Inflammation plays a significant role in human health. Optimal level of inflammation is required for immunity enhancement while chronic inflammation is associated with several metabolic disorders like type 2 diabetes, obesity, cardiovascular disease, etc. Though inflammation and type 2 diabetes are directly associated, the cause and effect is not well defined. Several reports have indicated that the progression and severity of the metabolic disorders are well correlated with increased level of inflammatory parameters. It has also been demonstrated both in animals models and human studies that lowering of end point markers (e.g., HbA1c) using pharmaceutical drugs like Thiazolidinediones and GLP 1 analogs also reduced the levels of inflammatory markers (e.g., IL-6, TNF \( \alpha \) etc).5-7

It may be hypothesized that in healthy population, change in pro-inflammatory markers should be compensated by altered anti-inflammatory markers. Any deviation from this profile would lead to systemic disorder. The two important regulators in this context of metabolic disorders are adiponectin and IL6. Adiponectin is a 244 amino acid long protein that is secreted from adipocytes with anti-inflammatory and insulin-sensitizing property. The other member is pro-inflammatory cytokine, IL-6 which is known to be elevated in diabetes.10,11 These markers though well understood in terms of their regulation in diabetes population are still lacking acceptance as clinical markers due to the variation of levels among various ethnic groups.12,13

In recent times, the number of diabetic and obese people has increased significantly in developing and emerging countries like India and China. Various metagenomic studies indicated the association of common gene variants encoding adipokines and inflammatory markers with adiposity in Asian Indians and role of truncal obesity accounting for the 2-fold excess incidence of diabetes in Asian Indians. Unfortunately, two main factors are not reported in these meta-genomic studies, i.e., gene function manifestations in pre-diabetic population and comparison of gene function with other ethnic groups. In this study, we focused on the importance of inflammation on type 2 diabetes and investigated its association with the progression from normoglycemic to hyperglycemic (type 2 diabetes) via pre-diabetic stage (impaired fasting glucose specifically) in Indian population. In addition, currently limited information is available on inflammatory parameters in type 2 diabetes and people with impaired fasting glucose in Indian population. In this manuscript we highlighted the role of balance of inflammatory regulators (adiponectin and IL 6) in progression of type 2 diabetes in Indian population.
Results

Table 1 lists the clinical and biochemical parameters of the study groups. Total cholesterol, triglycerides, LDL contents increased significantly and HDL content decreased significantly in serum of diabetic and pre-diabetic groups compared with the normoglycemic individuals. In addition, the liver function enzymes also increased significantly in pre-diabetic and hyperglycemic groups compared with normoglycemic individuals.

Serum IL 6 levels were significantly higher in IFG population compared with normoglycemic population (2.00 ± 0.14 pg/ml vs 1.77 ± 0.23 pg/ml, P < 0.05). On comparing the hyperglycemic population with normoglycemic population, the increase was statistically significant (2.84 ± 0.62 pg/ml vs 1.77 ± 0.23 pg/ml, P < 0.01). No significant difference was observed between IFG and hyperglycemic groups. The result is as shown in Figure 1.

Significant difference was observed in serum adiponectin levels as shown in Figure 2. The level was highest in the normoglycemic population (6.9 ± 0.45 μg/ml) and found to be lesser in the remaining 2 groups as per disease progression. Significant lowering in the adiponectin levels in serum was observed in IFG (5.57 ± 0.53 μg/ml) and hyperglycemic group (4.0 ± 0.42 μg/ml) compared with normoglycemic group (P < 0.05 and P < 0.001 respectively). In addition, the adiponectin levels in serum reduced significantly in hyperglycemic group when compared with IFG group (P < 0.05).

Diabetes is an inflammatory disease and when the ratios of adiponectin (anti-inflammatory adipokine) to IL 6 (pro inflammatory cytokine) were compared among the three groups, there was a significant decrease in the ratio (Fig. 3) with progression from normoglycemia to IFG (P < 0.005) to hyperglycemia (P < 0.001). The decrease in the ratio with the progression of IFG to hyperglycemia is a statistically significant (P < 0.05) change.

In addition, when the levels of inflammatory markers, i.e., high sensitivity C reactive protein and IL 6 were divided into tertiles, the largest proportion of normoglycemia individuals were in the first tertile of these markers (which is the normal physiological range) whereas the larger proportion of the IFG and hyperglycemia when compared with normoglycemia individuals were in the third tertile of the markers (higher level of inflammation) (Figs. 4 and 5).

In case of adiponectin, as expected, the reverse was observed with the largest proportion of normoglycemia individuals in the third tertile (maximum level of anti-inflammation) and larger
proportion of the IFG and hyperglycemic individuals in the first tertile of serum adiponectin levels, which means higher level of inflammation (i.e., lowest anti-inflammation or adiponectin level) (Fig. 6).

**Discussion**

The adipose tissue mass increase occurs by the expansion of pre-existing adipocytes (adipocyte hypertrophy) or by generating new small adipocytes. Chronic overfeeding results in adipocyte hypertrophy and is associated with decreased adiponectin levels and increased IL-6, and TNF-α production. In addition, the abnormal functioning of adipocytes like lipodystrophy and inability to store triglycerides and fatty acids may result in ectopic fat storage in liver, skeletal muscle etc., thus causing dyslipidemia and insulin resistance. This condition may also play an important role in the development of a chronic low-grade pro-inflammatory state associated with adipose tissue dysfunction and diabetes. Enlarged adipocytes commonly associated with South Asian population appear to lead to an imbalance between pro- and anti-inflammatory adipokines.\(^{15,16}\) The secretions of pro-inflammatory cytokines IL-6, IL-8, MCP-1, and GM-CSF have been positively correlated with adipocyte size.\(^{17}\)

Significant correlation with insulin resistance makes adiponectin a powerful prognostic marker for diabetic risk in patients who do not yet manifest T2DM. The protective effects of adiponectin in prevention of progression of insulin resistance and in cardiovascular events, and its potent influence in components of the metabolic syndrome, have made it a highly promising therapeutic target.\(^{18-21}\) Numerous clinical studies have reported the inverse relationship between adiponectin and insulin resistance.\(^{12}\) Low circulating adiponectin levels is associated with a decrease in whole body insulin sensitivity in humans and has been shown to be predictive of future development of diabetes in few studies.\(^{12}\) In diabetes prevention program studies, subjects who are at the risk of type 2 diabetes were followed up for the progression to type 2 diabetes. Low levels of baseline adiponectin had a strong correlation with the onset of T2DM. The subjects who progressed to T2DM within one year had very low baseline value of adiponectin. This study concluded adiponectin as a powerful marker of diabetes risk in subjects at high risk for diabetes, even after adjustment for weight. An increase in adiponectin in the lifestyle and placebo groups was associated with a reduction in diabetes risk.\(^{22}\)

The populations where adiponectin as marker were investigated were from various ethnic groups.\(^{12,23-25}\) There is a need to evaluate adiponectin levels in Indian population as there is limited population based evidence among Indians during the progression
of normoglycemic to hyperglycemic condition. A recent study done by Mohan et al. indicated profiling of serum adiponectin in healthy, IGT, and diabetic individuals. Adiponectin levels in IFG individuals were not evaluated in that study.

IL-6 is a major pro-inflammatory cytokine, secreted from leucocytes, adipocytes, skeletal muscle and endothelial cells. In vitro studies have shown that IL-6 treatment downregulates adiponectin mRNA suggesting a negative role of IL-6 in adiponectin regulation. Experimental studies and cross-sectional analyses have shown that circulating IL-6 is associated with hyperglycemia and insulin resistance. It has also been shown that circulating IL-6 increases with the degree of insulin resistance. Though these studies evaluated the levels of the cytokine in healthy, IGT, and diabetic individuals, individuals with impaired fasting glucose were not considered. The level of CRP and IL-6 reported is approximately 10-fold higher than what we have observed in our study population.

It has been reported in German populations that serum adiponectin, hsCRP, and IL-6 levels were not significantly different between IFG and IGT individuals but no information is available from other ethnic groups.

We also found metabolic dyslipidemia characterized by high circulating cholesterol, triglycerides, LDL, and low circulating HDL in our study. The increase in the lipid parameters during transition from pre-diabetes (IFG) to hyperglycemia clearly indicates that hepatic metabolism of cholesterol, triglycerides, and lipoprotein metabolism is impaired and hepatic insulin sensitivity is compromised which would definitely contribute to impaired fasting glucose.

Our results also indicate that the levels of liver enzymes increase significantly during disease progression. Various other studies have shown that increase in the levels of these hepatic enzymes is associated with later development of type 2 diabetes. Both adipocyte hypertrophy and lipodystrophy results in increase in inflammatory cytokines like IL-6 and release of fatty acids into the circulation. The excessive deposition of triglycerides inside the liver along with the inflammatory cytokines could induce hepatocellular damage which may lead to an increase in high levels of circulating liver enzymes. It is also hypothesized that elevation in ALT, a gluconeogenic enzyme whose gene transcription is suppressed by
insulin could indicate impairment in insulin signaling rather than hepatocyte injury. The ratio of AST to ALT showed a decreasing trend along with the disease progression. This is in congruence with similar study done in Thailand population where ALT levels were strongly indicative of future risk of developing IFG and type 2 diabetes. Another observation in our study was the increase in the high sensitivity C-reactive protein (hsCRP) in diabetics. High sensitivity CRP is synthesized in hepatocytes and is widely recognized as a marker for subclinical inflammation. Prospective studies have shown that high hsCRP is predictive of metabolic syndrome, type 2 diabetes, and coronary heart disease. Studies have also shown positive correlation with abdominal obesity and CRP in Asian Indians. In Japanese populations high levels of hsCRP was a strong predictor of non-alcoholic fatty liver disease. Recently increased levels of hsCRP have been reported in Indian population. In case of individuals with impaired glucose tolerance, the levels of hsCRP were higher than that of individuals with impaired fasting glucose. In addition it was also observed in that study that there were gender based differences in the levels of hsCRP across all groups. Similarly, we have observed that there are significant gender based differences in normoglycemic individuals both in the case of hsCRP and adiponectin levels (P < 0.05); in IFG, this was observed only in the case of adiponectin (P < 0.005) whereas in hyperglycemic condition, this was observed only in the hsCRP levels (P < 0.05). The gender-specific differences (increased levels in females) observed in this group of people are in line with a study conducted on a Finnish population. This study did not note any significant difference in the levels of hsCRP as in the case of our study. Similar to the Finnish study, we observed that adiponectin concentrations in women decreased relatively more compared with men across individuals with normoglycemia, IFG, and type 2 diabetes, whereas inflammatory markers increased relatively more in women.

In the case of IL 6, there was no significant difference between genders across all groups (Table 2). From our data it can be summarized that there is a significant change in both adiponectin and IL-6 levels in healthy, prediabetic, and diabetic population in Indian population. There is a significant but gradual change during the progression of healthy
toward diabetic population via pre-diabetic condition. This is a pilot study and needs to be validated in larger cohort.

**Materials and Methods**

The study was approved by the Unilever Independent Research Ethics Committee (UIEC), India. Pregnant or lactating females, subjects with renal diseases, smokers, and subjects consuming more than 60 ml alcohol/week or exercising more than 10 h/week were excluded from the study. Diagnosis of Type 2 diabetes was based on fasting blood glucose and American Diabetes Association guidelines. The cohort consisted of 162 volunteers (78 males, 84 females) aged between 28–60 y (mean age 46 y) and of Indian origin. Diabetic (n = 32) and prediabetic (n = 49) volunteers were gender and age matched with volunteers who are normoglycemic (n = 81). Informed consent was obtained from all the volunteers before initiation of the study.

The groups were classified as per ADA norm: Normoglycemia or healthy (fasting glucose < 100 mg/dl), impaired fasting glucose (IFG) or pre-diabetes (fasting glucose > 100 mg/dl to < 126 mg/dl), and hyperglycemia or diabetes (fasting glucose > 126 mg/dl).

Physical examination included height and weight measurements, and the body mass index (BMI) was calculated using the formula: weight (kg) divided by height in meters squared. Waist and hip measurements were made in the standing position using standard techniques. A fasting blood sample was taken and the serum was separated and used for the assay. Clinical measurements: total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides (TG), blood urea, glycated hemoglobin (HgbA1c), blood glucose, creatinine, serum bilirubin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), glutamyl transferase (GGT), alkaline phosphatase (ALP), and high sensitivity creative protein (hsCRP) were measured using Roche Hitachi 912 automatic analyzer. Serum adiponectin and serum IL-6 levels were quantified using ELISA kits (R&D Systems).

**Data Analysis**

As mentioned earlier, the volunteers were classified into 3 groups, namely normoglycemia or healthy, impaired fasting glucose (IFG) or pre-diabetes, and hyperglycemic or type 2 diabetes. The range of serum high sensitivity CRP, IL-6, and adiponectin quantified in our study samples were divided into tertiles and the proportion of individuals from the three groups falling in each of the tertiles were analyzed.

Data were assessed for normality and skewed data were logarithmically transformed before statistical analysis.

ANOVA adjusting for the effects of gender and age was performed to compare normoglycemia or healthy, impaired fasting glucose (IFG) or pre-diabetes and hyperglycemic or type 2 diabetes. All statistical analyses were performed using SAS software version 9.3.

Tables and graphs represents mean ± SE of absolute values and log transformed units respectively.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We are thankful to Prof P.R. Krishnaswamy and Dr Vilas Sinkar for providing critical inputs and suggestions during the planning and execution of this study.

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**Table 2. Gender based differences in the levels of inflammatory markers**

|                  | Normoglycemia | IFG | Hyperglycemia |
|------------------|---------------|-----|---------------|
| hsCRP (mg/L)     |               |     |               |
| M                | 0.19 ± 0.05   | 0.34 ± 0.05 | 0.35 ± 0.1   |
| F                | 0.41 ± 0.09a  | 0.44 ± 0.07 | 0.67 ± 0.08b |
| IL 6 (pg/ml)     |               |     |               |
| M                | 1.41 ± 0.17   | 2.11 ± 0.25 | 1.94 ± 0.39  |
| F                | 1.71 ± 0.13   | 1.89 ± 0.14 | 2.5 ± 0.26   |
| Adiponectin (μg/ml) |           |     |               |
| M                | 5.82 ± 0.48   | 4.05 ± 0.5 | 4.29 ± 0.82  |
| F                | 8.26 ± 0.78a  | 7.17 ± 0.85b | 3.74 ± 0.42c |

Data are represented as mean ± se. aP < 0.05 and bP < 0.005 when the two genders are compared. cP < 0.005 when compared with corresponding normoglycemic group. dP < 0.05 when compared IFG group.
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