# PMNL Status in Type-II Diabetic Patients with Multi-Vitamin Multi-Mineral Supplementation

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***Research Article***

***Abstract:*** **Introduction:** We have studied the effect of multi-vitamin multi-mineral supplementation on polymorphonuclear leukocyte(PMNL) membrane status in Type II Diabetes Mellitus patients. **Material and Methods**: The study was divided into four groups. Group A- Diabetic patients (DM0). Group B consisted of normal healthy controls (NHC), Group C of Diabetic patients given multi-vitamin multi-mineral supplementation capsules for 30 days (DM30). Group D consisted of diabetics withdrawn from multi-vitamin multi-mineral supplementation for next 30 days (DM60). Fasting blood samples were collected from the respective groups. PMNL Phospholipid phosphorous (PLp), cholesterol, triglyceride lipase, triglycerides, vitamin C, Magnesium and Zinc were estimated by standard methods. Analysis of variance was calculated by applying students "t" test. **Results and discussion:-** The PMNL cholesterol, triglycerides, phospholipids phosphorous were significantly high ($p<0.05$) whereas PMNL triglyceride lipase, vitamin C, Zinc and magnesium were significantly low ($p<0.05$) in DM without multi-vitamin multi-mineral supplementation as compared to NHC . The levels of Cholesterol, triglycerides, Phospholipid phosphorus significantly decreased after 30 days supplementation. Withdrawal of supplementation for next 30 days showed a slight increase in PMNL PLp. A significant increase in PMNL TG lipase activity was seen after multi-vitamin multi-mineral supplementation in diabetic patients. Supplementation of multi-vitamin multi-mineral capsules to diabetic patients showed significant elevation in PMNL Vitamin C, PMNL Mg and PMNL Zn. A significant change was observed in the levels of PMNL Vitamin C, Mg and Zn after withdrawing multi-vitamin multi-mineral supplementation for next 30 days.**Conclusion:-**This suggests that multi-mineral multi-vitamin supplementation in combination with conventional hypoglycemic treatment may modulate the PMNL function and provide better metabolic control in Type II Diabetes Mellitus patients.

***Keywords:*** Multi-vitamin multi-mineral supplementation, Type II Diabetes Mellitus, PMNL cholesterol, PMNL Phospholipid phosphorus.

## Introduction

Derangement of carbohydrate and lipid metabolism is prominently seen in diabetes. At least 100 million people world-wide suffer from Type II Diabetes Mellitus. This figure is projected to rise to 250 million by the year 2010 [1]. Insulin resistance in Type II Diabetes Mellitus predisposes to the development of central obesity, hypertension, impaired glucose tolerance and characteristic dyslipidemia [2,3]. This alteration in the lipid profile may possibly contribute for the increased susceptibility of infections in diabetic patients. It is known that the major part of cell membrane is constituted by lipids like phospholipids, glycosphingolipids and cholesterol [4]. Numerous studies [8,18] have reported alterations in lipid composition and metabolism of Polymorphonuclear leukocyte (PMNL) in diabetics. PMNL are metabolically most active and are primarily concerned with the body defense. Functions of polymorphonuclear leukocyte (PMNL) like bacterial adherence, chemotaxis and phagocytosis are also hampered with the change in the constitution of the cellular membrane in diabetics [6,7] probably accounting for susceptibility to infections. Adequate studies have also been shown to modulate glucose homeostasis across the cell membrane in type II diabetes mellitus [4,5] resulting in significant alteration in the concertrations of minerals like zinc (Zn) and magnesium (Mg). The depletion of zinc which is known to potentiate the activity of insulin may also be a result of altered PMNL status. It is therefore of interest to study the effect of supplementation with multi-vitamin and minerals in diabetic patients with regular anti-diabetic treatment. Multi-vitamin and multi-mineral capsules provided in our study contain L-carnitine, Vitamin A(2333IU), Vitamin D3 (600IU), VitaminE 20mg (natural sources), VitaminB 130 mg, Riboflavin 5mg, Thiamin 30mg, Niacin, Vitamin B6, Folic Acid 400microgm, Vitamin B12 9microgm, Biotin 20mg, Panthothenic acid 10mg, Iron 8mg, Magnesium 100mg, Zinc 15mg, Cromium 16microgm, selenium 100microgm, Copper 1mg, Mangnese 2mg and Iodine 100microgm. Our study aims at estimating the lipid composition of PMNL in diabetes and study the effect of one month treatment with multivitamin and mineral capsule on the composition of cell membrane.

## Material and Methods

The present study was carried out in the Department of Biochemistry at S. V. N. Govt. Medical College, Yavatmal. The study population was divided into four groups. Group A consisted of Type II diabetes mellitus patients (DM0) (n=16) undergoing regular treatment. Group B consisted of normal healthy controls (NHC) (n=12). Group C consisted of Diabetic patients given multi-vitamin multi-mineral supplementation capsules (one capsule daily after main meal) for 30 days (DM30). Group D consisted of randomly selected diabetic patients (n=8) who were withdrawn from supplementation (DM60). All the diabetic cases taking other nutrient supplementations were excluded from the study. 15 ml fasting blood sample was collected in plain bulb and EDTA bulb from the respective groups. PMNL were isolated and lipids were extracted by the method of Ways and Hanahor [9,10]. Phospholipid phosphorous was estimated by method of Bagade and Ways [11]. Cholesterol and triglycerides were estimated by the methods of Courchaine and Kaplan [12, 13]. Triglyceride lipase was estimated by method of Esbach and Rizack [14]. PMNL vitamin C, Magnesium and Zinc were estimated by ELISA based standard kit method of Randox company [15-17]. The study was approved by the ethical committee of the Institution. All the patients voluntarily participated in the study. Statistical analysis was done by applying students "t" test.

## Result

| Groups | Category | | |
|---|---|---|---|
| A | Type II Diabetes Mellitus patients | (DM) | (n=16) |
| B | Normal healthy controls | (NHC) | (n=12) |
| C | Type II Diabetes Mellitus cases supplemented Multivitamin- Multimineral capsule for 30 days | (DM30) | |
| D | Type II Diabetes Mellitus cases withdrawn from supplementation for next 30 days | (DM60) | |

**Table 1:** Status of PMNL in Diabetic patients when compared with Normal healthy controls

| | NHC | DM |
|---|---|---|
| **PMNL Cholesterol (mg/dL)** | 51.4 ± 2.88 | 58 ± 9.137 |
| **PMNL Triglyceride (mg/dL)** | 43 ± 1.581 | 50 ± 2.23 |
| **PMNL Phospholipid phosphorus (mg/dL)** | 47 ± 1.58 | 55.4 ± 3.13 |
| **PMNL TG-Lipase** | 27 ± 3.87 | 17.2 ± 3.271 |
| **PMNL Vitamin C (mg/dL)** | 0.68 ± 0.08 | 0.48 ± 0.36 |
| **PMNL Zinc (µg/dL)** | 73.8 ± 4.54 | 62.6 ± 4.66 |
| **PMNL Magnesium (mg/dL)** | 0.61 ± 7.4 | 0.56 ± 7.7 |

It is evident from our studies that the lipid profile was considerably altered in the diabetic PMNL compared to controls. The PMNL cholesterol (NHC – 51.4 ± 2.88, DM – 58 ± 9.137, t = 1.54). PMNL triglycerides (NHC – 43 ± 1.581, DM – 50 ± 2.23, t = 5.71) was considerably high as compared with NHC. PMNL phospholipid phosphorous (NHC – 47 ± 1.58, DM – 55.4 ± 3.13, t = 5.35) were significantly high ($p<0.05$) where as PMNL triglyceride lipase activity (NHC – 27 ± 3.87, DM – 17.2 ± 3.271, t = 4.32) was significantly low ($p<0.05$) when NHC and DM without multi-vitamin multi-mineral supplementation were compared. PMNL vitamin C (NHC – 0.68 ± 0.08, DM – 0.48 ± 0.36) and PMNL Zinc (NHC – 73.8 ± 4.54, DM – 62.6 ± 4.66) were significantly decreased ($p<0.05$) when DM patients were compared with NHC. No change was found in PMNL Magnesium levels (NHC – 0.61 ± 7.4, DM – 0.56 ± 7.7).

**Table 2:** Status of PMNL in Diabetic patients without multivitamin-multimineral supplementation, under multivitamin-multimineral supplementation for 30 days and withdrawing the supplementation for next 30 days

| | DM (without supplementation) | DM (after 30 days supplementation) | DM (after withdrawal for next 30 days) |
|---|---|---|---|
| **PMNL Cholesterol (mg/dL)** | 58 ± 9.137 | 55.63 ± 3.34*** | 60.63 ± 2.83 |
| **PMNL Triglyceride (mg/dL)** | 50 ± 2.23 | 45.13 ± 2.95*** | 48.0 ± 4.41 |
| **PMNL Phospholipid phosphorus (mg/dL)** | 55.4 ± 3.13 | 50.0 ± 3.7*** | 52.50 ± 4.11 |
| **PMNL TG-Lipase** | 17.2 ± 3.271 | 25.5 ± 2.68*** | 23.8 ± 2.17 |
| **PMNL Vitamin C (mg/dL)** | 0.48 ± 0.36 | 0.94 ± 0.09*** | 0.76 ± 0.11** |
| **PMNL Zinc (µg/dL)** | 62.6 ± 4.66 | 87.8 ± 6.06*** | 70.0 ± 5.0** |
| **PMNL Magnesium (mg/dL)** | 0.56 ± 7.7 | 1.75 ± 0.08*** | 1.36 ± 0.07** |

***p value<0.001 (Highly significant), **p value<0.05 (Significant).

It was observed that supplementation of micronutrients significantly decreased the PMNL Cholesterol and TG (DM0:DM30). However no alteration was seen in PMNL Cholesterol and TG levels after withdrawal of multi-vitamin multi-mineral supplementation for next 30 days. The levels of Phospholipid phosphorus (DM0:DM30, Dm30:DM60) significantly decreased after supplementation. Withdrawal of supplementation for next 30 days showed a slight increase in PMNL PLp. A significant increase in PMNL TG lipase activity was seen after supplementation of multi-vitamin multi-mineral capsules in diabetic patients. No significant change was found in PMNL TG lipase after withdrawal of multi-vitamin multi-mineral capsules for next 30 days. Supplementation of multi-vitamin multi-mineral capsules to diabetic patients showed significant elevation in PMNL Vitamin C (DM0:DM30, DM30:DM60), PMNL Mg (DM0:DM60), PMNL Zn (DM0:DM60). A significant change was observed in the levels of PMNL Vitamin C, Mg and Zn after withdrawing multi-vitamin multi-mineral supplementation capsules for next 30 days.

## Discussion

It is well recognized from our study that the leukocyte membrane structure, which is largely dependent on the lipid composition, is grossly altered in diabetes. Our study demonstrates an increase alteration of PMNL lipid status in diabetic patients. This is in accordance with the earlier studies [18]. In our study PMNL cholesterol, Triglyceride and phospholipid phosphorous showed significant decrease after 30 days of supplementation but there was no alteration observed with the continuation of supplementation for 60 days and or withdrawal of multi-vitamin multi-mineral capsules for next 30 days. Triglyceride lipase increased with supplementation and no change was observed after withdrawal. Although, our present finding did indicate small amount of triglyceride influx from the plasma, the altered membrane fluidity in diabetic PMNL can result in increased influx of triglycerides from the plasma, even if the concentration of plasma triglyceride is not increased. Whether, the overall alteration in the lipid composition of Diabetic PMNL would cause variation in the selective entrance of certain micro-nutrients conjectural but the changes in the morphological characteristics of the cell membrane seems to be certain. It is apparent from our results, that there was significant alteration in the composition of lipids in type II diabetes mellitus patients treated with oral hypoglycemic drugs along with multi-vitamin multi-mineral supplementation. Numerous vitamins and minerals have important roles in glucose metabolism [19-29]. Mg is involved in glucose homeostasis at various levels. Mg is cofactor for glucose uptake by cell membranes and also in several enzymatic pathways involved in glucose oxidation. In type II diabetes mellitus patients, hyperinsulinemic status increases renal Mg excretion, more ever hyperglycemia accelerates Mg turnover in pancreatic beta cells as well as in other cells resulting in reduced intracellular free Mg [30]. Zn is involved in the insulin uptake and therefore interacts in maintaining glucose homeostasis. Zn directly participates in the production, storage and release of insulin from the pancreatic beta cells. Also as a part of the structure of superoxide dismutase, Zn has a protective role against tissue damage caused by lipid peroxidation which in turn affects function of insulin or its cell membrane glucose transporter [31-34]. In type II diabetes mellitus, hyperzincuria induced by hyperglycemia together with impaired Zn absorption is mainly responsible for deficient or marginal Zinc status. Vitamin C inhibits glycosylation of proteins [35] and therefore serves as a means of preventing long term complications associated with diabetes. In type II diabetes mellitus, there is high turnover of vitamin C associated decreased intestinal absorption. Our study indicates that the levels of PMNL Zn and Vitamin C were significantly decreased in diabetic patients. The levels of Mg, Zn and Vitamin C showed restoration with the supplementation for 30 days. Our results revealed significant restoration in the PMNL Vitamin C, Mg, and Zn levels in Diabetic patients with supplementation of multi-vitamin multi-mineral capsules. Withdrawing the supplementation for 30 days showed a significant decrease in the levels of the vitamin C, Mg and Zn levels. This suggests that this multi-mineral and multi-vitamin supplementation in combination with conventional hypoglycemic treatment may modulate the PMNL function and provide better metabolic control in Type II diabetes mellitus patients and may also have a role in prevention of further complications of disease like increased susceptibility to infections and vascular complications involving eye, kidney, nerves and blood vessels. Moreover there is a place for judicious replacement of specific micro-nutrients in diabetic patients.

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