SUMMARY & CONCLUSIONS

Type II DM is frequently associated with an inflammatory status; there is a cytokine associated acute phase reaction, part of the innate immune response. The relationship between low grade inflammation and diabetic complications are still unclear. Whether imbalance between oxidants & antioxidant system aggravates the complications & what is their relation with the acute phase reactants in such diabetic patients, needs to be further explored. Therefore the present study “Association of markers of acute phase response to serum total sialic acid levels in type II DM patients with and without nephropathy and its correlation with the antioxidant levels” was undertaken to evaluate and establish the role of sialic acid and APP in type II DM along with the status of oxidants & antioxidant system & their correlation with micro vascular complications. Correlation between complications, acute phase reactants & decrease antioxidant levels may further help in establishing newer simpler parameters to monitor progression of diabetes and support the role of exogenous antioxidants in prevention & delaying of complications in diabetics.

The study was conducted in the Dept. of Biochemistry M.G.M. Medical College and Associated M.Y. Hospital Indore (M.P). The study comprised of 319 type II diabetes patients that included 162 patients without nephropathy (group A) and 157 diabetics with nephropathy (group C) grouped on basis of microalbuminuria. Hundred and sixty five age matched healthy non diabetic volunteers were taken as control group (group A).

Group C patients were further divided into two sub groups as diabetics with microalbuminuria, C-I (urine albumin 30-299 mg/day) (n=30) and diabetics with macroalbuminuria, C-II (urine albumin≥ 300mg/day)
For diagnosis of diabetes mellitus W.H.O. criteria was used. Relevant inclusion and exclusion criteria were applied for selecting cases and control subjects for the study.

Fasting venous blood sample was collected from all the subjects in EDTA, fluoride & plain vials. Post prandial venous samples were collected 2 hours after meals. Twenty four hour urine samples were collected from all patients and control group.

Serum sialic acid, ceruloplasmin, SOD, vitamin-C and MDA were analyzed by manual methods using computerized fully automated Shimadzu pharma spec (UV-1700) double beam UV-VIS spectrophotometer. Glycosylated hemoglobin in whole blood, serum high sensitivity C-reactive protein, plasma glucose fasting and post prandial, serum urea, serum creatinine, Lipid Profile, urine microalbumin/albumin and creatinine were measured by using Biosystems A-25 and Vitalab SELECTRA-E computerized fully automated random access clinical chemistry analyzer.

The present study was a case control study and the method of sampling used was non random-purposive. For comparing above said parameters in control and study groups, we applied independent samples t test. Correlations were calculated by Pearson’s correlation coefficients.

**From the present study following results were obtained:-**

**On comparison of biochemical parameters of control group (A) with the diabetics without micro albuminuria (gr. B) and diabetics with micro albuminuria (gr. C).**

1. The mean values of fasting & post prandial glucose were significantly higher in gr. B & C diabetic patients compared to controls (Gr. A) (P=0.000).

2. The mean value of urea was significantly higher in gr. C compared to control (gr. A) and group B (P=0.000). No statistical significant
difference was seen in mean values of urea among control group and group B (P=0.052).

3. The mean value of serum creatinine was significantly higher in gr. B & gr. C compared to control gr. A (P=0.029, 0.000).

4. The mean values of urinary micro-albumin were significantly higher in gr. C compared to gr. A and gr. B (P=0.000).

5. The mean values of glycosylated haemoglobin (HbA1c) and mean average glucose value of gr. B & C were significantly higher compared to mean values of gr. A (P=0.000).

6. On comparing the mean values of different parameters of lipid profile of group A with gr. B & C, significant difference was observed for triglycerides, HDL, Chol:HDL ratio, LDL:HDL ratio & VLDL. While no statistically significant difference was seen for cholesterol and LDL values.

7. The mean values of total sialic acid (TSA) & ceruloplasmin were significantly higher in diabetics with & without nephropathy (gr. B & C) compared to control group.

8. Vitamin C and SOD mean values were significantly lower in gr. B & C compared to gr. A (P=0.000).

9. On comparing the mean values of MDA and hs-CRP in all 3 groups, significantly higher values were seen in gr. B &C compared to gr. A (P=0.000).

On comparison of biochemical parameters among diabetics without nephropathy/microalbuminuria (gr. B) and diabetics with nephropathy/microalbuminuria (gr. C).

1. The mean values of FBS, PPBS, urea and creatinine were significantly higher in Gr. C compared to gr. B (P=0.000).
2. The mean values of glycosylated haemoglobin (HbA1c), mean average glucose and microalbuminuria of gr. C were significantly higher compared to mean values of gr. B (P=0.000).

3. On comparing the mean values of lipid profile parameters among gr. B & gr. C, significant difference was observed only in triglycerides & VLDL values.

4. The mean values of total sialic acid & ceruloplasmin were significantly higher in gr. C compared to gr. B cases (P=0.000).

5. Vitamin C and SOD mean values were significantly lower in gr. C compared to gr. B.

6. On comparing the mean values of MDA and hs-CRP significantly higher values were seen in gr. C compared to gr. B (P=0.000).

7. On comparing the mean values of duration of diabetes from onset in gr. B & gr. C significantly higher value was seen in diabetics with nephropathy as compared to diabetics without nephropathy.

Correlation between biochemical parameters among three groups of patients

1. The fasting and post prandial glucose levels showed highly significant and positive correlation in all 3 groups A, B & C with each other and with HbA1c.

2. Significant positive correlation of FBS & PPBS was seen in group B & C with triglycerides, VLDL, MDA, hs-CRP, total sialic acid and ceruloplasmin. Highly significant negative correlation of FBS & PPBS was seen with vitamin C and SOD in all three groups of patients. **Suggesting a role of hyperglycemia in acute phase reaction, oxidative stress and decreased antioxidants.**

3. On comparing glycosylated haemoglobin (HbA1c) with other parameters highly significant positive correlation was seen with FBS,
PPBS in all three groups and with microalbuminuria, triglycerides, VLDL, total sialic acid, MDA, ceruloplasmin and hs-CRP in groups B & C. Glycosylated haemoglobin showed strong negative correlation with vitamin C and SOD in diabetics with and without nephropathy (gr. B & C).

Thus increased blood glucose levels showed parallel increase in HbA1c, microalbuminuria, total sialic acid, MDA, ceruloplasmin, hs-CRP, triglycerides and VLDL. While increase in glucose levels showed corresponding decrease in vitamin C and SOD levels.

4. Blood urea and creatinine showed strong positive correlation with each other in all the 3 groups, while highly significant and significant positive correlation of urea and creatinine was seen with ceruloplasmin, MDA, hs-CRP and microalbuminuria, total sialic acid in diabetics with nephropathy (gr. C). Strong negative correlation of urea and creatinine was seen with vitamin C in group C.

5. Microalbuminuria showed highly significant positive correlation with FBS, PPBS, HbA1c, UACR, total sialic acid, ceruloplasmin, MDA and hs-CRP in all the 3 groups. Significant positive correlation of microalbuminuria was seen with creatinine, cholesterol, triglycerides, LDL, VLDL, LDL: HDL and duration from onset of diabetes in diabetics with nephropathy (gr. C).

6. Highly significant negative correlation of microalbuminuria levels was seen with vitamin C and SOD in all the three groups of patients.

7. Cholesterol showed highly significant positive correlation with triglycerides, HDL,LDL, VLDL, Chol:HDL and LDL:HDL in all the 3 groups and with sialic acid in group C. Significant positive correlation was seen of cholesterol with UACR, MDA and hs-CRP in group C.

8. Triglycerides showed strong positive correlation with other parameters of lipid profile in all the 3 groups. Highly significant positive
correlation of triglycerides was seen with FBS, PPBS, HbA1c in groups B & C and with sialic acid, ceruloplasmin, MDA, Microalbuminuria and hs-CRP in group C. Significant negative correlation of triglycerides with SOD & vitamin C was seen in groups B&C.

9. HDL showed significant positive correlation with cholesterol and LDL in all the 3 groups and highly significant negative correlation was seen with chol:HDL and LDL:HDL in group B & C.

10. LDL showed strong positive correlation with all lipid profile parameters in all the 3 groups while significant positive correlation was seen with microalbuminuria and sialic acid in group C.

11. Chol:HDL and LDL:HDL ratios showed highly significant positive correlation with all lipid profile parameters except for HDL with which strong negative correlation was seen in all the groups of patients.

12. VLDL showed highly significant positive correlation with lipid profile parameters, FBS, PPBS, HbA1c in group B & C and with sialic acid, ceruloplasmin, MDA and hs-CRP in group C. VLDL showed significant negative correlation with SOD and vitamin C in groups B&C.

Thus the values of triglycerides and VLDL were higher in diabetics with higher values of FBS, PPBS, HbA1c, microalbuminuria, ceruloplasmin, sialic acid, MDA and hs-CRP.

13. Total sialic acid showed strong positive correlation with microalbuminuria, ceruloplasmin, MDA, and hs-CRP in all three groups while highly significant positive correlation was seen with FBS, PPBS and HbA1c in diabetics with and without nephropathy. Total sialic acid showed highly significant negative correlation with SOD and vitamin C.

14. Ceruloplasmin showed highly positive correlation with microalbuminuria, sialic acid, MDA and hs-CRP in all the 3 groups.
Highly significant positive correlation of ceruloplasmin was seen with FBS, PPBS, UACR and HbA1c in group B & C. Strong negative correlation was seen of ceruloplasmin with SOD and vitamin C in all the groups of patients.

15. Vitamin C & SOD showed highly significant positive correlation with each other and negative correlation with FBS, PPBS, HbA1c, Microalbuminuria, UACR, triglycerides, VLDL, sialic acid, ceruloplasmin, MDA and hs-CRP in groups B&C.

16. Highly significant positive correlation of MDA and hs-CRP was seen with each other and microalbuminuria, sialic acid and ceruloplasmin in all the 3 groups, while positive correlation was seen with UACR, FBS, PPBS, HbA1c in group B & C. Strong negative correlation of MDA and hs-CRP was seen with Vitamin C & SOD in diabetics with and without nephropathy.

17. On comparing the duration of diabetes and various biochemical parameter levels, highly significant positive correlation was seen with sialic acid and significant positive correlation was seen with microalbuminuria, ceruloplasmin and hs-CRP.

On Comparison of biochemical parameters among diabetics with micro albuminuria (20-299 mg/day) (gr. C I n= 127) and diabetics with macro albuminuria(≥300mg/L) (gr. C II n=30).

1. The mean values of fasting & post prandial glucose were statistically significantly higher in diabetics with macroalbuminuria (urine albumin≥300 mg/day) (gr. C II) compared to diabetics with microalbuminuria (urine albumin 20-299 mg/day) (gr. C I).

2. No statistical significant difference was seen in mean values of urea and creatinine among subgroups C I & C II (P=0.444, 0.23).

3. The mean value of urinary albumin was very high in Gr. C II compared to gr. C I (P=0.000).
4. No statistical difference was seen in mean HbA1C & avg. glucose levels among gr. C I & gr. C II.

5. Among the Gr. C I & gr. C II mean values of lipid profile, significant difference was seen in mean values of cholesterol, triglycerides, LDL & VLDL (P=0.027, 0.045, 0.044, and 0.045).

6. Among the subgroups of gr. C i.e gr. I & gr. II significantly higher mean values of total sialic acid, ceruloplasmin and MDA were seen in gr. C II (P=0.000).

7. Statistically significant lower mean value of vitamin C was seen in gr. C II compared to gr. C I patients (P=0.000).

8. No significant difference was seen in mean values of SOD in gr. C I & gr. C II (P=0.151).

9. Higher mean value of duration from onset was seen in diabetics with macro albuminuria (gr. C II) compared to diabetics with micro albuminuria (gr. C I) (P=0.000).

**On comparison of biochemical parameters among diabetics and control group in whom urinary albumin creatinine ratio was obtained (A(uacr), B(uacr) and C(uacr))**

1. Significantly higher mean values of urea were seen in diabetics with nephropathy (gr. C) as compared to control group & gr. B made on basis of UACR(P=0.000).

2. In groups based on UACR, the mean value of serum creatinine in control group was not significantly higher compared to gr. B (P=0.36), while it was significantly lower compared to gr. C (P=0.000) and highest in gr. C compared to gr. B (P=0.000).

3. No significant difference between mean values of urinary creatinine of gr. A compared to gr. B & C was seen (P=0.070, 0.640). The mean
urinary creatinine values was significantly higher in gr. C compared to gr. B (P=0.015).

4. The mean value of urinary albumin creatinine ratio (UACR) in gr. B & C were statistically significantly higher compared to mean values of gr. A (P=0.024 &0.000). The mean value of UACR in gr. C was statistically significantly higher compared to mean values of gr. B (P=0.000).

On comparison of biochemical parameters in males & females of all three groups

1. Significantly higher mean values of urea were seen in males compared to females in control group (P=0.000), while no such difference was seen in gr. B and gr. C.

2. Significantly higher mean creatinine values were seen in males compared to females in control group and gr. C (P=0.000), with no significant difference in gr. B male and female values.

3. The mean value of microalbuminuria in females was significantly lower than males in control group (P=0.000), while no statistically significant difference was seen in mean values of males and females microalbuminuria levels in gr. B & gr. C.

4. On comparing the lipid profile mean values among males and females of all 3 groups, mean HDL values were significantly lower in males compared to females in all 3 groups (P-0.001, 0.000 and 0.027), while mean values of triglycerides & VLDL were significantly higher in males compared to females in gr. A (P=0.016) and Chol:HDL ratio showed significant difference in gr. B males & females (P=0.039).

5. On comparing the mean values of TSA, and ceruloplasmin in male and female subgroups of all 3 groups, significant difference was seen only in control group (gr. A) (P=0.019, 0.000 and 0.000).
6. On comparing the mean vitamin C values among males & females of all 3 groups, significantly lower value was seen in males only in control group (gr. A) (P=0.000).

7. No significant difference was seen in mean values of SOD among male & female subgroups of all 3 study groups.

8. Statistical difference in the mean values of MDA among male & female patients was seen in only control group (P=0.000).

9. Significantly higher mean value of Hs-CRP was seen in males compared to females in gr. A (P=0.000), while no significant difference was seen in gr. B & gr. C male & female patients.

CONCLUSIONS

1. The fasting and post prandial blood glucose levels are good markers of diabetes, with markedly higher levels in diabetics compared to non diabetics. Increased values of glucose were seen in diabetes with microangiopathy/ nephropathy. The levels of fasting & post prandial blood glucose correlate positively with Glycosylated hemoglobin and microalbuminuria.

2. Glycosylated Haemoglobin is a good parameter to monitor diabetes control and progression as it showed significant rise in diabetics with nephropathy compared to diabetics without nephropathy, indicating poor control of blood glucose levels leading to disease progression and development of complications. It correlates with duration of diabetes mellitus.

Glycosylated Haemoglobin correlates with markers of inflammation i.e high sensitive C-reactive protein, total Sialic acid and ceruloplasmin.

3. Estimated average blood glucose (eAG) compared to fasting & post prandial blood glucose correlates better with other parameters such as urine albumin creatinine ratio, Ceruloplasmin, Vitamin C,
malondialdehyde, high sensitive C-reactive protein and duration from
diagnosis of Diabetes.

4. Urea and creatinine levels do not distinguish diabetics without
nephropathy from non diabetic patients. They can be good markers to
confirm diabetic nephropathy, especially with increasing levels of
microalbuminuria with which they show positive correlation. Urinary
albumin:creatinine ratio (UACR) is a better marker of diabetic
nephropathy compared to alone urinary creatinine which may not be
significantly raised in initial stages. Urine albumin creatinine on
random urine sample can be used to overcome the difficulty of
collecting 24 hour urine sample for microalbuminuria. It has similar
sensitivity and specificity in diagnosing diabetic nephropathy as
compared to urinary microalbumin levels.

5. The markers of renal insufficiency (serum urea and creatinine), are
clinically correlated with increasing concentration of sialic acid and
high sensitive C-reactive protein. These findings strengthen the
hypothesis that an increase in circulating serum sialic acid and high
sensitive C-reactive protein is an early manifestation of diabetic
renal disease. Hence our study postulates the efficacy of total Sialic
acid to be at par with high sensitive C-reactive protein which is an
established marker for early detection of diabetic complications
like nephropathy.

6. The hypothesis of presence of dyslipidemia in diabetes is further
strengthened by present study showing strong positive correlation of
triglycerides, VLDL and to some extent LDL with blood glucose levels,
Glycosylated hemoglobin, microalbuminuria and high sensitive C-
reactive protein.

7. Urine microalbuminuria was positively correlated with dyslipidemia in
Group C (T2DMN) and therefore this suggests the possibility of
presence of cardiovascular disease in patients with diabetic
nephropathy and the potential use of urine microalbumin as a marker of cardiovascular risk.

8. Increasing concentration of Sialic acid is found to be positively correlated strongly with diabetes. It shows higher values with diabetic nephropathy and positive correlation with the markers of glycemic control (blood glucose and Glycosylated hemoglobin) and renal insufficiency (urea, creatinine and microalbuminuria). These findings strengthen the hypothesis that an increase in total Sialic acid is an early manifestation of diabetic renal disease. The strong positive correlation of high sensitive C-reactive protein with increasing levels of Glycosylated hemoglobin and microalbuminuria make them additional good prognostic markers of disease progression in diabetes. Total sialic acid can be estimated by simple manual biochemical test making it more handy, cheap and reliable parameter to monitor diabetes especially in primary set ups lacking sophisticated instrumentation and costlier test kits of high sensitive C-reactive protein and HbA1c.

9. Positive correlation of total Sialic acid with acute phase reactants like ceruloplasmin and high sensitive C-reactive protein, further establish the role of cytokines induced low grade inflammatory process in pathophysiology of diabetes.

10. Total Sialic acid was positively correlated with Lipids in diabetics and therefore this suggests the potential use of serum total Sialic acid as a marker for cardiovascular risk.

11. Ceruloplasmin is highly significantly correlated positively with Glycosylated hemoglobin, estimated Average glucose, Micral, urine albumin creatinine ratio, total Sialic acid, high sensitive C-reactive protein, malondialdehyde, duration from diagnosis of diabetes and negatively with superoxide dismutase and Vitamin C, in Diabetic patients (group B and C). This is in support of ceruloplasmin as an
APP that increases with hyperglycemia and furthermore with development of diabetic nephropathy and also its pro-oxidant role as it is correlated positively with malondialdehyde and negatively with the antioxidants studied.

12. The role of increased oxidative stress as the root cause underlying development of insulin resistance, beta cell dysfunction and type 2 diabetes has been supported by the increased levels of malondialdehyde. It is also implicated in long term micro and macro vascular complications of diabetes with raised levels in diabetic nephropathy.

13. MDA shows strong positive correlation with hyperglycemia (Glycated hemoglobin), development of microvascular complications (microalbuminuria) and markers of inflammation (total Sialic acid and high sensitive C-reactive protein).

14. The efficiency of antioxidant defense mechanism was accessed by studying the levels of super oxide dismutase (SOD) an endogenous agent & exogenous potent antioxidant agent in the form of vitamin C, both of which were significantly decreased in diabetics, emphasizing the decrease in antioxidants and an indirect evidence of increase in oxidative stress which depletes the antioxidants. Hence it may be hypothesized that apart from good glycemic control, supplementation by antioxidants nutrients in subjects with increased malondialdehyde levels in type 2 DM may help in reducing oxidative stress which is one of the causes of development of Diabetes and its complications.

15. Both the antioxidants studied show a strong negative correlation with markers of acute phase response such as sialic acid and high sensitive C-reactive protein levels.