



## EFFECT OF PUFA RICH OIL ON PLASMA TNF-ALPHA AND CALCIUM LEVELS IN TYPE 2 DIABETIC PATIENTS

**Uma Shanmugasundaram\*<sup>1</sup>, Dr. Anitha Achuthan<sup>2</sup>, Dr. Saravanan Selvaraj<sup>2</sup> and Prakash Selvaraj<sup>2</sup>**

<sup>1</sup>MSc., Assistant Professor, Department of Physiology, Chengalpattu Government Medical College and Hospital, Chengalpattu -603001, Tamilnadu, India.

<sup>2</sup>Department of Physiology, Chengalpattu Government Medical College and Hospital, Chengalpattu -603001, Tamilnadu, India.

**\*Corresponding Author: Uma Shanmugasundaram**

MSc., Assistant Professor, Department of Physiology, Chengalpattu Government Medical College and Hospital, Chengalpattu -603001, Tamilnadu, India.

Article Received on 21/09/2018

Article Revised on 11/10/2018

Article Accepted on 01/11/2018

### ABSTRACT

Glucose is a ubiquitous fuel in biology. When there is a lack of insulin secretion or cell resist to uptake glucose leads to Diabetes Mellitus (DM). Diabetes leads to both premature death and complications such as inflammation, blindness, amputations, renal disease and cardiovascular diseases. Many scientists put limits saturated fat intake at the top of the list because these fats are strongly linked with heart disease and Type 2 Diabetes Mellitus. Polyunsaturated fatty acids (PUFA's) cannot be synthesized in the body and it must be provided in the diet only. Generally plasma Tumor Necrosis factor alpha (TNF- alpha) levels increase in many inflammatory diseases. Plasma levels of TNF alpha and calcium levels were assessed in diabetic patients without PUFA therapy (n = 30), with PUFA therapy for 30 days (n = 30) and normal healthy individuals (n=30). The values were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's posthoc test for multiple comparison methods. The Significance level was kept at P<0.05. The TNF alpha levels significantly increased in both diabetic groups than the normal group, and TNF alpha levels was significantly decreased in the PUFA therapy group than the without PUFA therapy group. The serum calcium levels were assessed in this study, but there is no significant change between the groups. In conclusion the PUFA rich oil has the effect on the reduction of TNF alpha levels in diabetic patients.

**KEYWORDS:** PUFA, TNF-alpha, Diabetes mellitus and HbA1c.

### INTRODUCTION

The incidence of Type 2 Diabetes Mellitus (T2DM) is increasing at an alarming rate both nationally and worldwide. Diabetes is the fifth leading cause of death in the US and it is also a major cause of significant morbidity. The worldwide occurrence of diabetes has continued to increase significantly. Globally, as of 2011, an estimated 366 million people had DM, with type II makes up about 90% of the cases.<sup>[1][2]</sup> The risk of developing Type II diabetes increases with age, obesity, and lack of physical activity.<sup>[3]</sup> In India, estimates show that there were 31.7 million people with diabetes in the year 2000 and this is projected to increase to 79.4million by 2030.<sup>[4]</sup> Diabetes mellitus occurs throughout the world, but is more common (especially type II) in the more developed countries, where the majority of patients are aged between 45 and 64 years.

PUFA are fatty acids also called omega-3 fatty acids; it includes alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). PUFA has an effect on depression,<sup>[5]</sup> Prevention of

Cardiovascular Disease, Anti-inflammatory activity, hypertriglyceridemia and rheumatoid arthritis.<sup>[6]</sup> Tumor necrosis factor (is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction. Calcium is an essential trace element in living organisms; it is an important component of a healthy diet and a mineral necessary for life.

### MATERIALS AND METHODS

**Study area and protocol:** After the approval of Institutional Ethical Committee (IECNo: 20/09/2017), this study was conducted in Central Research Laboratory, Chengalpattu Government Medical College and Hospital, Chengalpattu between 8.00 AM and 10.00 AM. Three groups were used for this study and each group comprises about 30 individuals.

Group – I: Normal, healthy individuals

Group – II: Newly diagnosed and controlled Type II diabetic patient without PUFA Therapy

Group – III: Newly diagnosed and controlled Type II diabetic patient with PUFA Therapy.

**Drugs and sample collection:** The Type II diabetic patients (Group III) were instructed to take one pill of PUFA rich oil (Flax seed oil) (500 mg) three times a day after food for 30 days (Vestige Marketing Pvt. Ltd.,

India). After the PUFA oil therapy, with empty stomach 2 ml of blood were collected from all the groups (Group I, Group II and Group III) in cubital vein for Biochemical analysis.

**Table 1: Criteria for Individuals (according to American diabetic society).**

Inclusion criteria	Exclusion criteria
<ul style="list-style-type: none"> <li>• Age group: above 20 below 50 yrs,</li> <li>• Both sexes,</li> <li>• Normal, healthy individuals,</li> <li>• Non smoking,</li> <li>• HbA1c &lt; 7.0 %</li> <li>• Non alcoholic and not other serious illness.</li> </ul>	<ul style="list-style-type: none"> <li>• Age group: below 20 to above 50 yrs</li> <li>• Chronic inflammatory diseases,</li> <li>• Psychological and neurological problems,</li> <li>• Smoking,</li> <li>• Alcoholic,</li> <li>• HbA1c &gt; 7.0%</li> <li>• Uncontrolled diabetic and cardiac related problem individuals and other serious illness problems.</li> </ul>

### Biochemical parameters

#### Estimation of serum calcium level

Serum calcium ion was measured by using calcium (Arsenazo) reagents (OSR65117). In this method the serum calcium ions react with Arsenazo III reagents to form an intense purple colored complex, and then it was calibrated in a spectrophotometer at 660 nm wavelength.

#### Estimation of HbA1c level

The high quality enzymatic assay for the quantification of HbA1c in whole blood was performed in all the groups by Crystal Chem's Hemoglobin A1c (HbA1c) kit (C. No - 80099), according to the manual given by the manufacturer.

#### Estimation of plasma TNF-alpha level

EDTA containing blood sample vials were centrifuged at 1200 RPM for 10 min at 4°C. The levels of TNF- alpha was determined by using an ELISA Kit according to the manual given by the manufacturer. (Cusabio, Biotech Co Limited, China. C.No.CSB-E04740h)

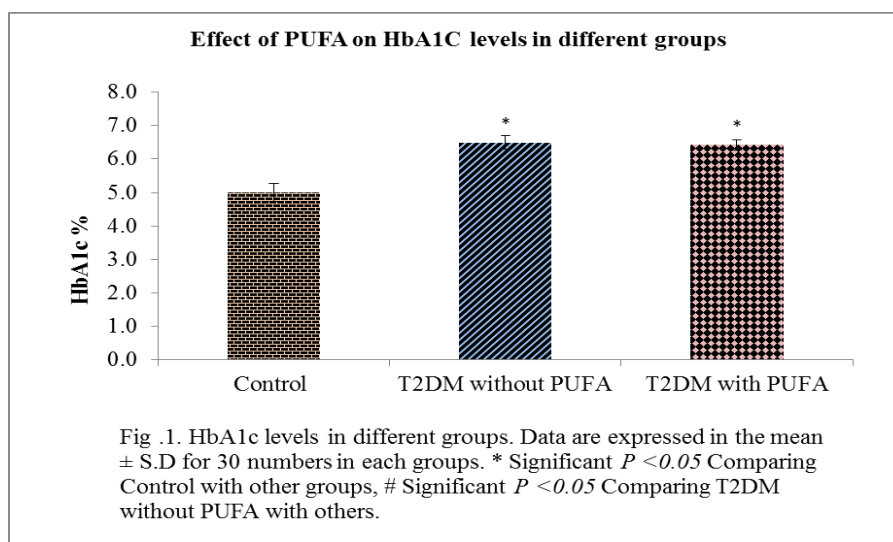
### Statistical Analysis

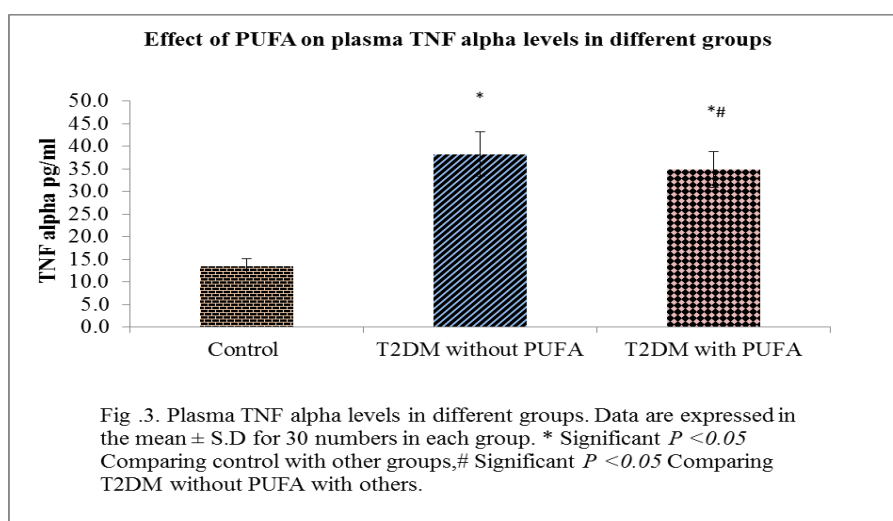
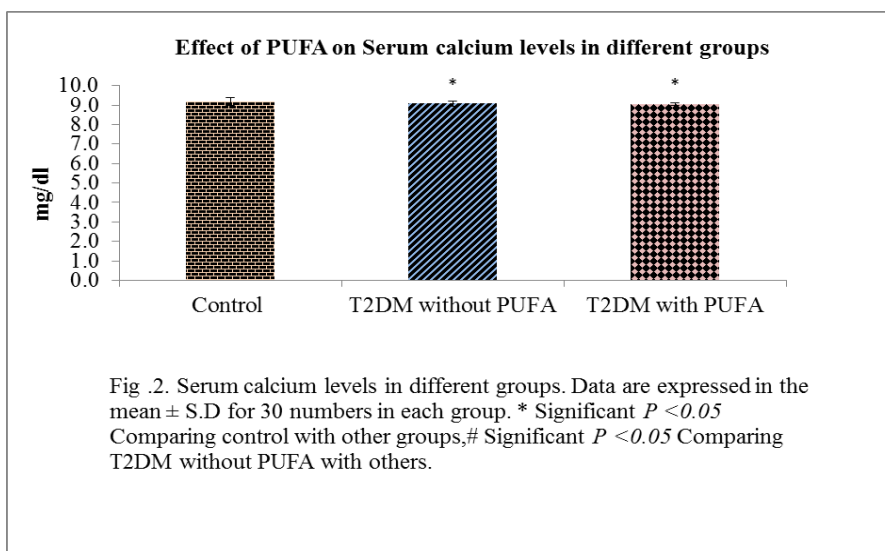
All data were expressed as Mean  $\pm$  S.D. The statistical significance was evaluated by one -way analysis of

variance (ANOVA) using SPSS statistical package version 20.0 (SPSS, Cary, NC, USA). When there was a significant difference, Tukey's multiple comparisons were performed by fixing the significance at level  $P < 0.05$ .

### RESULTS

The HbA1c levels "Fig.1" in T2DM without PUFA and with PUFA group are significantly changed when compared with control (d.f.=2,F=469.598) and there is no significant between Group II and Group III. Serum calcium levels "Fig. 2" in Group II and Group III are significantly changed when compared with control (d.f.=2,F=8.313) and there is no significant between T2DM without PUFA and with PUFA groups. The plasma TNF alpha levels "Fig. 3" in T2DM without PUFA and with PUFA therapy group are significantly increased when compared with control (d.f. =2, F=381.154) and also there is a significant change occurred between PUFA therapy and without PUFA therapy group.





## DISCUSSION

Diabetes - derived from the Greek word ‘**Diabaineine**’, refers a tubular organ that take-in or expels water - excessive urine discharge disease. In 1675 Thomas Willis added ‘mellitus’ in Latin means “honey” to the word diabetes and called it as Diabetes Mellitus, which refers to too much of sweet taste urine. **“Diabetes affects all people in the society, not just those who live with it”** India has the highest number of diabetic patients in the world; the sugar disease is posing an enormous health problem in the country. Calling India the diabetes capital of the world, the International Journal of Diabetes in Developing Countries says that there is an alarming rise in the prevalence of diabetes, which has gone beyond epidemic form to a pandemic one.

The International Diabetes Federation estimates that the number of diabetic patients in India is doubled from 19 million in 1995 to 40.9 million in 2007. It is projected to increase to 69.9 million by 2025. Various studies have shown that the highest incidence of diabetes in India is mainly because of a sedentary lifestyle, lack of physical activity, obesity, stress and consumption of diets rich in

fat (saturated), sugar and calories. Globally, diabetes affects 246 million people, which is about 6 per cent of the total adult population. Diabetes leads to both premature death and complications such as blindness, amputations, renal disease and cardiovascular diseases. Each year, over three million deaths worldwide is tied directly to diabetes and even greater number die from cardiovascular disease.

The mechanisms that trigger the activation of TNF-alpha in Type 2 diabetes are not fully understood, but it is likely that local hypoxia, induced by capillary occlusion, and high levels of advanced glycosylation end-products, which are associated with the development of diabetic complications, may induce TNF-alpha activation. [7][8][9] TNF-alpha, along with other cytokines, is a primary stimulant for the hepatic production of acute-phase proteins, such as fibrinogen and C-reactive protein. This suggests the possibility of an additional pathophysiological mechanism by which TNF-alpha activation may play a detrimental role in the development of diabetic complications. [10]

PUFA are fatty acids also called omega-3 fatty acids some of which have at least two carbon-to-carbon double bonds in a hydrophobic hydrocarbon chain, which typically includes X-Y carbon atoms and terminates in a carboxylic acid group.<sup>[11][12]</sup> There are three major types of omega-3 fatty acids that are ingested in foods and used by the body: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Once eaten, the body converts ALA to EPA and then to DHA. EPA and DHA are the two types of omega-3 fatty acids that serve as important precursors for lipid-derived modulators of cell signaling, gene expression and inflammatory processes. Omega-3 fats from fish are enriched in EPA and DHA and thus do not need to undergo the complex conversion steps required of ALA. In addition, the conversion of ALA to EPA and then EPA to DHA is inefficient in individuals consuming a typical Western diet rich in animal fats. Therefore, direct dietary intake of omega-3 fats rich in EPA and DHA are clinically more beneficial.<sup>[13][14]</sup> Consumption of flaxseed oil supplements should be based on the amount of EPA and DHA, not on the total amount of flaxseed oil. Supplements vary in the amounts and ratios of EPA and DHA. A common amount of omega-3 fatty acids in flaxseed oil capsules is 0.18 grams (180 mg) of EPA and 0.12 grams (120 mg) of DHA. Five grams of flaxseed oil contain approximately 0.17–0.56 grams (170–560 mg) of EPA and 0.072–0.31 grams (72–310 mg) of DHA.<sup>[15][16]</sup> Oils with PUFA inhibit histamine induced TNF-alpha production from monocytes and mast cells.<sup>[17]</sup> The anti-inflammatory activity of n3 containing PUFA oils reduced the TNF alpha levels and increased the PGE2 levels. PUFA rich oil ultimately inhibit tissue destruction by reactive oxygen showed good gastric protective anti-ulcer activity species.<sup>[18]</sup> Dietary supplementation with n-3 PUFAs improved colonic anastomoses healing and enhance the colonic wound healing in a rat model. Actually, it may prompt faster resolution of inflammation within the wound microenvironment, which leads to facilitated regeneration and re-epithelialization.<sup>[19]</sup>

## CONCLUSION

In conclusion, based on the data obtained from this study PUFA may account for the beneficial activity in T2DM patients by reducing TNF alpha levels. PUFA rich oils could effectively help to resolve the inflammation in diabetes mellitus. It also confirms that the PUFA has an anti-inflammatory activity. In future, there is a need to conduct more studies to elucidate their molecular mechanism in anti-inflammatory activity.

## ACKNOWLEDGEMENT

The authors are grateful to the Tamilnadu State Research Committee, King Institute of Preventive Medicine and Research, Guindy, Chennai- 600 032, for funding to conduct the research.

## Authors contributions

All the authors have contributed equally.

## Disclosure of Interests

The authors declare that they have no conflicts of interest concerning this research article.

## REFERENCES

1. Jumpup. Williams Textbook of endocrinology (12th ed.). Elsevier/ Saunders, 12<sup>th</sup> edition, Philadelphia, USA, 2007; 1371–1435.
2. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus present and future perspectives. *Nat Rev Endocrinol*, 2011; 8(4): 228-36.
3. Derek Yach, Corinna Hawkes, C. Linn Gould et al. The Global Burden of Chronic Diseases: Overcoming Impediments to Prevention and Control. *JAMA*. 2004; 291(21): 2616-2622.
4. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, 2004; 27: 1047-1053.
5. Alan C Logan ND, FRSH, MS (Cand.). Neurobehavioral Aspects of Omega-3 Fatty Acids: Possible Mechanisms and Therapeutic Value in Major Depression. *Altern Med Rev*, 2003; 8(4): 410-425.
6. GianLuca Colussi, Cristiana Catena, Sara Baroselli, Elisa Nadalini, Roberta Lapenna, Alessandra Chiuch and Leonardo A. Sechi, Recent Patents on Cardiovascular Drug Discovery, 2007; 2: 13-21.
7. Beisswenger PJ, Makita Z, Curphey TJ. Formation of immunochemical advanced glycosylation end-products precedes and correlates with early manifestations of renal and retinal disease in diabetes. *Diabetes*, 1995; 44: 824– 829.
8. Blakesley VA. The role of growth factors in the pathogenesis of diabetic microvascular complications. *Diabetes mellitus: A fundamental and clinical text*, 2000; 2: 1000–1008.
9. Pankewycz OG, Guan JX & Benedict JF. Cytokines as mediators of autoimmune diabetes and diabetic complications *Endocrine Reviews*, 1995; 16: 164–176.
10. Kulseng B, Vatten L and Espevik T. Soluble tumor necrosis factor receptors in sera from patients with insulin-dependent diabetes mellitus Relations to duration and complications of disease. *Acta Diabetologica*, 1999; 36: 99– 105.
11. Das UN. Essential fatty acids: Biology and their clinical implications. *Asian pacific J. Pharmacol*, 1991; 6: 317-330.
12. Das UN. Essential fatty acids: biochemistry, physiology and pathology. *Biotechnol. J.*, 2006; 1: 420-439
13. Kris-Etherton PM, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, et al. Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr.*, 2000; 71: 179S-88S.
14. Kris-Etherton PM, Harris WS, Appel LJ. American Heart Association. Nutrition Committee. Fish Consumption, flaxseed oil, omega-3 fatty acids and

- cardiovascular disease. *Circulation*, 2002; 106: 2747-57.
15. Yongsoon Park and William S. Harris. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. *Journal of Lipid Research*, 2003; 44: 455- 461.
  16. Scott D Doughman, Srirama Krupanidhi and Carani B Sanjeevi. Omega-3 Fatty Acids for Nutrition and Medicine: Considering Microalgae Oil as a Vegetarian Source of EPA and DHA Current *Diabetes Reviews*, 2007; 3: 198-203.
  17. Azuma Y, Shinohara M, Wang PL, Hidaka A, Ohura K. histamine inhibits chemotaxis, phagocytosis, superoxide anion production and the production of TNF alpha and IL-12 by macrophages via H<sub>2</sub> \_ receptors. *Int Immunopharmacol*, 2001; 1(9-10): 1867-75.
  18. Takeuchi T, Miura S, Wang L, Uehara K, Mizumori M , Kishikawa H et al .Nuclear Factor-B and TNF-Mediate Gastric Ulceration Induced by Phorbol Myristate Acetate. *Dig Dis Sci*, 2002; 47: 2070.
  19. Hunter B, Donald MC, Gibney M J. The effects of acute and chronic administration of n-6 and n-3 polyunsaturated fatty acids on ethanol-induced gastric haemorrhage in rats. *Br J Nutr*. 1992; 67: 501-7.