SUMMARY:

The present thesis entitled “Biochemical and Molecular Studies in Type 2 Diabetes Mellitus and Effect of Nutritional Interventions in Animal Models” comprises a human and an animal study. The human study is an observational, cross-sectional study carried out in type 2 diabetes mellitus (T2DM) patients and healthy individuals (100 each). The human study was undertaken to examine the effects of oral hypoglycemic agents on biochemical parameters and pattern of their associations with other biochemical markers in T2DM patients. The animal study was carried out to examine the effects of nutritional intervention of omega-3 fatty acids on various biochemical parameters in nicotinamide (NIC)-streptozotocin (STZ) induced diabetic Wistar rats. Additionally, expression of genes involved in lipid metabolism such as fatty acid synthase (FAS), long chain acyl CoA synthetases (ACSL), malonyl-CoA-acyl carrier protein transacylase (MCAT), transcription factors like peroxisome proliferator activated receptor (PPARγ), sterol regulatory element binding protein (SREBP1) and genes for inflammatory markers like nuclear factor kappa beta (NFκβ) and tumor necrosis factor alpha (TNF-α) were examined from peripheral blood mononuclear cells (PBMCs) both from human individuals and animals, to detect modulation of expressions of these genes.

Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Poorly controlled hyperglycemia is known to affect different tissues and organs which in turn results into micro- and macrovascular complications. Prolonged hyperglycemia is known to be associated with altered lipid metabolism, increased oxidative stress and alterations in the liver function. Poor glycemic control, duration of diabetes and secondary complications are considered as important factors contributing to mortality in diabetic individuals. The risk of diabetic complications is known to be dependent on the duration and severity of hyperglycemia and it varies with gender.

A number of oral antidiabetic agents currently available for better glycemic control play an important role in reducing blood glucose, improving lipid profile and in limiting oxidative stress and inflammation. Despite all the new treatments that have emerged in the last 20 years, diabetes sufferers remain challenged in effective long-term management of their disease, as some drugs lose efficacy and show adverse
effects with increase in the duration of disease. Most of the drug metabolizing genes show different activities in different populations, which are often major determinants of variations owing to drug exposure and response. In addition to this, drug exposure may vary between men and women due to differences in absorption, metabolism and excretion. There are very few studies which describe gender dependent effects of T2DM and hence it is necessary to focus on sex specific differences of the effects of T2DM.

Diabetic dyslipidemia is one of the risk factors for many diseases which accounts for about 52% mortality in diabetic people. Diabetic patients have 2-4 fold higher risk of development of cardiovascular disease (CVD). Indians are more prone to develop CVD than other complications. Prevalence of altered lipid profile is around 90% in type 2 diabetic Indian patients. Available treatments for dyslipidemia in modern medicine have various undesirable effects and hence alternative/complimentary treatments are one of the attractive approaches for better lipid management in diabetes. Recent reports suggest the use of nutraceuticals and functional foods as a supplementary treatment for lipid abnormalities. Omega-3 fatty acids are one of the recommended food components due to their anti-inflammatory, anti-atherogenic, vasodilatory and lipid lowering properties. The beneficial effects of these omega-3 fatty acids have been established in several chronic diseases including diabetes. However, there is lack of evidence for these effects of omega-3 fatty acid in T2DM and the underlying molecular mechanisms have not been well evaluated. The available literature also points to a need for studies to assess the effects of omega-3 fatty acids on lipid metabolism and its underlying mechanism.

In view of this, it is important to undertake studies to analyze different biochemical markers and their association in diabetic and healthy individuals. Since dyslipidemia is considered as one of the most frequent co-morbidities in T2DM, the comparative lipid normalizing effects of omega-3 fatty acids and metformin, in NIC-STZ induced diabetic rats were also studied in the present study.
HYPOTHESIS:

“Type of drug treatment, fasting blood glucose levels and duration of disease may alter the lipid levels in T2DM which can be improved by omega-3 fatty acid interventions through the modulation of expression of transcription factors and genes involved in lipid metabolism and inflammation”.

STUDY DESIGN:

Human Study: The present study was an observational, cross-section study. The present study assessed type 2 diabetic and healthy individuals (each 100). Diagnosis of type 2 diabetes was based on the American Diabetes Association (ADA) criteria i.e. fasting plasma glucose ≥126 mg/dl. Fasting venous blood samples were collected, blood components were separated and used for various biochemical analyses. Alterations in the expression of different genes were studied from PBMCs.

Animal Study: Animals were randomly divided in five groups (n=6). Nicotinamide (NIC)-streptozotocin (STZ) was used for induction of diabetes. After confirmation of stable hyperglycemia, metformin or flax/fish oil intervention was given for 30 days. Blood samples were collected at day 15 for various biochemical estimations. Animals were dissected at day 30 for collection of blood and liver. Blood components were separated and used for various biochemical estimations. PBMCs were used for gene expression analysis while liver tissues were subjected to histological analysis.

Statistical Analysis:

The data were analyzed by using SPSS/PC+ statistical package (Version 20.0, Chicago, IL). Skewed variables were transformed to normality using natural logarithm transformation. For human studies, two tailed unpaired t-test was used to compare means of different parameters. Mean values of various parameters from the patient with higher FBG levels were compared with those of the patient with normal FBG levels. In addition, mean values of various parameters from the disease duration groups were compared using Fischer’s least significance difference test. Correlation analysis was done using Pearson correlation coefficients to correlate HOMA-IR, FBG
levels and duration of disease with biochemical parameters. Correlation was considered significant if $P \leq 0.05$. Statistical differences between means in different groups of the animal studies were determined using one way analysis of variance (ANOVA) followed by Boneferroni multiple comparison test. Mean values of various parameters from each group at day 15 were compared with those at day 30 using Student’s t-test. $P \leq 0.05$ was considered statistically significant.

THE KEY FINDINGS AND CONCLUSIONS OF THIS STUDY INDICATE:

1. **Human Study:**

   Stringent management of metabolic risk factors is believed to be crucial in reducing morbidity and mortality in diabetic patients. This requires aggressive strategies for intensive glucose control.

   **The important findings from the human study are:**

   1. The alterations in lipid profile, liver function tests, antioxidant enzyme activities, inflammatory marker and adipocytokines are dependent upon the fasting blood glucose levels of diabetic individuals

   2. Insulin resistance and variation in biochemical parameters depend on the type of treatment

   3. Fasting glucose levels and chronicity of disease have gender dependent effects on biochemical parameters

   4. Frequently used OHAs significantly improve circulatory levels of adipocytokines

   5. Expression of genes involved in lipid metabolism and inflammation are altered in diabetic individuals

   **The results of the present study, demonstrate that oral hypoglycemic agents and injectable insulin treatment has gender dependent effects on insulin resistance, lipid profile, liver function test, antioxidant enzymes, inflammatory marker and adipocytokines.** The study, for the first time, **has reported that diabetes affects the expression patterns of genes from PBMCs.** The present study
suggests a need to select the best treatment option based on variations in biochemical parameters for each patient on a particular antidiabetic drug treatment to normalize the levels of important biochemical and molecular markers. ADA guidelines and 2012 European Association for the Study of Diabetes (EASD) recommend individualized treatments based on socioeconomic and lifestyle factors of the patients.

2. Animal Study:

The important findings from the animal study are:

1. The treatment with metformin and flax/fish oil intervention in diabetic rats improves serum lipid profile

2. Metformin and flax/fish oil intervention improves the expression of lipid metabolism genes like PPARγ and SREBP1 and lowers atherogenic cytokines like NFκβ and TNF-α

3. Flax oil and/or fish oil exhibit protective effects on hepatic architecture under uncontrolled hyperglycemic states

The results of the present study, for the first time, demonstrate expressions of transcription factors involved in the lipid metabolism from rat PBMCs. The results of the present animal study demonstrate comparative effects of metformin and omega-3 fatty acids on the expressions of transcription factors involved in the lipid metabolism. Omega-3 fatty acid supplementation was found to normalize lipid profile through modulation of transcription factors involved in lipid metabolism and inflammation. The combination therapy of metformin and omega-3 fatty acid intervention is worth investigating in T2DM patients.

IMPLICATIONS:

Epidemiological studies indicate high prevalence of diabetes all over the world with rapid increase in India. The human study presented in the thesis has demonstrated variations in biochemical parameters and their associations in diabetic and healthy individuals. The current study establishes an association of insulin
resistance with biochemical markers which depends on the type of treatment i.e. OHAs or injectable insulin treatment. In addition, the animal study demonstrates that omega-3 fatty acid supplementation has beneficial effect on lipid profile and expression of genes involved in lipid metabolism and inflammation. These findings may help to design the future studies with omega-3 fatty acid interventions in diabetic individuals on metformin monotherapy.

**SOCIETAL RELEVANCE:**

The current work is useful to understand gender specific effects of antidiabetic drugs. This study may provide clues to clinicians about variations in important biochemical parameters based on antidiabetic drug treatments. Data from animal study may be used to design intervention studies in dyslipidemic type 2 diabetic individuals with a focus to normalize lipid abnormalities.