ABSTRACT

The high prevalence of diabetes mellitus and its increasing incidence worldwide is becoming a public health issue of great concern, especially in developing countries. Hyperglycemia, the characteristic of diabetes, is considered to be one of the major reasons for the establishment of micro- and macrovascular diseases associated with it. Hyperglycemia mediated overproduction of superoxide radicals by the mitochondrial electron transport chain, is the key event in the activation of several pathways which are involved in the pathogenesis of diabetic complications. Excess generation of superoxide radicals lead to the formation of several secondary radicals creating a state of imbalance between their production and the intrinsic antioxidant defense mechanism of a cell, referred to as oxidative stress (OS). In addition to the role of OS in development of secondary complications, OS has also been suggested to cause abnormalities of β-cell dysfunction and insulin secretion or insulin action resulting in insulin resistance (IR).

There exist several reports demonstrating enhancement of OS in diabetic patients, however, serial and comprehensive measurements of oxidative stress parameters (OSP) in newly diagnosed diabetic patients from the time of diagnosis and during follow-up visits after starting anti-diabetic treatment is still missing. In the present study, a comprehensive profiling of OSP was done which included antioxidant enzymes namely, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD); antioxidant molecules namely, glutathione (GSH), vitamin C, bilirubin and uric acid; and oxidative damage markers namely, protein carbonyls, protein sulphhydryls, TBARs and 8-hydroxy-2’-deoxyguanosine (8-OHdG). Since, OS plays an important role in the pathogenesis of both β-cell dysfunction and IR, inter-relation between oxidative stress status, β-cell function and IR was investigated.

Fifty-four newly diagnosed diabetic patients were studied at diagnosis and four and eight weeks after anti-diabetic treatment. A group of fifty non-diabetic subjects served as controls. The baseline difference between the two groups was tested using multiple linear regression analysis, adjusted for age and BMI, and differences in serial measurements was analysed by repeated measure ANOVA. Oxidative stress score (OSS) was computed as gender specific Z score of each
parameter and its association with changing glucose concentrations, HOMA-IR and HOMA-β was analysed.

The results obtained indicate that there was a significant impairment in antioxidant defense and an increase in oxidative damage markers in newly diagnosed diabetic patients compared to non-diabetic subjects. This was clearly reflected in lower activities of antioxidant enzymes, lower concentrations of antioxidants vitamin C & GSH, and elevated concentrations of oxidative damage markers despite elevation in endogenous antioxidants (bilirubin and uric acid). Eight weeks of anti-diabetic treatment improved glucose and HbA₁C concentrations in newly diagnosed diabetic patients. Additionally, in diabetic patients, antioxidant defense status improved in response to anti-hyperglycemic treatment. This was reflected in elevated activity of antioxidant enzymes and concentrations of antioxidant molecules and decreased concentration of oxidative stress markers. Improvement in OSP in diabetic patients was not related to the different anti-diabetic drugs received by them. Non-diabetic subjects showed a stable status of OSP over eight weeks. Among OSP, concentrations of GPx, uric acid, bilirubin and TBARs became comparable to those in non-diabetic subjects. Level of glycemia was a strong predictor of OS at baseline in diabetic patients, and fall in glucose concentration over the study duration was a strong predictor of improved antioxidant status.

Twelve different OSP were combined into a single comprehensive oxidative stress score (OSS), which reflected total OS state in diabetic patients. Improvement in OSS in diabetic patients was strongly associated with improvement in β-cell function which is indicative of restoration of β-cell function in response to reduced OS. However, improvement in hyperglycemia and reduction in OSS were not associated with improvement in insulin sensitivity, suggesting that insulin sensitivity could not be reversed in eight weeks. Anti-diabetic treatment for a longer duration is likely to further restore the activity of antioxidant enzymes and consequently reduce the oxidative damage to cells and improve insulin sensitivity. Therefore, it is interesting to study the effect of stricter and longer glycemic control regimen on OS and its potential benefits on β-cell function and other forms of tissue damage.

Considering the involvement of OS in diabetes, it is suggested that drugs having anti-hyperglycemic and antioxidant activity would serve as a better therapeutic approach towards treatment and management of diabetes. Antioxidants of plant origin such as resveratrol, curcumin, quercetin, rutin, etc have been reported to possess anti-
diabetic potential both in animal and clinical studies. Of these resveratrol, a naturally occurring stilbenoid has been widely studied for its beneficial effects in preventing and/or managing several diseases. Pterostilbene is a methoxylated analogue of resveratrol and it is gradually gaining more importance as a therapeutic drug owing to its higher lipophilicity, bio-availability and biological activity in comparison to resveratrol. With this background, pterostilbene, a compound with known anti-diabetic activity was studied with respect to its antioxidant potential. Antioxidant activity of pterostilbene was characterized by evaluating its ability to scavenge free radicals such as superoxide, hydroxyl and hydrogen peroxide and to protect biomolecules within a cell against oxidative insult by modulating its antioxidant defense mechanism.

Antioxidant activity of pterostilbene was evaluated extensively by employing several *in vitro* radical scavenging/inhibiting assays and pulse radiolysis study. In addition to this, its ability to protect rat liver mitochondria against tertiary-butyl hydroperoxide (TBHP) and hydroxyl like radicals (generated via Fenton reaction) generated oxidative damage was determined by measuring the damage markers such as protein carbonyls, protein sulphhydrils, lipid hydroperoxides, lipid peroxides and 8-OHdG. Pterostilbene was also evaluated for its ability to inhibit hydroxyl-like radical induced single strand breaks in pBR322 plasmid DNA. Further the mechanism by which pterostilbene alleviates OS was elucidated by using HepG2 cells as a model system and TBHP, as a pro-oxidant.

Pterostilbene exhibited strong antioxidant activity against various free radicals such as DPPH, ABTS, hydroxyl, superoxide and hydrogen peroxide in a concentration dependent manner. Pulse radiolysis studies also revealed that pterostilbene exhibited ability to scavenge ABTS$^-$, $^\cdot$OH and CO$_3^{2-}$ radicals within a short span of time. Results obtained in pulse radiolysis study are physiologically more relevant as most of these free radicals are short lived and need to be neutralised instantly before they interact with molecular entities in a cell. The antioxidant activity of pterostilbene, as observed in the present study, could be attributed to the hydroxyl group in the 4′ position as well as trans-configuration of the ethylene functional group in its stilbene skeleton.

Pterostilbene also conferred protection to proteins, lipids and DNA in isolated mitochondrial fractions against oxidative damage induced by TBHP and hydroxyl-like radicals (generated via Fenton reaction). It also prevented the formation of single
strand breaks in pBR322 plasmid DNA against oxidative assault. Further, its ability to modulate antioxidant defense mechanism under OS was studied using HepG2 cells as a model system. Exposure to TBHP significantly led to a reduction in GSH and increased the activity of antioxidant enzymes. This consequently resulted in increase in the oxidation of lipids measured in terms of formation of malondialdehydes. Treatment with pterostilbene reduced the formation of ROS in HepG2 cells and also maintained the redox buffer by restoring the concentration of GSH. Pterostilbene also effectively decreased the activity of antioxidant enzymes which was concurrent with the down-regulated expression of genes coding antioxidant enzymes.

In conclusion, decline in the antioxidant defense along with increase in the oxidative damage markers was evident in newly diagnosed diabetic patients in comparison to non-diabetic subjects. Glucose control in response to anti-diabetic treatment over the duration of study partially restored the antioxidant defense and decreased the extent of oxidative damage in diabetic patients. This decline in OS was associated with improvement in β-cell function. Having realised the importance of controlling OS in diabetes in conjunction with controlling glucose concentration, Pterostilbene, a known anti-diabetic agent, exhibited ability to confer protection to cellular biomolecules by modulating the expression of genes encoding antioxidant enzymes. These results potentiate the importance of pterostilbene in the treatment of diabetes owing to its anti-diabetic and antioxidant potential.