap et al., 2001). Various studies have shown that anthropometric parameters such as BMI, waist circumference (WC), waist-hip ratio (WHR), and waist height ratio (WHR) are useful indicators for predicting incidence of type 2 diabetes in populations (Stevens et al., 2001; Lincoln et al., 2002; Nahar et al., 2012; Sandhya et al., 2015).

Accumulation of fatty deposits lead to the narrowing of blood vessels (e.g., atherosclerosis) obstructing blood supply to the vital organs like brain, heart etc (Kolodgie et al., 2003) and causing stroke, cardiac failure etc. Lipid changes can cause deposits of fatty plaque in arteries and results in the CVD (Reaven et al., 2004). Nevertheless, glucose plays a crucial role by glycating the proteins in whole body including LDL. Therefore, any disturbance in the normal metabolism of glucose and/or glycation, particularly that resulting from altered insulin functionality has serious consequences (Nosheen et al., 2012).

Patients with type 2 diabetes mellitus associated dyslipidemia remain exposed to a high residual risk of CVD complications, even if they are treated with current standards of care, making this one of the major unmet needs in the treatment of patients with diabetes. An understanding of the complex interplay of how treating dyslipidemia reduces the risk for CVD events in patients with type 2 diabetes mellitus and an ability to assess at-risk patients is necessary to ensure the most appropriate treatment strategies are implemented (Kamatreddy et al., 2013). Along with dyslipidemia, elevated HbA1c was regarded as risk factor for CVD. In diabetic population for every 1% increase in absolute HbA1c risk of CVD was increased by 18% (Selvin et al., 2004).

As the prevalence of DM is rising, it will be a major contributory risk factor of increased CVD events. Measurement of surrogate markers of CVD is of utmost importance for circumventing CVD end-points, especially in diabetic subjects (Tabatabaei et al., 2014). Anthropometric and biochemical parameters have evolved into reliable indicators for predicting the incidence of cardiovascular disease as they are inexpensive and easy to monitor. The present study describes anthropometric and biochemical profiles of Diabetes Patients and its risk for Cardiovascular Disease.

Material and Methods:-
Seventy two study subjects were diagnosed as diabetes mellitus by ADA criteria and sixty healthy subjects without any chronic illness were selected as control for the present study. The study was conducted at Diabcare India Ltd, Calicut, Kerala. After taking descriptive history, demographic and anthropometric characters were recorded using a proforma. Four ml of fasting venous blood was collected after obtaining proper written consent from the subjects. One ml of blood sample transferred to a fluoride tube and performed the blood sugar estimation and, HbA1c is estimated using the Biorad D10 HPLC analyser. The remaining 3 ml blood transferred to a plain tube and allowed to clot, the serum was used to estimate the lipid profile using Beckman AU 480 analyser. HbA1c is estimated using the Biorad D10 HPLC analyser. Biorad quality control materials were used for monitoring the quality.
Results:-
Table 1: Anthropometric and biochemical characteristics among subjects:-

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study subjects</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>27.4±4.44</td>
<td>24.6±3.36</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>129.5±11.97</td>
<td>98.9±12.28</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.4±2.55</td>
<td>6.0±1.52</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>210.8±43.71</td>
<td>202.9±46.02</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>36.7±16.51</td>
<td>40.6±15.53</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>148.6±29.80</td>
<td>131.3±33.61</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>161.7±26.16</td>
<td>143.7±35.04</td>
</tr>
</tbody>
</table>

Among the seventy two study subjects, 51.92% (n=37) were males and 48.08% (n=35) were females. In the case of sixty control subjects 68.06% (n=40) were males and 31.94% (n=20) were females. The age of the study subjects ranged from 30 to 72 years with a mean age of 58.90. The age of the control subjects ranged from 38 to 65 with a mean age of 53.48.

Majority of the study subjects belonged to rural area (69.23%) (n=50), 21.15% (n=16) belonged to urban area and the rest belonged to coastal area (7.69%) (n=6). Majority of the study subjects belonged to average socioeconomic status (73.07%) (n=53). History of infertility/subfertility was reported by 7.69% (n=6) of study subjects. 61.53% (n=44) of the study subjects reported history of chest pain in early years.

The observed mean BMI of the study subjects was 27.4 kg/m² and the control subjects showed a mean BMI of 24.67 kg/m². The mean FBS of the study subjects was 129.5±11.97 mg/dl and that of the control subjects was 98.9±12.28 mg/dl (p<0.001). The mean HbA1c of the study subjects shown 9.4±2.55 and that of the control subjects shown 6.0±1.52 (p<0.001). The mean TC level of the study subjects was 210.8±43.71 mg/dl and that of control subjects was 202.9±46.02 mg/dl. This shows a slight difference among the study and control subjects but this difference was not statistically significant (p=0.142). The mean value of HDL was 36.7±16.51 mg/dl in study subjects and the mean value of HDL was 40.6±15.53 mg/dl in control subjects (p<0.001). Similarly the mean value of LDL was 148.6±29.80 mg/dl and 131.3±33.61 mg/dl in study and control subjects respectively. No statistically significant difference was shown between the study and control subjects regarding the mean LDL level (p=0.021). The study subjects shown a mean triglycerides value of 161.7±26.16 mg/dl and control subjects showed a mean triglycerides value of 143.7±35.04 mg/dl (p<0.001):Table1.

Discussion:-
The diabetic condition contributes for initiation and progression of micro and macro vascular complications in diabetes (Maritim et al., 2003). Of all, cardiovascular complications are the leading cause of mortality and morbidity in diabetics. Framingham heart study (1974) demonstrated a direct association between diabetes and heart failure (Saxena, 2002). Epidemiological studies published in recent years suggest that postprandial blood glucose might be an independent risk factor of cardiovascular disease (Bonora and Muggeo, 2001). In the present study the fasting blood glucose levels were higher in majority of patients constituting a risk factor for CAD.

The most important risk factors contributing to the development of CHD include lipid disorders. Elevated levels of cholesterol, LDL and triglycerides as the risk factors in diabetes have been demonstrated in several studies (Castelli, 1988; Meckes e al., 1989; Gopinath et al., 1994; Surekha Rani et al., 2005). In the present study the estimated levels of cholesterol, LDL, triglycerides were found to be significantly high compared to controls and the levels of HDL were lower than that of the controls.

Rural populations who consume low fat and high carbohydrate diets have low HDL cholesterol and high triglyceride levels (Gupta et al., 2002). Gupta et al. (2009) has shown that total cholesterol and triglycerides were higher while HDL levels were lower in individuals with ischemia heart disease (IHD) than in those without IHD. A higher prevalence of low HDL cholesterol and an elevated triglyceride level was found in the rural than in the urban
population by Chadha et al., (1997). The findings of the present study agree with those of Gupta et al., (2009) and Panwar et al., (2011) who reported high cholesterol and triglycerides, with low HDL in their study subjects.

The results of the United Kingdom Prospective Diabetes Study (UKPDS) showed a significant association between increased risk of CVD in diabetics with hyperlipidaemia and increased HbA1c levels (Turner, 1998). In the present study, it was observed that the mean HbA1c level of the study subject was 9.42 ±5.15 and in control subject the mean value was 6.06 ±1.52.

In the present study, anthropometric measurements (Height, Weight, BMI) showed a significant level of risk for CVD. The available data from the present study also suggest a significant cardiovascular burden in patients with diabetes.

Conclusion:-
In short, the present study has shown that diabetes is correlated with higher serum lipid concentrations, which indicates increased cardiovascular risk. The correlation between anthropometric and biochemical profile shows the importance of CVD in people with diabetes. In order to achieve this, it is necessary to define specific anthropometric cutoff points. Furthermore, it is essential that serum lipids be monitored if adequate metabolic control is to be achieved and maintained. The present data confirm that a high degree of CVD risk factors were prevalent in patients with diabetics, as shown by anthropometric and biochemical variables. Therefore, the current study suggests that these parameters be integrated in regular assessment to determine various CVD risks.

References:-


Altered Baseline Somatic DNA Damage in Patients With Type 2 Diabetes After A Non-Experimental Intermittent Fasting.

Sandhya AM1 Shakuntha GA2 Suresh Kumar P3 Mukkadan JK4

1Research Scholar, Little Flower Medical Research Centre, Angamali
2Reader, Department of Oral Medicine and Radiology, Mah Institute of Dental Sciences and Hospital, Mahé.
3Consultant Diabetologist, DIABECARE India Ltd, Calicut.
4Research Director, Little Flower Medical Research Centre, Angamali

ABSTRACT

Diabetes mellitus can lead to the generation of ROS leading to increased oxidative stress which worsen diabetic complications and DNA damage. A change in circadian pattern of food intake and sleeping has a major role in progression of this disease. Ramadan fasting is considered as an intermittent fasting therefore it is important to assess the somatic DNA damage in type 2 diabetic patients who observe this fast. The present study assessed extent of somatic DNA damage in type 2 diabetic patients who observe Ramadan fasting by cytokinesis block micronucleus assay method. The micronucleus frequency was increased in test group and control groups. However comparisons of the difference in test group with control group was not significant. In diabetic non fasting group there was a marked increase in the micronucleus frequency. But similar change was not noticed in diabetic fasting group. The possible reason could be that contribution of fasting to DNA damage is negligible in diabetic patients. It is also revealed that diabetic subjects have more DNA damage when compared to nondiabetic subjects. Ramadan fasting does not alter DNA damage in Type 2 diabetic patients.

Keywords: DNA damage, Ramadan fasting, CBMN assay, Diabetes Mellitus.

*Corresponding author
INTRODUCTION

Diabetes Mellitus (DM) refers to a group of metabolic disorders with multiple etiologies. It is characterized by chronic hyperglycemia which results from defects in insulin secretion, insulin action, or both. The defect in the insulin action and secretion leads to various metabolic derangements in carbohydrate, protein, and lipid metabolism as well as water and electrolyte regulation. There are mainly two types of DM based on etiology, type 1 and 2. In type 1 DM there is a complete absence or severe deficiency of insulin secretion or the insulin is defective. Type 2 diabetes is characterized by insulin resistance, a relative insulin deficiency and increased glucose production, which encompasses 90-95% of the diabetic individuals [1].

Reactive oxygen species (ROS) which is generated in the body is normally defended by the antioxidant system of the body [2, 3]. DM can lead to the generation of ROS leading to increased oxidative stress which has a crucial role in diabetic complications and DNA damage [4-6]. Several factors such as lifestyle modifications can lead to the development of DM [7]. The change in the number, timing and portioning of the meals can affect various metabolism [8]. Ramadan fasting, observed by Muslims, is considered as intermittent fasting, because the abstinence from food and drink is only between dawn and sunset [9]. Not only food and drinks, drug intake is also restricted during fasting hours of Ramadan [8]. It is important to assess the somatic DNA damage in type 2 diabetic patients while observing this type of non-experimental intermittent fasting as there are alarmingly increasing number of diabetic observants of Ramadan fasting [9].

MATERIALS AND METHODS

The study subjects were selected from the patients and their relatives who visited Dr. Suresh’s Diabcare India, a holistic centre for diabetes and diabetic foot care, Calicut, Kerala, India. Informed consent was obtained from the volunteers of the study and detailed demographic data were collected. Relevant medical history and medications if any were also recorded. Height and weight measurement for calculation of body mass index (BMI) were also done in subjects. The blood pressure of the subjects was also recorded to verify hypertension. The study was approved by the Institutional Ethics Committee. The blood samples collected from the subjects were considered as the material for the present study. Clinically proven diabetic and non-diabetic subjects for the present study were categorized as following.

Group A: Patients who observe Ramadan fasting (considered as test group for the present study)

Group B: Diabetes patients who do not observe Ramadan fasting (considered as diabetic control group for the present study)

Group C: Non-diabetic people who observe Ramadan fasting (considered as fasting control group for the present study)

Group D: Non-diabetic people who do not observe Ramadan fasting (considered as normal control group for the present study).

Non-smoking, non-alcoholic male subjects above 18 yrs old, without any auto immune diseases, cancer or any chronic or acute infections, who follow a non-vegetarian diet were included in this study. Subjects above 60 yrs of age and females were excluded from the study.

SAMPLE SIZE

One hundred and fifty three subjects participated in the present study. There were 41 subjects in Group A, 49 subjects in Group B, 34 subjects in Group C and 38 subjects in Group D. The blood samples were collected three days before and three days after Ramadan fast (sample I and II respectively). Five ml of venous blood was collected aseptically from all the subjects by venipuncture in heparinized vacutainers.
METHODS

CYTOKINESIS BLOCK MICRONUCLEUS ASSAY (CBMN ASSAY) [10, 11]

The micronucleus (MN) assay detects DNA damage and considered as a best established biomarkers of chromosome damage. After isolating the lymphocytes, the cell pellet was suspended in RPMI 1640 medium and centrifuged for 10 minutes. Removed the supernatant and cultured the lymphocytes in sterile bottles using RPMI 1640 medium containing 15% foetal calf serum and incubated for 72 hours at 37°C. Lymphocytes were stimulated to divide with phytohaemagglutinin (PHA). Forty four hours after PHA stimulation, cytochalasin-B was added to the cultures to give a final concentration of 4.5μg/ml and this block the cytokinesis. After 70 hrs, the cells were harvested and treated with hypotonic solution of 0.075 M KCl. Then it is fixed with a mixture of methanol-glacial acetic acid (3:1) and dropped onto clean coded microscopic slides followed by Giemsa staining technique.

SCORING OF MICRONUCLEI

A total of 1000 binucleated cells were scored for each subject on coded slides to determine the frequency of binucleated lymphocytes with micronuclei. The following criteria were satisfied while scoring the micronuclei.

- The diameter of the MN was less than one-third of the main nucleus
- MN was either separated from or marginally overlapped with main the nucleus with a clear nuclear boundary.
- The staining of MN was similar to that of main nuclei.

STATISTICAL ANALYSIS

The data analysis was performed using SPSS version 22.0. Quantitative Variables were expressed as Mean ± SD. Comparison of quantitative variables of sample I and II were analysed by paired t test. Comparison of quantitative variable among more than two groups were analysed by ANOVA. A p-value of <0.05 was considered as the level of significance.

RESULTS

There were 153 male subjects in the study population in which the mean age as well as the BMI of the subjects in different groups was comparable. None of the subjects selected were hypertensive [Table 1].

<table>
<thead>
<tr>
<th>Variables</th>
<th>A (N=41)</th>
<th>B (N=40)</th>
<th>C (N=34)</th>
<th>D (N=38)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>52.2±18.2</td>
<td>49.4±7.0</td>
<td>46.0±10.4</td>
<td>41.8±12.0</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1±3.0</td>
<td>24.5±2.9</td>
<td>25.2±3.8</td>
<td>25.1±4.0</td>
<td>NS</td>
</tr>
<tr>
<td>SBP</td>
<td>132±9±19.5</td>
<td>123±0±11.3</td>
<td>127±5±15.2</td>
<td>123±3±18.6</td>
<td>NS</td>
</tr>
<tr>
<td>DBP</td>
<td>86±6±19.0</td>
<td>82±3±10.1</td>
<td>82±4±8.3</td>
<td>81±6±16.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI- Body mass index, SBP- systolic blood pressure, DBP- diastolic blood pressure; A=diabetic fasting group, B=diabetic non fasting group, C= non diabetic fasting group, D= non diabetic non fasting group, NS=non significant

The mean CBMN frequency in group A in sample I and II was 14.2±1.1 and 13±0.9 respectively with mean increase of 0.1 in sample II as compared to sample I, which is statistically not significant. In group B mean CBMN frequency value was 13.6±0.9and 14±0.8 in sample I and II respectively, with a significant increase of 1.8(p<0.01). Mean CBMN frequency in group C subjects was 9.3±2.8 and 9±0.8 in sample I and II respectively, with non significant increase in CBMN frequency of 0.2 in sample II as compared to sample I. No significant difference in the micronuclei frequency was observed between sample I and II of group D (Table 2). The difference in mean CBMN frequency level in sample I as compared to sample I in group A and group B was compared; the difference was statistically significant(p<0.002). Similarly the difference in MN of I and II of
group A and group C were compared and found statistically non significant. The difference in mean CBMN frequency level in sample II as compared to sample I in group A and group D was compared; the difference was statistically not significant [Table 3].

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>Group</th>
<th>Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14.21 ± 1.1</td>
<td>B</td>
<td>14.39 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>B</td>
<td>13.61 ± 0.9</td>
<td>C</td>
<td>9.3 ± 2.8</td>
<td>0.01</td>
</tr>
<tr>
<td>C</td>
<td>9.3 ± 2.8</td>
<td>D</td>
<td>9.6 ± 2.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

A = diabetic fasting group, B = diabetic non-fasting group, C = nondiabetic fasting group, D = nondiabetic non-fasting

In sample before fasting, In sample after fasting *NS = non-significant

Table 3: Post Hoc test – Multiple comparison of the difference in I & II of micronuclei frequency in test group vs control groups

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Comparison among groups</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micronuclei Frequency in 1000 cells</td>
<td>A vs B</td>
<td>&lt;0.05 (0.02)</td>
</tr>
<tr>
<td></td>
<td>A vs C</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td></td>
<td>A vs D</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

A = diabetic fasting group, B = diabetic non-fasting group, C = nondiabetic fasting group, D = nondiabetic non-fasting

DISCUSSION

The present study assessed the difference in the extent of somatic DNA damage in diabetic and healthy males before and after Ramadan fast which extended for 30 days. Average duration of fasting was 14±1 hrs per day. There were two meals per day and were no restrictions on the energy intake during non-fasting hours. CBMN assay was used to measure the extent of somatic DNA damage in this study. The main question of interest in this study was to analyze the alterations in the cellular oxidative functions as the Ramadan intermittent fasting completely alter circadian pattern of an individual abruptly which continue for a longer term. The present study observed that there was no significant change in DNA damage in diabetic patients after fasting and this coincide with other studies which support that intermediate fasting reduce oxidative stress in type 2 diabetes patients [12] and enhances the ability of nerve cells to repair DNA [13]. In healthy adults no alteration in the markers of oxidative stress was observed in many studies [14, 15] and alleviation in oxidative stress was noted by Faris et al., [16]. The present study is in agreement with these studies as it was also observed that no alteration in DNA damage occurred in healthy males. Fasting has beneficial role in the prevention of cancer as well by protecting the cells from DNA damage and promoting the programmed death of the damaged cells [17]. Previous studies also reported a reduction in markers of oxidative stress with a decrease in body weight [18]. This disparity may be due to the difference in sample size, or lifestyle difference in the study group. Various factors such as timing of sample collection and meals can affect the oxidant concentration [18, 19].

In the present study it was also noticed that diabetic patients without fasting has shown a marked increase in baseline DNA damage after 30 days. This result supports the findings of a previous observation in which significant increase in DNA strand breaks has found in diabetic patients than normal people [20]. Diabetes leads to the increased production of reactive oxygen species which intensifies the oxidative stress leading to DNA damage. The reactive oxygen species damage the cellular macromolecules and modify the DNA [20].

CONCLUSION

It is a well known fact that diabetes has an association with DNA damage and intensifying the diabetic complications [21]. Intermittent fasting enhances defense mechanism of antioxidant system and reduces proinflammatory cytokines [22]. The present study found that Ramadan fasting does not have a major role in augmenting DNA damage in diabetic patients.
REFERENCES


APPENDIX XII

PUBLICATION 3

ISSN: 2320-5407

Journal Homepage: www.journaliar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2139
DOI URL: http://dx.doi.org/10.21474/IJAR01/2139

RESEARCH ARTICLE

EFFECT OF RAMADAN FASTING ON ANTHROPOMETRIC, HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF TYPE 2 DIABETIC PATIENTS - A STUDY ON INDIAN MALE DIABETIC PATIENTS.

Sandhya A M 1*, Suresh Kumar P 3 and Mukkadan JK 2.
1. Research Scholar, department of physiology, Little Flower Medical Research Centre, Angamaly, Kerala, India.
2. Consultant diabetologist and Managing Director, Dr. Suresh’s DiabCare India, Calicut, Kerala, India.
3. Research Director, Little Flower Medical Research Centre, Angamaly, Kerala, India.

Abstract

Manuscript Info

Received: 25 September 2016
Final Accepted: 27 October 2016
Published: November 2016

Key words: Ramadan fasting, Metabolic alterations, Diabetes, Intermittent Fasting

Ramadan fasting is an excellent example of intermittent fasting. During Ramadan not only the food intake but the sleep pattern also alters. In spite of the exemption by the Islamic law many of the type 2 diabetic patients observe Ramadan fasting every year. The sudden change in the lifestyle which includes the change in eating and sleeping pattern can affect the various metabolic parameters in type 2 diabetic patients. The present study evaluated the anthropometric, hematological and biochemical parameters of type 2 diabetic males before and at the end of Ramadan fasting month. There was a significant reduction in the body weight, but blood pressure, total WBC count, total RBC count, hemoglobin, platelet count, fasting blood glucose, post prandial blood glucose, and HbA1c did not alter after Ramadan fasting. Total cholesterol remained unchanged while HDL increased at the end of Ramadan fasting. Triglycerides, LDL and VLDL decreased after Ramadan fasting. Renal function was affected by a significant increase in urca, creatinine and uric acid but was within physiological range. Total and direct bilirubin, total protein, albumin, globulin, ALT and AST did not altered during Ramadan fasting but there was a significant increase in the ALP. In conclusion observing Ramadan fasting does not harmfully affect type 2 diabetic patients.

Copy Right, IJAR, 2016. All rights reserved.

The annual observance of Ramadan fasting is considered as one of the five pillars of Islam. Ramadan fasting differs from other experimental fasting as there is involvement of psychological and sociological aspects. During Ramadan fasting the observers must completely abstain from food, fluids, medications through any route, smoking and sexual activities. Fasting extends from pre-dawn to sunset - the length of the fasting day and month may vary by geographical location and season. There are two major meals consumed during Ramadan month, first one is the preparatory meal taken before pre-dawn and the second one is to break the fast which is taken after sunset. There is no restriction on the calorie and type of food during the non-fasting hours of Ramadan [Azizi et al. 2013]. Even though Ramadan fasting is obligatory for all adult Muslims, the Islamic rules exempt the sick and pregnant ladies from observing fasting. Despite of this exemption majority of the type 2 diabetic patients insist to fast by

Corresponding Author: Sandhya A M.
Address: Research Scholar, department of physiology, Little Flower Medical Research Centre, Angamaly, Kerala, India.
ignoring the advice by the medical practitioners (Al-Arouj et al., 2010). One of the major and modifiable risk factors of diabetes is lifestyle especially the diet, sleep pattern and physical activities (Risserus et al., 2009). During Ramadan fasting Muslims tend to alter their dietary habits which involve changes in eating times and content of food, in addition to fluid deprivation. The altered metabolism in the type 2 diabetic patients may again be affected by the altered diet and sleep pattern during Ramadan fasting (Pinar, 2002; Beshyah, 2009). It is well understood that the reduced fluid intake, the altered food timing and type may affect the metabolism especially the renal and liver functions as there are chances of dehydration and increase in the intake of fat and proteins. There are many studies on the effect of Ramadan fasting on various parameters in diabetic patients. But the results are conflicting. The results may vary according to the ethnicity, dietary habits, and season. Most of the studies on the effect of Ramadan on diabetes were carried out in the Middle East countries. The socio economic status, culture, race and food habit of Middle East countries has a drastic difference from that of the Indian context. The present study for the first time in India assessed the anthropometric, hematological and biochemical parameters of diabetic males during Ramadan fasting without altering their food habits and medications.

Materials and Methods:-
The present study was conducted during the Ramadan month of 2014. The study subjects were selected from Dr. Suresh’s Diabetare India, (A holistic centre for diabeto-cardiology and diabetic foot care) Calicut, kerala, India. Clinically proven type 2 diabetic males, above 18 yrs of old and below 60 yrs old, who informed their willingness to observe Ramadan fasting were included in the present study. The subjects with any auto immune diseases, cancer or any chronic or acute infections, or on prolonged medication for any other purposes and who had recently undergone radiological procedures were excluded from the current study. Those who consume alcohol and ex- and current smokers were also excluded from the current study. Sixty subjects were identified for the present study. Seven of them could not complete their fasting for the whole month. Fifty three subjects appeared for the follow up after Ramadan fasting. All the subjects were from a middle class socioeconomic background, who were having jobs which required physical exertion. All the subjects were informed about the present study and a written consent was obtained from each subjects.

Height and weight measurement for calculation of body mass index (BMI) were done for all the subjects. The blood pressure of the subjects was also recorded to verify hypertension. Urine and blood samples collected from subjects were considered as the materials for the present study. The urine and blood samples were collected one to three (3) days before Ramadan (R1) and on 30th day (R2) of Ramadan. Total 7 ml of venous blood was collected aseptically from all the subjects by venepuncture in two different sessions. The first session was after a minimum of 8 hours fasting and the second session was 2 hours after meal. Blood glucose levels, glycated hemoglobin (HbA1c), lipid profile, markers of renal function, and markers of liver function were estimated using AT-112 PLUS semi-automated clinical chemistry analyzer manufactured by Accurex Biomedical Pvt Ltd. Hematological tests were done by SYSMEX XE-2100 Haematology Automated Analyser. Quality control was done with BIO-RAD Lyphochek Assayed Chemistry Control Level 1 and 2 of Bio-Rad Laboratories. The study was approved by the Institutional Ethics Committee.

The data analysis was performed using SPSS version 22.0. Quantitative Variables were expressed as Mean ± SD. Comparison of quantitative variables before Ramadan fasting and after Ramadan fasting were analysed by paired t test. A p-value of <0.05 was considered as the level of significance.

Results:-
A total of 53 male diabetics were participated in the present study. The blood and urine samples were tested before (R1) and after (R2) Ramadan fasting. All the results were expressed as Mean±SD.

Table 1 shows the changes in anthropometric parameters of diabetic males during Ramadan fasting. There was significant reduction in the mean body weight of the subjects after Ramadan fasting when compared to before Ramadan (p=0.001). From the table 1 it is also clear that the alteration in systolic blood pressure and diastolic blood pressure after observing Ramadan fasting was not significant. Table 2 shows the changes in hematological parameter of diabetic patients after Ramadan fasting. After Ramadan mean values of total WBC count (p= 0.415), RBC (p=0.328), hemoglobin concentration (p= 0.485), hematocrit (p=0.453) and platelet count (p=0.166) did not show any significant change from pre-Ramadan values.
Table 1: Comparison of anthropometric parameters before and after Ramadan fasting (mean±sd)

<table>
<thead>
<tr>
<th>Variables</th>
<th>R1</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>66.6±6.3</td>
<td>65.6±5.9</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>132.9±18.5</td>
<td>132.1±15.62</td>
<td>0.568</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.1±6.9</td>
<td>84.9±6.3</td>
<td>0.513</td>
</tr>
</tbody>
</table>

In this BW = Body weight, SBP= Systolic blood pressure, DBP= Diastolic blood pressure, R1= Values before Ramadan fasting, R2=Values after Ramadan fasting.

Table 2: Comparison of hematology parameters before and after Ramadan fasting (mean±sd)

<table>
<thead>
<tr>
<th>Variables</th>
<th>R1</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (cells/cumm)</td>
<td>7571.6±1904</td>
<td>7272.3±1864</td>
<td>0.415</td>
</tr>
<tr>
<td>RBC (million cells/cumm)</td>
<td>5.02±0.67</td>
<td>5.2±0.71</td>
<td>0.328</td>
</tr>
<tr>
<td>HB (gm%)</td>
<td>14.1±1.1</td>
<td>14.3±1.4</td>
<td>0.485</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>41.3±3.3</td>
<td>41.9±4.1</td>
<td>0.453</td>
</tr>
<tr>
<td>PLT (lakh cells/cumm)</td>
<td>2.48±0.45</td>
<td>2.66±1.01</td>
<td>0.166</td>
</tr>
</tbody>
</table>

In this WBC= White Blood Cells, RBC = Red Blood Cells, HB= Hemoglobin, HCT = Hematocrit, PLT= Platelets, R1= Values before Ramadan fasting, R2=Values after Ramadan fasting.

From table 3 the glycemic status of the test subjects before and after Ramadan fasting can be understood. The mean value of fasting blood glucose (FBG) has slightly decreased from R1 (166.1±32) to R2 (158.6±30.6). But the decrease was not very significant (p= 0.074). The mean values of post prandial blood glucose (PPBG) (p=0.554), glycated hemoglobin (HbA1c) (p=0.565) and mean blood glucose (MBG) (p=0.429) did not change significantly from R1 to R2. Table 4 shows the lipid profile of test subjects before and after Ramadan fasting. The decrease shown in mean value of total cholesterol (TC) after Ramadan fasting was not statistically significant when compared to the pre-Ramadan value (p=0.997). Triglyceride (TG) has shown a significant decrease in the R2 (151.1±40.7) when compared to R1 (164.4±43.0). The mean high density lipoprotein (HDL) cholesterol has increased significantly in R2 (48.2±8.3) when compared to R1 (44.0±4.9) while a significant reduction in low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol was seen in R2 (93.1±35.9, 30.2±8.1 respectively) when compared to R1 (108.8±32.7, 32.8±8.6 respectively).

Table 3: Comparison of blood glucose level before and after Ramadan fasting (mean±sd)

<table>
<thead>
<tr>
<th>Variables</th>
<th>R1</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG (mg/dl)</td>
<td>166.1±32</td>
<td>158.6±30.6</td>
<td>0.074</td>
</tr>
<tr>
<td>PPBG (mg/dl)</td>
<td>242.2±45.6</td>
<td>238.5±54.2</td>
<td>0.554</td>
</tr>
<tr>
<td>HbA1c(gm%)</td>
<td>8.4±0.9</td>
<td>8.3±0.5</td>
<td>0.565</td>
</tr>
<tr>
<td>MBG Mean Blood Glucose(mg/dl)</td>
<td>198.9±41.5</td>
<td>193.1±30.3</td>
<td>0.429</td>
</tr>
</tbody>
</table>

In this FBG= Fasting Blood Glucose, PPBG= Post Prandial Blood Glucose, HbA1c= Glycated hemoglobin, R1= Values before Ramadan fasting, R2=Values after Ramadan fasting.

Table 4: Comparison of lipid profile before and after Ramadan fasting (mean±sd)

<table>
<thead>
<tr>
<th>Variables</th>
<th>R1</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dl)</td>
<td>179.4±39.8</td>
<td>170.5±37.9</td>
<td>0.997</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>164.8±43.0</td>
<td>151.1±40.7</td>
<td>0.013</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>44.0±4.9</td>
<td>48.2±8.3</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>108.8±32.7</td>
<td>93.1±35.9</td>
<td>0.039</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>32.8±8.6</td>
<td>30.2±8.1</td>
<td>0.013</td>
</tr>
</tbody>
</table>

In this TC=Total Cholesterol, TG =Triglycerides, HDL = High density lipoprotein cholesterol, LDL= Low density lipoprotein cholesterol, VLDL= Very Low density lipoprotein cholesterol, R1= Values before Ramadan fasting, R2=Values after Ramadan fasting.

Table 5 describes the status of renal function of type 2 diabetics before and after Ramadan fasting. The markers of renal function like urea, creatinine and uric acid has shown a significant increase the mean values after Ramadan fasting when compared to the pre-Ramadan while microalbumin level decreased significantly after Ramadan fasting. The R1 values of urea, creatinine, and uric acid were 22.5±5.2, 1.03±0.1, 6.1±1.5 respectively, and in R2 the values

734

190
The values of bilirubin levels - total and direct, did not change from R1 to R2 significantly. Total protein (TP) as well as albumin and globulin values also did not show much difference from R1 to R2. The alanine amino transferase (ALT) and aspartate aminotransferase (AST) remained unchanged in R2 when compared with R1. The mean alkaline phosphatase (ALP) has increased in R2 (201.7±43.0) when compared with R1 (154.7±35.4) and the change was significant (p<.001).

**Table 5:** Comparison of markers of renal function before and after ramadan fasting (mean±sd)

<table>
<thead>
<tr>
<th>Variables</th>
<th>R1</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>23.5±5.2</td>
<td>26.2±6.4</td>
<td>0.011</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.03±0.1</td>
<td>1.1±0.3</td>
<td>0.024</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>6.1±1.5</td>
<td>5.5±1.0</td>
<td>0.027</td>
</tr>
<tr>
<td>Microalbumin (mg)</td>
<td>20.4±8.7</td>
<td>15.9±7.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

In this R1= Values before Ramadan fasting, R2= Values after Ramadan fasting.

**Table 6:** Comparison of markers of liver function before and after ramadan fasting (mean±sd)

<table>
<thead>
<tr>
<th>Variables</th>
<th>R1</th>
<th>R2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bil D (mg/dl)</td>
<td>0.3±0.2</td>
<td>0.3±0.1</td>
<td>.270</td>
</tr>
<tr>
<td>Bil T (mg/dl)</td>
<td>0.9±0.2</td>
<td>1.0±0.3</td>
<td>.212</td>
</tr>
<tr>
<td>TP (mg/dl)</td>
<td>6.4±0.6</td>
<td>6.5±0.5</td>
<td>.323</td>
</tr>
<tr>
<td>ALB (mg/dl)</td>
<td>3.6±0.2</td>
<td>3.5±0.3</td>
<td>.126</td>
</tr>
<tr>
<td>GLB (mg/dl)</td>
<td>2.8±0.4</td>
<td>2.9±0.2</td>
<td>.234</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>27.4±9.0</td>
<td>27.9±8.2</td>
<td>.769</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>27.249.1</td>
<td>27.7±11.7</td>
<td>.817</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>154.7±35.4</td>
<td>201.7±43.0</td>
<td>.000</td>
</tr>
</tbody>
</table>

In this Bil D= Direct bilirubin, Bil T= Total bilirubin, TP= Total protein, ALB= Albumin, GLB= Globulin, ALT= Alanine amino transferase, AST= Aspartate amino transferase, ALP = alkaline phosphatase.

R1= values before Ramadan fasting, R2= Values after Ramadan fasting.

**Discussion:**

The present study evaluated the anthropometric, hematological and biochemical parameters of 53 type 2 diabetic male patients who observed Ramadan fasting. The body weight was significantly decreased after observing Ramadan fasting. This is in line with Khaled et al., 2006 and Mafiouzy et al., 1990 who observed decrease in body weight after fasting in Algerian and malaysian diabetic population respectively. Increase in body weight (kamar et al., 2015) and no change in body weight is also reported (Aliwan and Al Banyan, 2010; Bouzid et al., 2016).

Usually during Ramadan, the frequency of food intake decreases and along with this, the fluid restriction which leads to dehydration might have caused weight reduction. The weight reduction did not lead to significant alteration in the arterial blood pressure in the present study. Eventhough there was slight decrease in the systolic blood pressure, it was not statistically significant. Norouzy et al., 2012 also reported an unaltered blood pressure in diabetic patients after Ramadan fasting. All the subjects in the present study were normotensive. The increase in the dietary fibre and rehydration in the evening could have a role in maintain the blood pressure in the normal level.

The current study indicates that there was no development of anemia during Ramadan fasting on the light of results of the hematological parameters. The hematological parameters such as total WBC count, total RBC count, hemoglobin, hematocrit and platelet did not show any significant difference after observing Ramadan fasting. Norouzy et al., 2012 observed a consistent WBC count and HCT, while RBC, hemoglobin and platelet increased. Decrease in RBC and platelet was observed by Mcguill et al., 2007. Eventhough fluid and food intake is restricted during fasting hours; the food items used in the non fasting hours of Ramadan are usually rich in iron and fibers like dates, vegetable salads and fruit juices. The redistribution of micronutrients might have helped to maintain the normal distribution of plasma and the blood cells.

The glycemic status was well maintained by the diabetic people during Ramadan fasting as evidenced by the results of the present study. There was a non-significant decrease in the fasting blood sugar while the parameters like post prandial blood sugar, mean blood glucose and HbA1c did not altered. Khatib and Shafaghi, 2004 also found that
blood sugar level and HbA1c did not change in normal weight people. Weight reduction has shown to improve the insulin resistance and improve the glycemia in diabetics (Ludwig, 2002).

In the present study Ramadan fasting has shown a favorable effect on lipid profile. The triglycerides decreased and the total cholesterol remained unchanged while HDL has increased and LDL and VLDL decreased. Uysal et al., 1998 found an increased HDL, while LDL, TG and TC did not alter. Saada et al., 2010 has found an increase in HDL, and decreases in TC, TG LDL and VLDL. The disparity in the results may be due to the difference in the type of food and the physical activities by the subjects. In the present study the subjects were having physical exertion as part of their job during the Ramadan fasting period. These physical activities require more energy for which the body might have used fat as a substrate. This might have led to decrease in LDL and TG.

The increase in renal function markers, the urea, creatinine and uric acid seen in the present study is possibly due to a hypohydration resulted from the reduced fluid intake during day time which is reinforced by the osmotic diuresis of diabetic patients. Similar results have been observed by Azwany et al., 2004. But the increase seen was under physiological limit and the microalbuminuria was improved after fasting which indicate that glomerular membrane health was not harmfully affected by Ramadan fasting. Reducing body weight with normal blood pressure and well controlled blood glucose in diabetic patients may reduce the microalbumin level in urine (Koroshi et al., 2007). No previous studies have been reported on the microalbumin level of diabetic after Ramadan fasting.

Liver function in type 2 diabetic also was not affected adversely by Ramadan fasting except there was a significant increase in the alkaline phosphatase level. Bilirubin levels, total protein, albumin, globulin, ALT and AST did not change after Ramadan fasting. In a previous study by Mguire et al., 2008 has noticed decrease in total protein, ALT, AST, and increase in bilirubin but ALP was not altered. This is in contrast with the present result. ALP is not a specific marker of liver function. Any biliary duct disease or malnutrition can alter the ALP levels. More studies are required for the exact reason of increase in ALP after fasting.

Conclusion:-
The present study has observed that Ramadan fasting has a beneficiary effect on body weight, lipid profile and microalbuminuria. Ramadan fasting does not altered the glycemic status and hemotological parameters. Ramadan fasting has adversely affected the renal function but not clinically relevant as the values were within physiological limit. The liver function was also not adversely affected by Ramadan fasting. These results point that Ramadan fasting is safe for type 2 diabetics.

Reference:-
Original Article

A study on the glycemic, lipid and blood pressure control of type 2 diabetes patients of Kerala

Suresh Kumar P.1,4, Sandhya A.M., Research Scholar2

1Dr. Sreevidya DiabCare India, Thrissur, Kerala 680661, India
2Asian Flower Medical Research Center, Kannur, Kerala, India

ABSTRACT

Aim: Aim of the study was to detect the level of comprehensive diabetes control among the diabetic patients of Kerala, India.

Method: Patients (200) were randomly selected from a diabetes care center. Their blood sugar and other biochemical and anthropometric measurements were done and statistically analyzed.

Result: Only 28.3% had their A1C at or below 7% and 45% above 9%. 70% of the male patients had CVD. The prevalence of hypertension was almost equal in both sexes. However, there was a statistically significant higher systolic BP (mean 162.12 mm Hg vs 147.49 mm Hg, p = 0.01044) among females. The total cholesterol was above 200 mg/dl in 42.1% of males and 45.81% of females. The triglyceride was > 150 mg/dl in 38.6% males and 59.88% females. Low HDL cholesterol levels were found in 20.07% of males and 41.32% of females (p=0.00145). The mean LDL was 120.75 (±32.30±).

Discussion: The mean blood sugar values are found to be high which will lead to a predictable increase in cardiovascular disease, which in turn will affect the quality of health and productivity of the individual and the economic growth of the society as a whole. Studies suggest that therapeutic interventions to improve glycemic control may reduce the risk of CVD and microvascular disease.

Conclusion: This study shows that the level of diabetes control in Kerala is unsatisfactory. We need more medicines, better strategies, and more emphasis on glycemic management than we are currently able to apply.

© 2016 Diabetes India. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The incidence of Diabetes is alarming in both developed and developing countries. In US, the incidence of diabetes in 2010 was 1.7 million new diagnoses/year, in 2012, it is increased to 1.9 million [1]. This means that we are going to have increasing numbers of cardiovascular events, cerebral vascular events, peripheral vascular and a number of other cardiovascular illnesses [2]. For the most part, diabetes has become the leading risk factor for cardiovascular disease in most clinical cardiology settings. Proper control of hyperglycemia is imperative and significant in preventing both microvascular and macrovascular complications in diabetes, and reduced control means an even more alarming increase in the complication rates [3]. The mean glycated Hb (HbA1c) levels as per the available Indian data are around 9%, which is at least 25% higher than the goal prescribed by international bodies [4]. Aim of our study was to identify whether we have achieved a satisfactory level of diabetes control or not in our diabetic population. This study aims to determine the level of diabetic control among a group of diabetic patients visiting a North Malabar diabetic clinic of Kerala to assess the mean glucose burden among the diabetic population as it will help give a direction for the future planning of diabetes management.

2. Materials and methods

Type-2 diabetic patients were recruited from the OP clinic of the “DiabCare” diabetes care center. Manjeshi is an important primary care center for diabetes for the whole of Malappuram district, which in turn represents a cross section of Malabar. All newly detected diabetics and diabetics with established organ failures like renal impairment, cardiac failure, and hepatic failure were excluded. Samples for the fasting blood sugar, Lipid Profile, HbA1c, Uric acid, Calcium and Fasting insulin levels were collected after at least 8 h of overnight fasting. Samples for post-prandial blood sugars was also collected after 2 h from the time of starting breakfast, after the patients taking their usual medicines or insulin if he/she is already on any. The study was conducted after getting informed consent. The study was approved by the IEC.

http://dx.doi.org/10.1016/j.diabres.2016.07.005

Please cite this article in press as: S.K.P., S.A.M., A study on the glycemic, lipid and blood pressure control of type 2 diabetes patients of Kerala, Diab Met Syndr Clin Res Rev (2016), http://dx.doi.org/10.1016/j.diabres.2016.07.005

194
The patients were examined for assessment of height, weight, Body Mass Index (BMI) and waist circumference and Waist – Hip Ratio (WHR). The BMI (according to the WHO criteria, <18.5 is underweight, 18.5–24.9 is healthy, 25–29.9 is overweight and 30 and above is obesity). However, the modified Asian criteria defines it differently with <18.5 underweight, 18.5–22.9 is healthy or acceptable risk, 23–24.9 is overweight or high risk and ≥25 is obese or very high risk was calculated after body weight and height and WHR were measured and BMI = Weight in Kg/Height in M² were measured with subjects in light clothing and without chapal. Waist circumference was measured on standing subjects midway between the lowest rib and the iliac crest. Hip circumference was measured at the widest area in the gluteal region and the waist to hip ratio (WHR), according to the WHO criteria, for males normal was <1 and for females <0.9 and according to the modified Asian standards normal for men is <0.90 and for females it was <0.85 was calculated, as a measure of fat distribution (central obesity), whereas BMI was considered a measure of over all adiposity. Two blood pressure readings were obtained from the right arm of the patients in a sitting position after 30 min of rest at 5 min intervals and their mean value was calculated. Systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg (or current use of anti-hypertensive medication) is defined as Hypertension [21]. Relevant medical history data was collected from the patients including the family history of diabetes and CAD in first-degree relatives. CAD was defined as using nitro glycerine experiencing typical chest pain or having a history of previous Myocardial Infarction (MI). The information was validated against ECG changes (Minnesota codes 1–3, 4–1, 5–3) compatible with ischemic heart disease.

Blood Glucose was estimated by glucose oxidase-peroxidase enzymatic (GOD-PX) end point colorimetry single reagent chemistry method. Cholesterol estimation was done by enzymatic (Cholesterol Oxidase- peroxidase), end point colorimetry, single reagent chemistry, with liquid clearing factor (LCF). Triglyceride estimation was done by enzymatic (Glycerol 3-Phosphate Oxidase (GPO)/Trinder) end point colorimetry, single reagent chemistry with liquid clearing factor (LCF). HDL cholesterol was estimated using Polyethylene glycol-cholesterol oxidase-peroxidase (PAP) end point colorimetry, two reagent chemistry with liquid clearing factor. Auto span semi auto analyzer was used for all the above procedures and colorimetric measurements. HbA1c was measured using Bio-Rad “multi” HbA1c analyzer using ‘boronate Affinity Chromatography’ method. Statistical Analysis of the data was done with the help of the SPSS v17.

3. Results

When the patients were categorized according to the blood sugar levels (fasting and post-prandial) (Figs 1 and 2), it was found that the majority of patients were controlled. The mean fasting blood sugar was 156.73 (±54.0), and the postprandial was 212.94 (±64.3). Among them, 42.15% males and 63.9% females had a fasting blood sugar in the range of 141–200 mg/dL and 51.72% of males and 54.54% of females had post-prandial blood sugar in the range of 201–300 mg/dL. Only around 1/3 of the cases had reasonably good (FBS <140 mg/dL and PPBS <200 mg/dL) control of blood sugar with only 28.3% of patients having their A1c at or below 7% and 45% had their A1c above 6% which shows that majority of the study population had poor blood sugar control.

The analysis of prevalence of coronary artery disease (CAD) (Table 1) showed that 1/3rd of the female and 1/6th of the male patients had CAD. This showed that females had a significantly higher incidence of CAD. However, the prevalence of hypertension was almost in both sexes (males = 62.21% and females = 27.9%, p = 0.00044) among females compared to their male counterparts. Regarding family history of diabetes, more than 50% of patients both among males and females had first degree relatives with diabetes (37.38% males vs 52.54% females).
Regarding lipid abnormalities (Table 3), the mean total cholesterol was 201.20 (±8.32) and was above 260 mg/dL in 42.1% of males and 45.01% of females, whereas the average triglyceride was 193.25 (±81.14). The triglyceride level was higher than 150 mg/dL in 38.6% of males and 50.88% of females. Low HDL cholesterol levels were found in 20.07% of males (≤40 mg/dL) and 43.12% of females (≤50 mg/dL) (mean 50.6 ± 13.78). This difference was found to be statistically significant (p = 0.0445). Among the various lipid fractions analyzed, high LDL cholesterol (≥100 mg/dL) was the most prominent abnormality (71.93% of males and 82.46% of females) found among the study population. The mean LDL was 121.74 (±32.29 mg/dL). Lower HDL and higher LDL cholesterol were found more among female diabetics compared to males.

A total of 1200 patients (610 males and 590 females) were categorized according to their HbA1C value. Among males 50.8% and among females 38.9% are having an HbA1C value above 6% indicating uncontrolled diabetes. 13.3% Patients have a value between 7.1% and 8% (reasonably stable control), and 11.7% of patients have values between 8.1% & 9% (poor control). This shows an overall poor control of diabetes among the majority of the study population. Among them, only an aggregate of 28.3% of patients showed a good control of their diabetes with an HbA1c value of ≤7% (Fig. 4). From the results, among either sex, females show a better control of their diabetes than males. (Table 4 and Fig. 3). The mean values of Calcium were slightly lower in both sexes, and was 8.13 mg/dL among males and 8.14 mg/dL among females, (p value = 0.71872-NS). Similarly, the mean Uric acid values were 0.14 mg/dL in males and 0.55 mg/dL in females (p value = 0.71872-NS).

4. Discussion

The mean blood sugar values are found to be high in our study group. Mean fasting blood sugar value was 156.57 mg/dL, and the average post-prandial blood sugar was 232.95 mg/dL. This is quite high, indicating poor control of blood sugar in the diabetic population. Fifty three percentage of males and 42.3% of the males had fasting blood sugar values between 141 and 200 mg/dL. Among the males, 51.72% and among females 54.54% had PPGS between 201 mg/dL and 300 mg/dL. Among males 50.8% and among females 38.9% are having an HbA1c value above 6% indicating uncontrolled diabetes. 13.3% Patients have a value between 7.1% and 8% (reasonably stable control), and 11.7% of patients have values between 8.1% & 9% (poor control). This shows an overall poor control of diabetes among the majority of the study population. Among them, only an aggregate of 28.3% of patients showed a good control of their diabetes with an HbA1c value of ≤7%. Similar trend was seen in the KRM-INDIAB study where there was only 31.1% of patients with HbA1c <7% from the three Indian states of Tamil Nadu, Maharashtra and Jharkhand, and one Union Territory- Chandigarh [12]. Some of the other studies show a lesser levels of blood sugar in their diabetic study population. Most of them had fasting blood sugar values between 130 and 140 mg/dL [4-6]. These data indicate that the majority of Indian diabetic population is poorly/poorly controlled.

Studies show that elevated blood glucose levels and the development of atherosclerosis are linked, suggesting that therapeutic interventions to improve glycemic control may reduce the risk of CVD [19]. Studies have also shown that post prandial

Table 3

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Distribution of different Lipid components among diabetics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S/D (N = 570)</td>
</tr>
<tr>
<td>Male Normal</td>
<td>530 (57.0%)</td>
</tr>
<tr>
<td>Male Abnormal</td>
<td>240 (42.1%)</td>
</tr>
<tr>
<td>Female Normal</td>
<td>380 (53.1%)</td>
</tr>
<tr>
<td>Female Abnormal</td>
<td>200 (48.1%)</td>
</tr>
</tbody>
</table>

TC = Total Cholesterol, TG = Triglycerides, HDL = HDL Cholesterol, LDL = LDL Cholesterol, IR = insulin resistance.

Fig. 3. distribution of diabetics according to the HbA1c value. It is found that the HbA1c value is high majority of the diabetic patients, in both sexes (females fared better than males).

% of Patients with good control of HbA1c and BP

Fig. 4. Distribution of patients with Diabetes, lipid and BP control within the goals prescribed by world bodies. HbA1c, LDL Cholesterol and BP are well below a third of the goal set by them.

Table 4

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Distribution of HbA1c in males and females.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>Male(n=570)</td>
</tr>
<tr>
<td>≤7</td>
<td>140 (23.95%)</td>
</tr>
<tr>
<td>7-8</td>
<td>60 (10.64%)</td>
</tr>
<tr>
<td>8-9</td>
<td>100 (16.85%)</td>
</tr>
<tr>
<td>≥9</td>
<td>310 (55.81%)</td>
</tr>
</tbody>
</table>

Please cite this article in press as: S.K. P., S.A.M. A study on the glycemic, lipid and blood pressure control of type 2 diabetes patients of Kerala, Diab Met Syndr: Clin Res Rev (2016), http://dx.doi.org/10.1016/j.dsx.2016.07.005
hyperglycemia is a risk factor for atherosclerosis and CVD [7]. This is caused by direct endothelial damage [8], through similar mechanisms as with insulin resistance and central obesity, like increased oxidative stress, reduced nitric oxide synthesis, and bioavailability [9, 10]. In addition, hyperglycemia may cause LDL-glycation and oxidation, activate coagulation pathways, increase circulating levels of adhesion molecules involved in early atherosclerosis, and increase levels of some of the inflammatory markers [11]. Several large epidemiologic studies have shown that postprandial hyperglycemia is associated with increased incidence of cardiovascular disease [10, 11-13]. In our study, total 259 (10.67% males and 30.51% females) of the patients have ECG evidence of CAD which is slightly higher than that recorded in 2001 in the Chennai urban population study (CUPS), a population based study in Chennai in South India, which showed a prevalence of 21.4% among their diabetic patients [13]. This substantiates the growing epidemic evidence for the association of postprandial hyperglycemia and macrovascular complications in diabetic individuals [14, 22-24].

In the present study, the prevalence of hypertension was 69.17% (males - 67.21% and females - 71.19%) (Table 2). This was higher than in the reports by Mohan et al. in 2007 on their CURES-38 study report which reported a prevalence of Hypertension of 36.2% among their diabetes population [25]. On further analysis, it was found that only 53.1% patients with HbA1C >9% have Hypertension, whereas 62.7% have hypertension in the HbA1C ≤9% group (Fig. 5, Table 3). In case of coronary artery disease, only 8.5% of patients with HbA1C >9% are having CAD, whereas 19.7% of patients with HbA1C ≤9% have CAD. So, unlike postprandial hyperglycemia, no significant causal relationship of elevated glyated hemoglobin level with CAD or Hypertension was identified in the present study. Though, targeting associated risk factors is much more cardioprotective than controlling the glucose level alone; good glycemic control is warranted to reduce the risks of neuropathy, retinopathy, and nephropathy [26]. The importance of tighter glycemic control is underscored by the American Diabetes Association decision to change the definition of “impaired fasting glucose” by lowering the glucose threshold to 100 mg/dl from 105 mg/dl [27].

Glycemic control in diabetics, around the world, seems to be worsening despite the newer additions to the medical armamentarium to treat diabetes. In contrast, hypertension control and cholesterol control have got better, not worse, in the same interval. So, there is an absolute absence of progress, in fact, a reversal, in the area of glycemic control.

### Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>HbA1C &gt;9</th>
<th>HbA1C ≤9</th>
<th>CHD</th>
<th>HTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD</td>
<td>53.1</td>
<td>62.7</td>
<td>8.5</td>
<td>19.7</td>
</tr>
<tr>
<td>No CAD</td>
<td>53.1</td>
<td>62.7</td>
<td>8.5</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Percentage of HbA1C and CAD group with HbA1C >9 and below 9: standard error.


