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Convergence of adipocyte hypertrophy, telomere shortening and hypoadiponectinemia in obese subjects and in patients with type 2 diabetes

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ABSTRACT

Objective: Although telomere shortening has been linked with type 2 diabetes and most variables of adiposity, a shortcoming of such studies is the measurement of telomere length in leukocytes. Therefore, we tested the association among adipocyte cell size, telomere length (both subcutaneous and visceral adipose tissue) and systemic levels of adiponectin in obese subjects and patients with type 2 diabetes compared to control subjects.

Methods: Human subcutaneous and visceral adipose tissues were obtained from the subjects who have undergone bariatric surgery or other abdominal surgeries. The study groups comprised: i) control subjects, ii) type 2 diabetes patients, iii) obese subjects without diabetes and iv) obese subjects with diabetes. Adipocyte cell size was measured by histological staining. Adiponectin levels were measured by ELISA. Telomere length was determined by Real-time PCR and lipid peroxidation was assessed by fluorimetry.

Results: Compared to control subjects, adipocyte size (both subcutaneous and visceral) from obese, diabetic and obese–diabetic subjects was significantly larger [$p<0.001$]. Individuals with adipose hypertrophy also exhibited shortened telomeres and hypoadiponectinemia. Pearson correlation analysis revealed that both visceral and subcutaneous fat cell size showed a positive correlation with FBS, HbA1c, HOMA-IR, LDL, total cholesterol, triglycerides and negatively correlated with HDL and adiponectin. Regression analysis revealed that the association between shortened telomeres and hypoadiponectinemia was lost when adjusted for adipocyte cell size.

Conclusion: Adipocyte hypertrophy appears to be strongly associated with shortened telomeres, hypoadiponectinemia and poor glycemic and lipid control. Interestingly, these molecular alterations seen in lean diabetics reflect a state of 'metabolic obesity'.

1. Introduction

South Asians are at the higher risk of getting obesity related-non communicable diseases (OR-NCDs) compared with white Caucasian counter parts, which include insulin resistance, the metabolic syndrome, type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD). The main reason for this accelerated increase in number of OR-NCDs is the rapid change in diet and more sophisticated life style [1]. Recent evidence also suggests that South Asians develop alterations in metabolic risk factors such as glucose, insulin, lipid levels and adipokines at significantly lower body mass indices than white Caucasians [2]. When introspecting the above statements, it is very much evident that there is a potential involvement of adipose tissue. It has been recently recognized that adipose cells have important endocrine function whereby they play a central role in the pathogenesis of diabetes and obesity. An increased proportion of visceral adipose tissue (VAT) is frequently reported to be associated with various metabolic diseases including type 2 diabetes [3]. In contrast with VAT, there is less consensus regarding the association between abdominal and peripheral subcutaneous adipose tissue (SAT) with disease risk, and both negative and positive associations have been reported [4]. Recent studies emphasize that adipocyte size is of importance in determining metabolic (i.e., lipolytic and lipogenic activities) and endocrine (i.e., leptin and adiponectin release) functions. Increased adipocyte size has been shown to predict the incidence of type 2 diabetes in women [5]. Moreover, adipocyte hypertrophy per se has been reported to be related to insulin resistance in type 2 diabetic patients independent of

Abbreviations: OR-NCDs, obesity related-non communicable diseases; T2DM, type 2 diabetes mellitus; CHD, coronary heart disease; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; BMI, Body mass index; LDL, Low density lipoprotein; HbA1c, Glycated hemoglobin; HOMA-IR, homeostasis assessment model-insulin resistance; MDA, Malonodialdehyde; TBARS, thiobarbituric acid reactive substances.

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total body fat [6]. Since, South Asians were reported to develop alterations in metabolic risk factors at significantly lower body mass indices, there is an imperative need to study the impact of adipocyte hypertrophy in relation to diabetes and its associated complications.

Recent evidence also suggests that accumulation of oxidative damage to various cellular macromolecules may be causally associated to cellular senescence and different age-related diseases. Indeed, recent studies propose that telomere shortening is a marker of biological aging, and that individuals with shorter telomeres than might be expected based on their chronological age, are more prone to develop chronic diseases including diabetes and atherosclerosis [7,8]. While the relationship between the oxidative damage and telomere shortening in various metabolic and cardiovascular diseases is known [9], in all these studies, telomere length was measured only in white blood cells. In order to obtain a causal relationship between adiposity and metabolic diseases, we need to study the telomere length in various depots of adipose tissue and to delineate how senescence mechanisms could compromise the cellular function. As the obesity epidemic increases, it is critically important to more fully understand the adipocyte cell type, and its potential as a major therapeutic target. In particular, understanding the relationship between metabolic disease and adipocyte size, number and distribution will drive the development of novel strategies to minimize the health impacts of obesity and related metabolic diseases. Therefore, the aim of this study was to test the cross-sectional association between adipocyte hypertrophy and telomere length alterations in both subcutaneous and visceral adipose tissue along with systemic levels of adiponectin in obese subjects and patients with type 2 diabetes compared to control subjects.

2. Methods

2.1. Sample collection

Human subcutaneous and visceral adipose tissues were obtained from subjects who have undergone bariatric surgery and other abdominal surgeries at the Lifeline Multi Speciality Hospital, a tertiary care center in Chennai. The present study was performed according to the Declaration of Helsinki and approved by the Institutional Ethics Committee. Written informed consent was obtained from all subjects. The study groups comprise i) non-obese control subjects (n = 20), ii) obese subjects without diabetes (n = 22), iii) non-obese type 2 diabetes patients (n = 10) and iv) obese subjects with diabetes (n = 7). The subjects were classified as diabetes and obese according to the WHO criteria. A part of the tissue samples collected was immediately snapped frozen in liquid nitrogen for molecular measurements and a small portion was stored in 4% paraformaldehyde for adipocyte cell size measurement.

2.2. Anthropometric measurements

Anthropometric measurements including weight and height were obtained using standardized techniques. Height was noted with a tape measured to the nearest centimeter. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. Body mass index (BMI) was calculated using the formula weight (kg)/height $(m)^2$.

2.3. Biochemical parameters

Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method) serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method) and HDL cholesterol (direct method–polyethylene glycol-pretreated enzymes) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). The intra and inter assay co-efficient of variation for the biochemical assays ranged between 3.1% and 7.6%. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Glycated hemoglobin (HbA1c) was estimated by high-pressure liquid chromatography using the Variant analyzer (Bio-Rad, Hercules, Calif., USA). The intra and inter assay coefficient of variation of HbA1c was <10%. Insulin resistance was calculated using the homeostasis assessment model (HOMA-IR) using the formula: fasting insulin (μU/mL) * fasting glucose (mmol/L)/22.5.

2.4. Adipocyte size measurement

The cross-sectional areas of adipose tissue in histological sections were determined using image analysis of digital photomicrographs as a measure of adipocyte size. Briefly, tissue samples stored in phosphate-buffered formalin solution were subjected to hematoxylin and eosin staining. Three sections (separated by 50 μm each) were photographed for analysis. The histology sections were viewed at 10× magnification, images were captured with a digital camera, the data was converted into a binary format with Adobe PhotoShop 6.0.1 (Adobe Systems, San Jose, CA) and Image Pro plus software was used to measure the cell size.

2.5. Telomere length measurement by Real-time PCR method

Telomere length was determined by Real-time PCR as previously described by Cawthon [10] using the genomic DNA obtained from adipose tissues of both subcutaneous and visceral fats. This method measures the factor by which the ratio of telomere repeat copy number to single-gene copy number differs between a sample and that of a reference DNA sample. PCR amplification was achieved using telomere (T) and single copy gene, 36B4 (encodes acidic ribosomal phosphoprotein) primers (S) which serves as a quantitative control. The mean telomere repeat gene sequence (T) to a reference single copy gene (S) is represented as T/S ratio which was calculated to determine the relative telomere length. Briefly, PCR reactions were performed in triplicate in 20 μl reaction volumes (using 25 ng DNA sample per reaction) for all the samples studied. The PCR mixture contained 10 pmol of each of the primers, 100 μm of each dNTPs and 0.3 × SYBR green dye and 0.5 Units of fast taq DNA polymerase (Fermentas, USA). The PCR thermal conditions for relative telomere length assay using telomeric primers (T) and single copy gene primers (S) consisted of an initial denaturation of 5 min at 95 °C, followed by a total of 40 cycles at 95 °C for 5 s, 56 °C for 30 s, and 72 °C for 30 s and fluorescence acquisition. Crossing points (Cp) were determined using the ABI 7000. A standard curve derived from serially-diluted reference DNA was generated in order to check PCR efficiency between the plates. The average of telomere versus single copy gene (T/S) ratio was calculated which is proportional to telomere length of each sample. For quality control purposes, we have repeated many samples that were separately PCR amplified. All measurements were performed in a blinded fashion without knowledge of the clinical data.

2.6. Adiponectin measurement by ELISA

Adiponectin levels were measured in serum from the study subjects by ELISA methodology (R&D Systems). In brief, samples were added to a 96 well microplate which was precoated with specific antibody against adiponectin. After incubation for 2 h at room temperature, wells were washed and antibodies were allowed to conjugate with horse radish peroxidase. Incubation was continued for 2, plates were washed, color development was achieved using substrate solution and the optical density was measured at 450 nm.

2.7. Lipid peroxidation measurement

Plasma levels of Malonodialdehyde (MDA), a marker of lipid peroxidation were measured by thiobarbituric acid reactive substances (TBARS) using a fluorescence methodology [7]. Absolute MDA levels

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were calculated by regression parameters using different concentrations of the standard, 1,1′,3,3′,-tetramethoxypropane. Inter- and intra-assay coefficient of variation of the above method were <5 and 10%, respectively.

2.8. Statistical analysis

All data are represented either as Mean ± SD or Mean ± SE. Comparison between groups was performed using one-way ANOVA with $p<0.05$ as the criterion for significance. Pearson correlation was done between variables and the risk factors. Linear regression analysis was done using telomere length (both subcutaneous and visceral fat) as dependent variable and adiponectin as independent variable. All analyses were done using windows based SPSS statistical package (version 12.0, Chicago, IL).

3. Results

Age-adjusted clinical and biochemical characteristics of the study subjects are presented in Table 1. Compared to control subjects, patients with type 2 diabetes were presented with significantly increased levels of fasting plasma glucose, HbA1c, triglycerides, total cholesterol, LDL and insulin resistance. Compared to control subjects, obese individuals also exhibited significantly increased levels of triglycerides, total cholesterol, LDL and insulin resistance and decreased levels of HDL cholesterol.

Representative histological images of the adipocyte cell size from both the fat depots from all the study groups were depicted in Fig. 1A & B. Compared to control subjects (67.5 ± 2.8 μm), the mean cell size of subcutaneous fat (Fig. 1C) from obese (90.4 ± 3.2 μm), diabetic (93.2 ± 3.1 μm) and obese–diabetic subjects (92 ± 4.4 μm) was significantly increased ($p<0.001$). The mean cell size of visceral fat (Fig. 1D) was also significantly increased in the obese (85.7 ± 3.1 μm) ($p=0.05$), diabetic (96 ± 3.1 μm) and obese-diabetic subjects (106.4 ± 5.6 μm) ($p<0.001$) compared to control subjects (66 ± 4.1 μm). In both subcutaneous and visceral adipose tissues, the mean cell size was also significantly ($p<0.05$) increased in obese–diabetic subjects compared to obese subjects without type 2 diabetes. While the increase in the mean cell size in subcutaneous fat was additive in obese subjects and in patients with type 2 diabetes, adipose hypertrophy exhibited a synergistic effect in visceral fat from obese subjects and in patients with type 2 diabetes.

Serum adiponectin levels (Fig. 2) were significantly decreased in obese (26.6 ± 4.5 ng/mL), diabetic (31.3 ± 6.5 ng/mL), and obese–diabetic subjects (16.6 ± 3.3 ng/mL) compared to control subjects (63.6 ± 3.5 ng/mL) ($p<0.001$). Lipid peroxidation as measured by plasma levels of TBARS (Fig. 3) was significantly increased in obese (9.4 ± 0.4 nM/mL), diabetic (12 ± 0.5 nM/mL), and obese–diabetic (12.1 ± 0.6 nM/mL) subjects compared to control subjects (6.2 ± 0.5 nM/mL) ($p<0.001$). The TBARS levels were also increased significantly ($p<0.01$) in diabetic and obese–diabetic subjects compared to obese subjects. Fig. 4 represents the telomere length measurements in both subcutaneous (Fig. 4A) and visceral adipose (Fig. 4B) tissues. Compared to control subjects, the shortening of telomeres in subcutaneous fat was to the extent of 28%, 24% and 20% in obese, diabetic and obese–diabetic subjects, respectively. Similarly, the shortening of telomeres in visceral fat was to the extent of 23%, 22% and 24% in obese, diabetic and obese–diabetic subjects, respectively.

Table 2 shows the Pearson correlation analysis of human adipose tissue cell size with metabolic risk factors in the study subjects. Both the subcutaneous and visceral fat cell size showed a significant and positive correlation with TBARS, BMI, FPG, HbA1c, insulin resistance, LDL, triglycerides and total cholesterol and exhibited negative correlation with serum adiponectin, adipose telomere length and HDL levels. Since shortened telomeres are related to accelerated senescence and functional compromise in adipocytes, we performed a linear regression analysis using telomere length (both subcutaneous and visceral adipose) as dependent variable and systemic levels of adiponectin as an independent variable. This analysis revealed a positive association of shortened telomeres with hypoadiponectinemia in the study subjects but this association was lost when adjusted for adipocyte cell size.

4. Discussion

This study is the first to report the association between adipocyte hypertrophy and shortened telomeres in both subcutaneous and visceral adipose tissues along with decreased systemic levels of adiponectin in obese subjects and in patients with type 2 diabetes compared to control subjects.

A pioneering study by Salans et al. [11] revealed that adipocyte size was shown to vary inversely with adipocyte insulin sensitivity. Studies have shown functional differences in large and small adipocytes from the same subjects, including altered gene expression profiles [12]. A blunting of GLUT-4 translocation in response to insulin stimulation was also seen in larger adipocytes [13]. Adipocyte size has also been shown to influence adipokine secretion, with increasing adipocyte size resulting in a shift towards dominance of pro-inflammatory adipokines [14]. Adipocyte hypertrophy per se has been reported to be related to insulin resistance in type 2 diabetic patients independent of total body fat [6] via, in part, the decreased secretion of a subset of adipokines, such as adiponectin [15]. Dietary weight loss combined with exercise was found to improve insulin sensitivity, decrease adipocyte size and increase plasma adiponectin concentrations in obese individuals [16]. Furthermore, large and small adipocytes prepared from the same subjects/animals differed in terms of adipokine secretion [14] and basal lipolysis [17]. A negative correlation between cell size and adiponectin secretion was also elucidated by Pasarica et al. [18]. A recent study also demonstrated that the size of large adipose cells is a

Table 1
Clinical and biochemical parameters (Mean ± SD) of the study subjects.

Parameters	Control (n = 20)	Obese (n = 22)	Diabetes (n = 10)	Obese and diabetes (n = 7)
Age (years)	34.25 ± 8	40.9 ± 8.5	47.3 ± 10*	47 ± 8
Male n (%)	6(30)	8(36)	8(80)	3(43)
BMI (kg/m^2)	19.3 ± 3.6	37.4 ± 11.5*	23 ± 5.3#	40.5 ± 11*
Fasting plasma glucose (mg/dl)	79 ± 17	98 ± 23	196 ± 74*#	200 ± 27*#
HOMA-IR	1.1 ± 0.8	3.3 ± 1.9*	6.8 ± 3.2*#	7.6 ± 3.5*#
HbA1c (%)	5.2 ± 0.6	6 ± 0.8	7.6 ± 1.3*#	7.6 ± 3.4*#
Serum triglycerides (mg/dl)	96.4 ± 32.2	195.1 ± 87*	183.3 ± 62*	260.7 ± 60*
Serum cholesterol (mg/dl)	138.7 ± 37.6	192.1 ± 50.7*	189.3 ± 31*	210.7 ± 58.2*
HDL cholesterol (mg/dl)	38.6 ± 8.3	30.4 ± 6*	32.3 ± 8.5	30.3 ± 5.7
LDL cholesterol (mg/dl)	79.1 ± 26.1	108 ± 27.7*	100.8 ± 18.7	129 ± 24.3*

* $p<0.05$ compared to control.
\# $p<0.05$ compared to obese.

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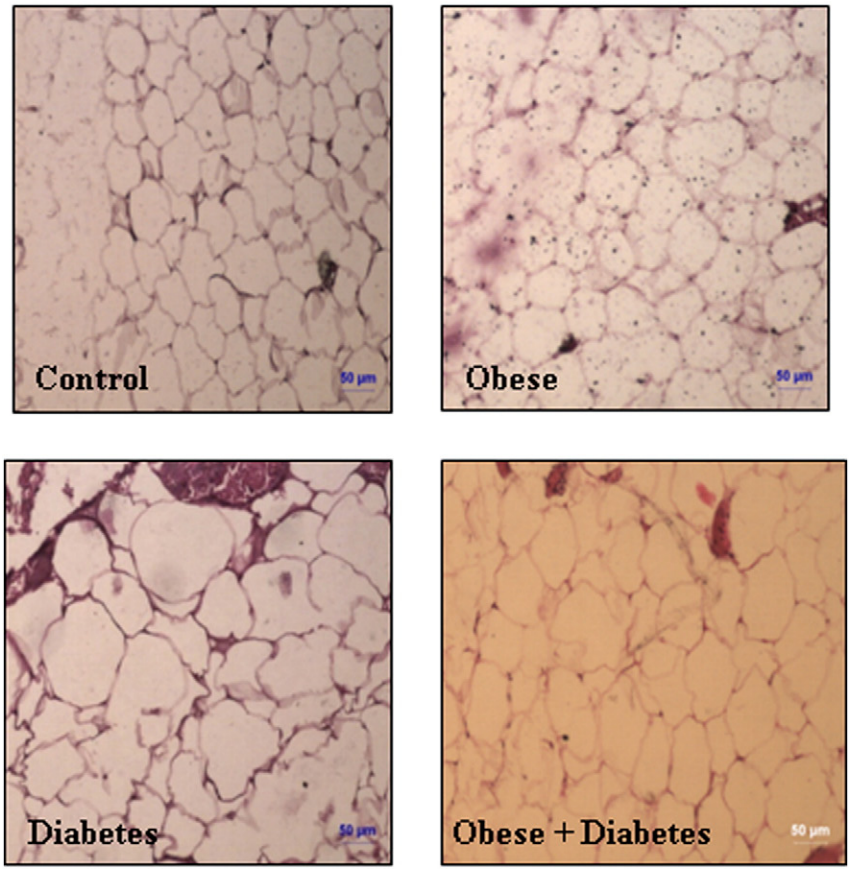
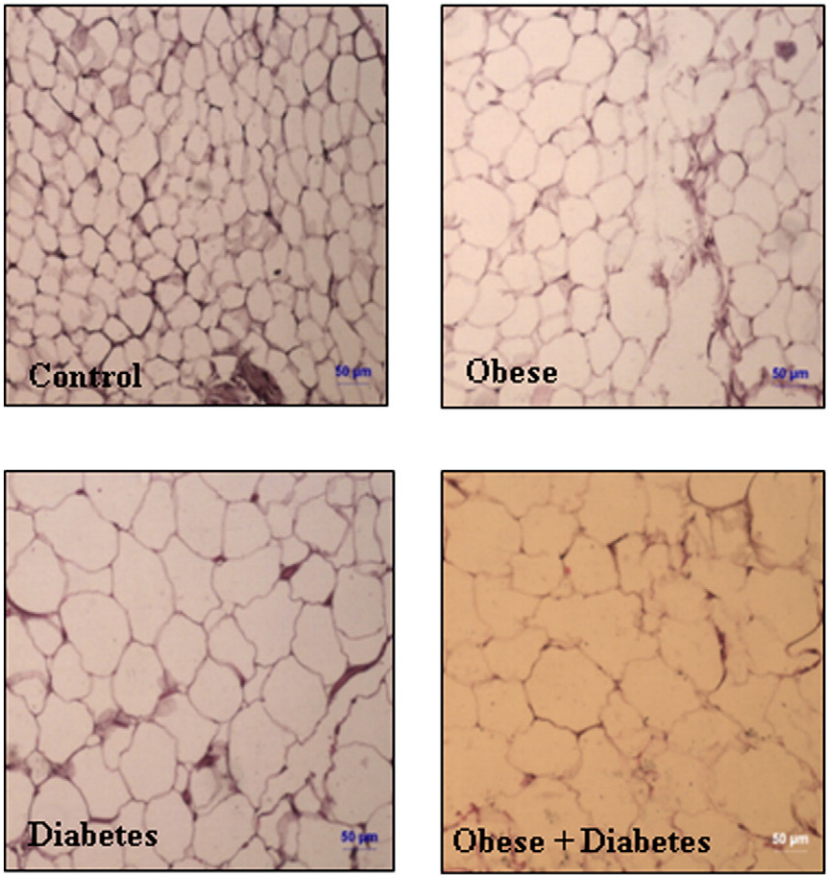
A) Subcutaneous adipose cell size



B) Visceral adipose cell size



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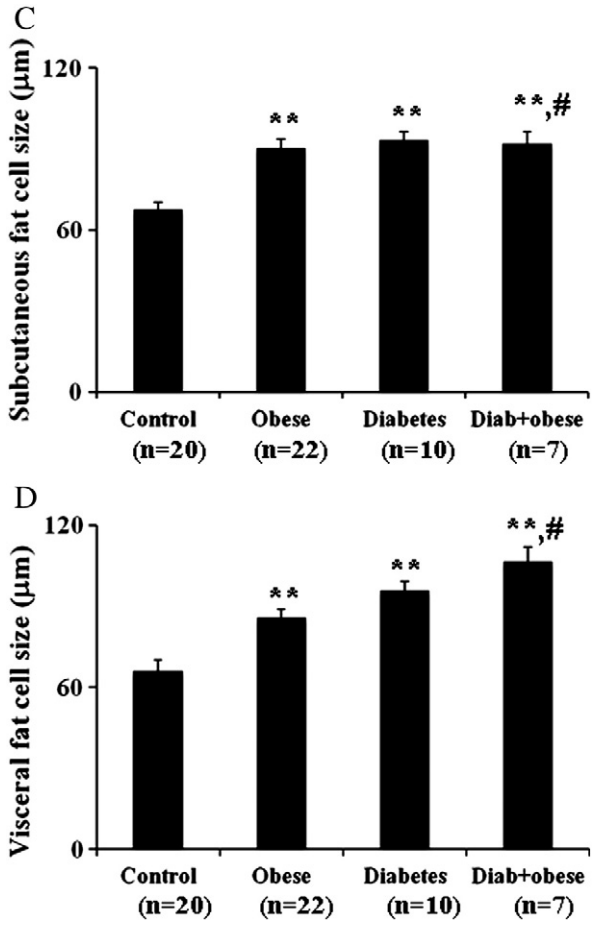

Fig. 1 (*continued*).

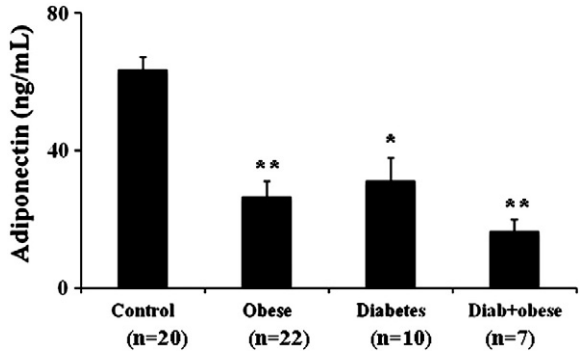

Fig. 2. Serum adiponectin levels (Mean ± SE) from the study subjects. $^{**}p<0.001$ compared to control, $^{*}p<0.01$ compared to control subjects.

predictor of insulin resistance in first-degree relatives of type 2 diabetic patients [19]. In a prospective study, increased adipocyte cell size has been shown to predict insulin resistance and type 2 diabetes independent of obesity [20]. While our results are in consonant with the above findings, we have demonstrated that increased adipocyte size of not only visceral adipose tissue but also subcutaneous adipose tissue from obese subjects and patients with type 2 diabetes positively correlated to dysglycemia, dyslipidemia, insulin resistance, oxidative stress and shortened telomeres and negatively correlated to the adiponectin levels.

Several studies have shown that increased oxidative stress is a manifestation of obesity-related metabolic derangement [21]. In our study, adipose hypertrophy in both subcutaneous and visceral adipose tissue was positively correlated to oxidative stress as evident from the increased levels of lipid peroxidation. Apart from the lipid and protein oxidation, the guanine sites of the DNA are more vulnerable to damage by oxidative stress. Free radicals in excess, damage the DNA and telomere ends of the DNA which protects the genomic integrity are enriched with guanine sites and hence are most susceptible to free radical attack leading to shortening of telomeres [22]. Significant negative relationships between adiposity measures and telomere length have been reported in the literature [23,24]. In a recent study by Lee et al. [25], it has been shown that BMI, waist circumference, hip circumference, total body fat, and visceral adipose tissue volume were all inversely associated with telomere length. Telomere length measured from subcutaneous adipocytes in formerly obese patients was significantly lower than in never obese patients [26]. Using both cross-sectional and prospective follow-up data, Njajou et al. [27] have recently demonstrated that shorter telomeres are a risk factor for increased adiposity. In our study, shortened telomeres were found in both subcutaneous and visceral fats from obese, diabetic and obese–diabetic individuals compared to control subjects. While several studies that reported association of shortened telomeres with adiposity and dysglycemia were related to the measurement of telomere length in leukocytes, our study is unique in that we measured telomere length in adipose tissue from both subcutaneous and visceral locations.

Adiponectin is produced from subcutaneous and visceral adipocytes and the exact proportion of circulating adiponectin attributed to each depot is debated [28]. Hypoadiponectinemia is a good marker of insulin resistance and an independent risk factor for type 2 diabetes and coronary artery disease [29,30]. Apart from its insulin-sensitizing, antiatherogenic and anti-inflammatory actions, adiponectin also appears to offer antioxidant function [31] and also has a protective effect against metabolic abnormalities that accelerate aging [32,33]. There is recent evidence demonstrating that adipocyte dysfunction is caused by cellular hypoxia of adipocytes secondary to adipocyte hypertrophy which results in lowered adiponectin synthesis [34]. Large adipocytes are also characterized by a reduced production and secretion of adiponectin compared with small newly differentiated adipocytes. Earlier, it was shown that subcutaneous adipocyte cell size was significantly higher in South Asians and was inversely correlated with both glucose disposal rate and plasma adiponectin concentrations [35]. South Asians compared to white Caucasians also had higher fasting insulin, lower HDL cholesterol and lower adiponectin and this "ethnic difference" in adiponectin was shown attenuated after adjustment for adipocyte area [36], implying that adipocyte hypertrophy has a significant influence on reduced adiponectin levels. In our study, adipocyte hypertrophy was positively correlated to oxidative stress, hypoadiponectinemia and shortening of telomeres. Interestingly, the association of shortened telomeres with hypoadiponectinemia was lost when adjusted for adipocyte cell size. Therefore, adipocyte hypertrophy irrespective of whether it is in the visceral or subcutaneous fat appears to be an independent risk factor for dysglycemia, dyslipidemia and metabolic disorders.

South Asians have a higher amount of total body fat for a given body mass index (BMI) compared with whites [37]. At a given level of total body fat, South Asians also have a higher amount of total abdominal adipose tissue, subcutaneous abdominal adipose tissue and visceral adipose tissue compared with white Europeans [1,38]. Moreover, adiposity has been linked to obesity related diseases and increased mortality in developing countries, including India. Although adiposity or obesity indicates an excess of fat tissue, the pathogenic potential of adipose tissue depends on its distribution and functional status. The adipocyte overflow

Fig. 1. Representative adipocyte cell size images of histology sections of human subcutaneous (Fig. 1A) and visceral (Fig. 1B) fat tissue from the study subjects. Histology was done on all the samples from the study subjects (as mentioned in Table 1), Image Pro plus software was used to measure the cell size, and the cumulative data of adipocyte cell size (Mean ± SE) of human subcutaneous (Fig. 1C) and visceral (Fig. 1D) fat tissue was expressed in μm. $^{**}p<0.001$ compared to control subjects, $^{\#}p<0.05$ compared to obese subjects.

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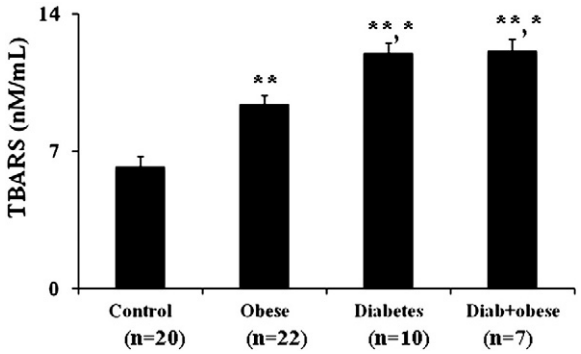

Fig. 3. Lipid peroxidation as measured by plasma levels of TBARS (Mean ± SE) from the study subjects. $^{**}p<0.001$ compared to control subjects, $^{*}p<0.01$ compared to obese subjects.

Table 2
Correlation of human adipose tissue cell size with metabolic risk factors.

Parameters	Subcutaneous		Visceral	
	r	p	r	p
BMI (kg/m^2)	0.578	0.001	0.456	0.001
Fasting plasma glucose (mg/dl)	0.455	0.001	0.483	0.001
HbA1c (%)	0.512	0.001	0.485	0.001
HOMA-IR	0.565	0.001	0.632	0.001
LDL cholesterol (mg/dl)	0.370	0.009	0.509	0.001
HDL cholesterol (mg/dl)	−0.386	0.006	−0.313	0.020
Serum cholesterol (mg/dl)	0.419	0.002	0.475	0.001
Serum triglycerides (mg/dl)	0.463	0.001	0.332	0.001
Serum adiponectin	−0.466	0.001	−0.628	0.001
Vis telomere	−0.415	0.002	−0.452	0.001
Sc telomere	−0.452	0.001	−0.395	0.003
TBARS	0.477	0.001	0.457	0.001

hypothesis suggests that as an adipocyte increases in size it eventually reaches a state where it can no longer store lipids. This causes an 'overflow' of fatty acids into ectopic sites such as the liver and muscle, promoting insulin resistance [39]. Therefore as hypothesized by Henry et al. [40], it may be plausible that individuals exhibiting adipose hypertrophy or impaired lipid storage in fat depots may be more susceptible to metabolic disorders because they experience "overflow" at a lower level of body fat than individuals with superior lipid storage potential. In our study, even in lean diabetics, the functional status of adipocytes was seen impaired as indicated by adipose hypertrophy and shortened telomeres in both subcutaneous and visceral fat tissue and this reflects a state of 'metabolic obesity' in non-obese patients with type 2 diabetes.

To conclude, adipose hypertrophy (in both visceral and subcutaneous adipose tissue) was strongly associated in our study with shortened telomeres, hypoadiponectinemia and oxidative stress in obese subjects and in patients with type 2 diabetes. It appears that prevention of adipocyte hypertrophy and telomere shortening either by pharmacological means or lifestyle modification will have promising therapeutic potential for type 2 diabetes and associated disorders.

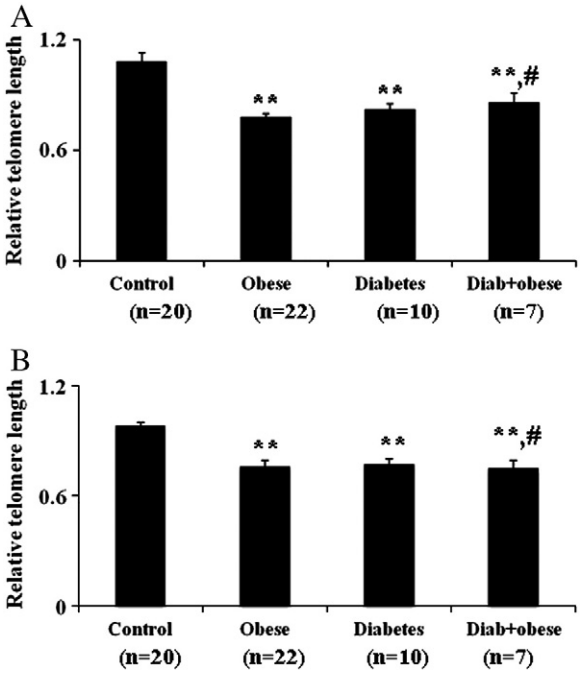

Fig. 4. Telomere length as measured by Real-time PCR. Cumulative data of telomere length (Mean ± SE) of human subcutaneous (Fig. 4A) and visceral (Fig. 4B) fat tissue from the study subjects. $^{**}p<0.001$ compared to control subjects, $^{\#}p<0.05$ compared to obese subjects.

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Human Genomics

PRIMARY RESEARCH Open Access

Linking a role of lncRNAs (long non-coding RNAs) with insulin resistance, accelerated senescence, and inflammation in patients with type 2 diabetes

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Abstract

Background: Studying epigenetics is expected to provide precious information on how environmental factors contribute to type 2 diabetes mellitus (T2DM) at the genomic level. With the progress of the whole-genome resequencing efforts, it is now known that 75–90% of the human genome was transcribed to generate a series of long non-coding RNAs (lncRNAs). While lncRNAs are gaining widespread attention as potential and robust biomarkers in the genesis as well as progression of several disease states, their clinical relevance and regulatory mechanisms are yet to be explored in the field of metabolic disorders including diabetes. Despite the fact that Asian Indians are highly insulin resistant and more prone to develop T2DM and associated vascular complications, there is virtually lack of data on the role of lncRNAs in the clinical diabetes setting. Therefore, we sought to evaluate a panel of lncRNAs and senescence-inflammation signatures in peripheral blood mononuclear cells (PBMCs) from patients with type 2 diabetes (T2DM; $n = 30$) compared to individuals with normal glucose tolerance (NGT; $n = 32$).

Results: Compared to control subjects, expression levels of lncRNAs in PBMCs from type 2 diabetes patients showed significantly ($p < 0.05$) increased levels of HOTAIR, MEG3, LET, MALAT1, MIAT, CDKN2BAS1/ANRIL, XIST, PANDA, GAS5, Linc-p21, ENST00000550337.1, PLUTO, and NBR2. In contrast, lncRNA expression patterns of THRIL and SALRNA1 were significantly ($p < 0.05$) decreased in patients with T2DM compared to control subjects. At the transcriptional level, senescence markers (p53, p21, p16, and β-galactosidase), proinflammatory markers (TNF-α, IL6, MCP1, and IL1-β), and epigenetic signature of histone deacetylase-3 (HDAC3) were significantly ($p < 0.05$) elevated in patients with type 2 diabetes compared to control subjects. Interestingly, mRNA expression of Sirt1 and telomere length were significantly ($p < 0.05$) decreased in patients with type 2 diabetes compared to control subjects. Majority of the altered lncRNAs were positively correlated with poor glycemic control, insulin resistance, transcriptional markers of senescence, inflammation, and HDAC3 and negatively correlated with telomere length. Logistic regression analysis revealed a significant association of altered lncRNA signatures with T2DM, but this association was lost after adjusting for insulin resistance (HOMA-IR) and senescence markers.

Conclusion: Our study provides a clinically relevant evidence for the association of altered lncRNAs with poor glycemic control, insulin resistance, accelerated cellular senescence, and inflammation.

Keywords: lncRNA, SASP, HDAC3, Type 2 diabetes, Insulin resistance, Inflammation

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Introduction

According to the latest edition of International Diabetes Federation Atlas [1], around 425 million people worldwide have diabetes and India alone harbors more than 73 millions of people with diabetes. While more than 90% of the diabetic population is affected majorly by type 2 diabetes mellitus (T2DM), it is a complex multi-factorial disease involving genetic, epigenetic, and environmental components. Several studies imply that accelerated aging, cellular senescence, and proinflammation are closely linked to the etiology of type 2 diabetes and insulin resistance [2, 3]. Although the association between the proinflammation and senescence in the development of insulin resistance and type 2 diabetes is well known, the underlying molecular mechanisms and upstream regulatory networks are only poorly understood. Epigenetics appears to play a major role in the regulation of inflammation and cellular senescence—the dual pathological features commonly associated with type 2 diabetes [4]. Aberrant epigenetic modifications such as DNA methylation, histone modification, and non-coding RNA alterations are well-recognized drivers for the cancer phenotype, but the accumulating evidence also implies their role in the etiology of diabetes and cardiovascular diseases.

Of the total genome that is transcribed, only 2% codes for proteins, whereas the vast majority of it is transcribed as non-coding RNAs which include long non-coding RNAs (lncRNAs), microRNAs, and others [5]. Of late, lncRNAs have gradually come into the spotlight for the increased appreciation of their functional importance both in health and disease [6]. lncRNAs were also found next to protein-coding genes that are under tight transcriptional control, and often, their expression pattern correlates with tissue differentiation, development, and disease [7]. The widespread dysregulation of lncRNA expression in several disease states and the finding that many lncRNAs are enriched for SNPs that associate with human traits/diseases have highlighted their role as master regulators [8, 9]. Challenging the concept that protein-coding genes are the sole contributors to the development of human disease, recent studies emphasize that lncRNAs mediate disease pathogenesis and hence should be studied and targeted for therapeutic benefits [10]. Accumulating literature on genetic, experimental, and epidemiological studies also highlights a growing list of lncRNAs that control glucose homeostasis and contribute to the pathogenesis of diabetes and its complications. Despite the fact that Asian Indians are highly insulin resistant [11] and more prone to develop T2DM and associated vascular complications [12], there is lack of data on the role of lncRNAs in the clinical diabetes setting and this is the rationale behind our study. Therefore, we planned to study the potential interactions among insulin resistance, cellular senescence, and proinflammation with a central focus on lncRNAs so as to better understand the clinical significance of these molecular perturbations in type 2 diabetes.

Research design and methods

Recruitment of the study subjects

Study participants with normal glucose tolerance (NGT; $n = 32$) and patients with type 2 diabetes (T2DM; $n = 30$) were recruited from Dr. Mohan's Diabetes Specialties Centre, Chennai, India, and from the ongoing epidemiological cohorts. The study was approved by the institutional ethics committee of the Madras Diabetes Research Foundation and conducted according to the principles of Declaration of Helsinki. Written informed consent was obtained from all the study participants prior to the start of the study. All the study participants were clinically well characterized into respective groups according to the World Health Organization (WHO) classification criteria. While all the diabetic patients were on oral hypoglycemic agent (OHA) treatment, < 10% were also on insulin, in addition to OHA.

Anthropometric measurements

Anthropometric measurements including weight, height, and waist circumference were obtained using standardized techniques. Height was noted down with a tape measured to the nearest centimeter. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. Body mass index (BMI) was calculated using the formula: weight (kg)/height (m^2). Waist circumference was measured using a non-stretchable fiber measuring tape. Blood pressure was recorded from the right arm in a sitting position to the nearest 2 mmHg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 min apart, and the mean of the two readings was represented as the blood pressure.

Biochemical and clinical investigations

Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase–peroxidase–amidopyrine method), serum triglycerides (glycerol phosphate oxidase–peroxidase–amidopyrine method), and HDL cholesterol (direct method–polyethylene glycol-pretreated enzymes) were measured using Hitachi-912 Autoanalyser (Hitachi, Mannheim, Germany). The intra and inter assay co-efficient of variation for the biochemical assays was < 5%. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [13]. Glycated hemoglobin (HbAlc) was estimated by high-pressure liquid chromatography using the variant analyzer (Bio-Rad, Hercules, Calif., USA). Serum insulin was estimated using enzyme-linked immunosorbent assay (Calbiotech, CA). Insulin resistance was calculated using the homeostasis

assessment model (HOMA-IR) using the formula: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5.

Blood collection and isolation of peripheral blood mononuclear cells (PBMCs)

Fasting blood (5–8 mL) was collected into the vacutainer tube and processed immediately for cell isolation within 2 h from the time of collection. Blood was processed for peripheral blood mononuclear cell (PBMC) isolation using Histopaque-1077 (Sigma-Aldrich) according to the standard protocol by overlaying the blood on density gradient solution and centrifugation at 1500–1800 rpm for 30 min. The buffy coat layer containing the PBMCs was aspirated, washed thrice with phosphate-buffered saline (PBS; pH 7.2–7.4), and aliquoted for various experiments.

RNA extraction and cDNA synthesis

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's protocol. RNA quantity and quality were assessed by NanoDrop 2000 (Thermo Scientific) instrument. For the first-strand cDNA synthesis reaction, total RNA (1 μg) was adjusted with nuclease-free water and mixed with the cDNA synthesis master mix containing 100 units of RevertAid M-MuLV reverse transcriptase enzyme and 2× buffer, random hexamer primers (1×), 20 units of RNase inhibitor, and 10 mM dNTP solution mix. The resultant samples were incubated at 42 °C for 60 min for the first-strand cDNA synthesis followed by a 5-min incubation at 95 °C for enzyme deactivation. cDNA reaction negative control without reverse transcriptase enzyme (−RT) was also performed.

lncRNA/mRNA expression by Q-PCR

A panel of lncRNAs was chosen for this study based on their involvement in metabolic disorders as well as their emerging roles in senescence [14, 15]. The relative expression of the lncRNA/mRNA signatures were analyzed by preparing reaction mixer with FastStart Universal SYBR Green Master (Roche) and the corresponding gene-specific primers (Sigma) with diluted cDNA and final volume made up to 20 μL using nuclease-free water. Quantification and analysis were carried out in LightCycler® 96 real-time PCR System (Roche). The target gene expression was normalized to the house-keeping gene 18SrRNA (lncRNA) and β-actin (mRNA), and relative expression was determined using $2^{-\Delta\Delta CT}$ method. Non-template control (NTC) was also performed for each reaction assay plate.

DNA isolation and measurement of telomere length

For the measurement of telomere length, DNA was isolated from the whole blood by phenol–chloroform extraction and ethanol precipitation [16]. Relative telomere length was determined by real-time PCR approach as previously described by Cawthon [17] with a minor modification in the PCR temperature conditions. This method measures the factor by which the ratio of telomere repeat copy number to single-gene copy number differs between a sample and that of a reference DNA sample. PCR amplification was achieved using telomere (T) and single copy gene, 36B4 (encodes acidic ribosomal phosphoprotein) primers (S), which serves as a quantitative control. The mean telomere repeat gene sequence (T) to a reference single copy gene (S) was represented as T/S ratio—a reflection of relative telomere length [3].

Statistical analysis

All data are represented as mean ± standard error mean (SEM) unless otherwise mentioned as standard deviation (SD). Based on our pilot study on the expression levels of lncRNAs and using the SPSS software, the minimum sample size required for the study was calculated as 28 in each group considering the level of significance set at 0.05 and the statistical power at 0.90. Comparison between groups was performed using the independent sample Student *t* test with $p < 0.05$ as the criterion for statistical significance. Pearson correlation analysis was done between variables and the risk factors. Binary logistic regression analysis was performed to show the association between lncRNAs (independent variable) and diabetes (dependent variable). All analyses were done using SPSS Statistics (version 20.0) and GraphPad Prism (version 6).

Results

Clinical and biochemical characteristics of the study groups

Clinical and biochemical characteristics of the study subjects are depicted in Table 1. BMI and waist circumference were slightly and significantly higher in patients with type 2 diabetes compared to control subjects. Patients with type 2 diabetes exhibited significantly ($p < 0.001$) increased fasting plasma glucose and HbA1c compared to the control subjects. T2DM patients were also hyperinsulinemic and insulin resistant as characterized by significantly elevated fasting insulin levels and HOMA-IR values, respectively. Blood pressure and lipid parameters did not differ significantly between the groups.

Altered lncRNA signatures in T2DM

Compared to control subjects, expression profiling of lncRNAs in PBMCs from type 2 diabetes patients showed significantly ($p < 0.05$) increased levels of HOTAIR, MEG3, LET, MALAT1, MIAT, CDKN2BAS1/ANRIL, XIST, PANDA, GAS5, Linc-p21, ENST00000550337.1, PLUTO, and NBR2 (Fig. 1). In contrast, lncRNA expression patterns of THRIL and SALRNA1 were significantly ($p < 0.05$) decreased in patients with T2DM compared to control subjects (Fig. 1).

Table 1 Clinical and biochemical characterization of the study subjects

Parameter	Normal glucose tolerance [NGT] ($n = 32$)	Type 2 diabetes mellitus [T2DM] ($n = 30$)	p value
Age (years)	44 ± 8	46 ± 8	0.218
Gender—male (female)	18 (14)	18 (12)	–
Body mass index (kg/m^2)	25 ± 3.1	27 ± 4	*0.015*
Waist circumference (cm)	85 ± 8	94 ± 9	*< 0.001*
Fasting plasma glucose (mg/dL)	87 ± 9	136 ± 24	*< 0.001*
Glycated hemoglobin—HbA1c (%)	5.6 ± 0.34	8.1 ± 1.9	*< 0.001*
HOMA-IR	1.8 ± 0.8	6.9 ± 3	*< 0.001*
Fasting insulin (μIU/mL)	8.6 ± 3.5	22 ± 7.2	*< 0.001*
Systolic blood pressure (mmHg)	120 ± 25	131 ± 21	0.079
Diastolic blood pressure (mmHg)	79 ± 13	80 ± 8	0.795
Total cholesterol (mg/dL)	174 ± 28	169 ± 37	0.545
Serum triglycerides (mg/dL)	132 ± 71	138 ± 49	0.737
HDL cholesterol (mg/dL)	41 ± 10	39 ± 7	0.352
LDL cholesterol (mg/dL)	107 ± 21	102 ± 34	0.568
VLDL	27 ± 14	28 ± 10	0.732

Data represented as mean ± SD. Italicized value represents statistically significant compared to NGT

Augmentation of HDAC3 and impaired Sirt1 expression in T2DM

Transcriptional profiling revealed that mRNA expression of HDAC3 was significantly ($p < 0.05$) increased while the Sirt1 level was significantly ($p < 0.05$) decreased in patients with type 2 diabetes compared to control subjects (Fig. 2).

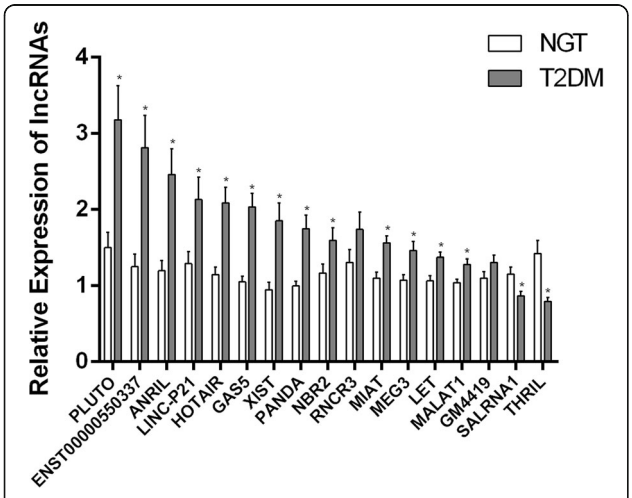

Fig. 1 Quantitative real-time PCR analysis of a panel of lncRNA expression levels in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

Altered senescence, inflammation, and telomere length in T2DM

At the transcriptional level, senescence markers, viz., p53, p21, p16, and β-galactosidase 1 (GLB1), were significantly ($p < 0.05$) elevated in patients with type 2 diabetes compared to control subjects (Fig. 3a). As a final read-out of augmented cellular senescence, patients with T2DM were also characterized by significantly ($p < 0.05$) shortened telomeres compared to control subjects (Fig. 3b). Interestingly, mRNA expression levels of proinflammatory gene mediators, viz. TNF-α, IL6, MCP1 and IL1-β, were also significantly upregulated ($p < 0.05$) in PBMCs from patients with type 2 diabetes, implying an acquisition state of senescence-associated secretory phenotype (Fig. 4).

Correlation analysis

A detailed correlation analysis of lncRNAs with various clinical and biochemical parameters (Additional file 1: Table S1) and molecular parameters (Additional file 2: Table S2) of the study subjects were summarized in the supplement tables. Majority of the altered lncRNAs were positively correlated with poor glycemic control, insulin resistance, transcriptional markers of senescence, inflammation, and HDAC3 and negatively correlated with telomere length. In contrast, expression levels of lncRNAs, viz., SALRNA1 and THRIL, were negatively correlated to glycemic control, insulin resistance, markers of senescence, inflammation, and HDAC3 and positively correlated to telomere length.

Logistic regression analysis

Logistic regression analysis using type 2 diabetes as dependent variable revealed that altered expression levels of lncRNAs, viz., PLUTO, ENST00000550337.1, CDKN2BAS1, LincRNA-P21, HOTAIR, GAS5, XIST, PANDA, NBR2, MIAT, MEG3, LET, MALAT1, SALRNA1, and THRIL, were associated significantly with T2DM, and this statistical significance was persisted even after adjusting for confounding factors like age and BMI. Interestingly, this statistical association was lost when adjusted for HOMA-IR and senescence markers. This implies that the association between lncRNAs and T2DM could be closely linked to insulin resistance and accelerated senescence with downstream inflammatory signaling (Table 2).

Discussion

Recent literature implies that the dysregulation of lncRNA expression and functionality contributes to several pathophysiological states as several lncRNAs get

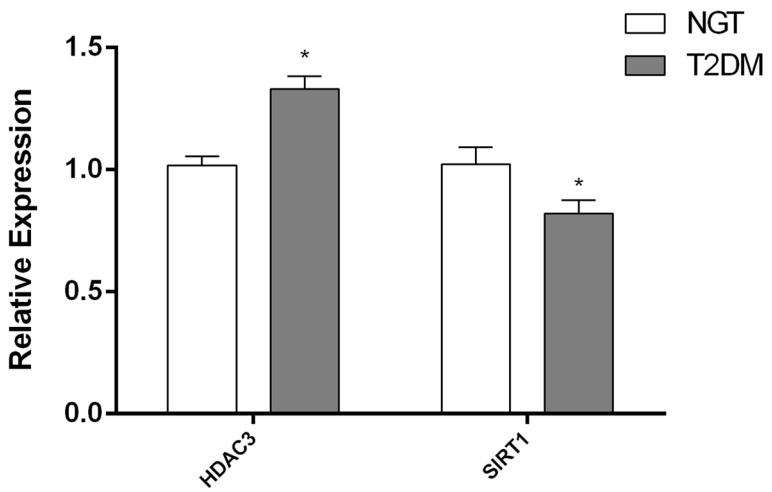

Fig. 2 Quantitative real-time PCR analysis of HDAC3 and SIRT1 in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

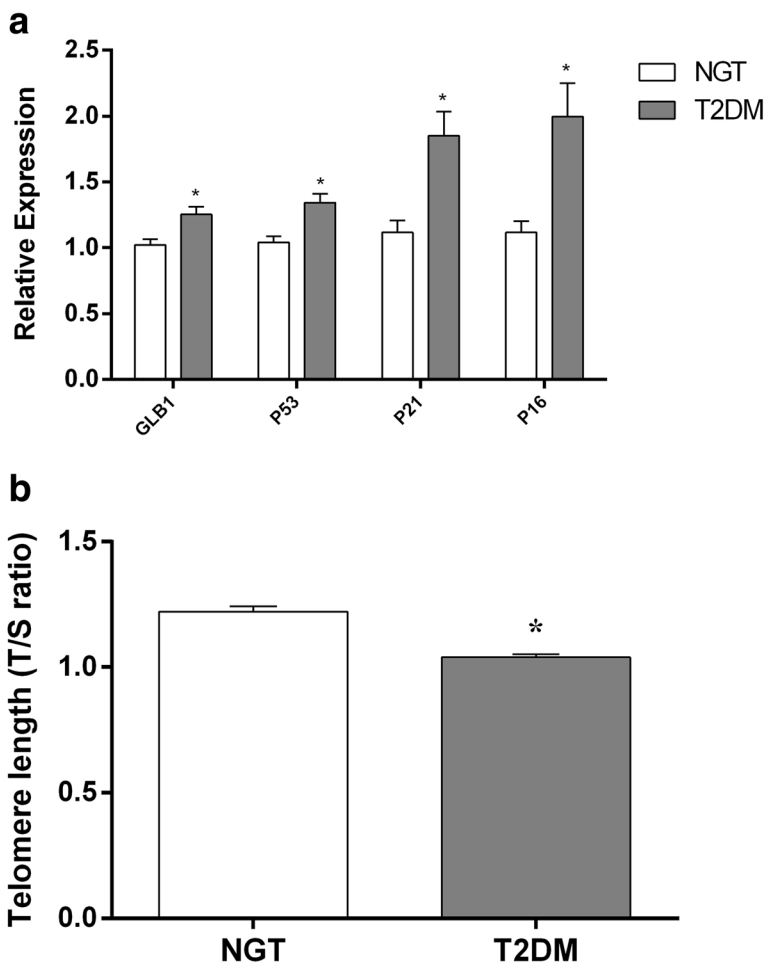

Fig. 3 Quantitative real-time PCR analysis of senescence marker gene expression levels, viz., GLB1, P53, P21, and P16 (**a**), and telomere length (**b**) in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

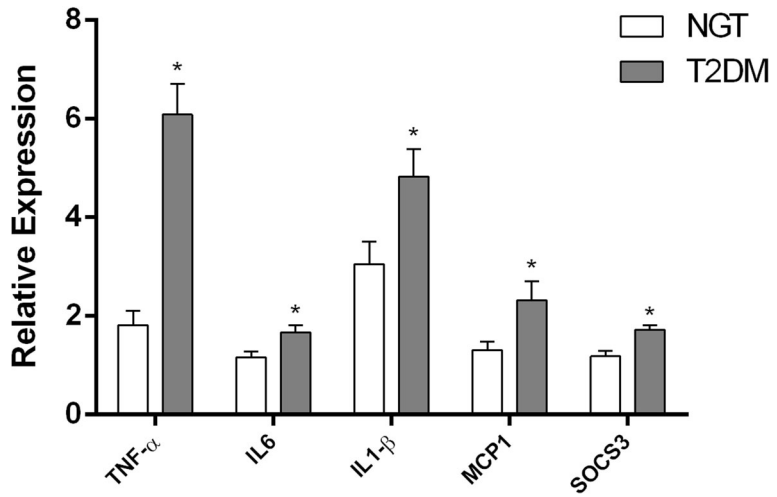

Fig. 4 Quantitative real-time PCR analysis of inflammatory signature gene expression levels, viz., TNFα, IL6, MCP1, IL1β, and SOCS3 in PBMCs from the study groups (NGT vs T2DM). Bars represent the mean ± SEM; *p value < 0.05 compared to control subjects

validated as bona fide prognostic/diagnostic markers and drug targets [9, 18, 19]. The role of lncRNAs in the pathogenesis of type 2 diabetes mellitus and related complications has only recently been recognized, but there is already some evidence for their involvement in many of the pathophysiological mechanisms that are linked to the genesis and progression of disease [20, 21]. Despite the fact that Asian Indians are highly insulin resistant [10], more prone to develop type 2 diabetes mellitus (T2DM) and associated vascular complications [11], and exhibit increased susceptibility to early β-cell dysfunction [22], there is virtually lack of data on the role of lncRNAs in the clinical diabetes setting. Our study is the first report from India to show an association of altered signatures of lncRNAs in T2DM with pathological connectivity reflected by poor glycemic control, insulin resistance, accelerated cellular senescence, and meta-inflammation.

Table 2 Binary logistic regression analysis using type 2 diabetes as dependent variable

	Unadjusted		Adjusted for age and BMI		Adjusted for HOMA-IR		Adjusted for senescence markers (GLB, P53, P21, P16, and TL)		Adjusted for inflammatory markers (TNF-α, IL6, MCP1, IL1-β, and SOCS3)	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
PLUTO	1.721	*0.005*	1.827	*0.003*	23.673	0.063	1.848	0.210	2.204	*0.025*
ENST00000550337.1	2.023	*0.004*	1.925	*0.013*	2.984	0.132	33.73	0.184	4.026	*0.038*
CDKN2BAS1	3.173	*0.006*	4.925	*0.002*	4.188	0.068	2.741	0.100	2.995	*0.044*
lincRNA-p21	1.867	*0.021*	1.970	*0.033*	3.492	0.096	2.283	0.311	6.395	*0.013*
HOTAIR	4.348	*0.001*	5.256	*0.001*	2.556	0.342	1.651	0.524	8.125	*0.015*
GAS5	10.642	*0.001*	14.054	*0.001*	20.820	0.226	22.512	0.128	12.214	0.069
XIST	0.388	*0.003*	3.824	*0.004*	3.677	0.145	2.318	0.386	3.166	*0.024*
PANDA	7.960	*0.003*	15.737	*0.002*	30.052	*0.041*	27.726	0.151	3.548	0.253
NBR2	2.045	*0.041*	1.728	0.159	1.496	0.522	1.869	0.443	2.675	0.141
RNCR3	1.464	0.144	1.650	0.065	1.795	0.394	1.582	0.410	1.740	0.225
MIAT	6.591	*0.002*	5.293	*0.012*	8.383	0.235	8.753	0.181	5.388	0.118
MEG3	5.669	*0.013*	6.444	*0.017*	17.060	0.141	12.830	0.063	57.903	*0.023*
LET	7.116	*0.014*	5.806	*0.036*	6.736	0.068	4.399	0.534	2.079	0.584
MALAT1	9.945	*0.008*	5.156	*0.046*	4.712	0.193	12.858	0.343	33.033	0.086
GM4419	2.468	0.104	2.142	0.242	3.254	0.232	4.832	0.293	1.937	0.433
SALRNA1	0.161	*0.013*	0.114	*0.009*	0.029	0.127	0.072	0.057	0.092	0.100
THRIL	0.047	*0.001*	0.529	*0.001*	0.013	0.084	0.063	0.139	0.333	0.271

Our study is in consistent with the recent literature of several lncRNAs upregulated in diabetes state. In support of our findings, increased expression of GAS5 [23] and lncRNA ENST00000550337.1 [24] was reported in type 2 diabetes even with high diagnostic claim and biomarker value. A role for lncRNAs XIST [25] and GM4419 [26] was implicated in diabetic nephropathy while alterations in PANDA [27] and NBR2 [28] P21 [29] were linked to cellular senescence, AMPK regulation, and liver fibrosis, respectively. Expression levels of lncRNA-LET was shown to be decreased in a certain type of cancers [30], but we observed it to be upregulated in patients with type 2 diabetes. MIAT is identified to be involved in various diseases, particularly myocardial infarction, diabetic retinopathy, and various other microvascular complications [31]. Similarly, lncRNA RNCR3 was shown to be increased in retinal vasculature of an animal model as well as in vitro cell model [32]. While lncRNA PLUTO has been shown to be downregulated in islets from donors who are patients with type 2 diabetes and pre-diabetes subjects [33], our study observed a highly significant upregulation of PLUTO in patients with type 2 diabetes. Previous studies also reported that upregulated expression of lncRNA MALAT1 was linked to hyperglycemia-induced inflammation and endothelial dysfunction [34], diabetic nephropathy [35], and gestational diabetes mellitus [36]. In vitro studies demonstrated that HOTAIR interacts with the various chromatin-modifying enzymes and thereby participates in the regulation of gene expression [37]. A functional role for HOTAIR in the diabetes pathogenesis is yet to be established; however, its role has been hinted to be associated with regional adiposity [38]. lncRNA MEG3 has an important regulatory role in beta cell function [39], and the knock-down of MEG3 has been shown contributing to the pathology of diabetic microvascular complication [40]. In contrast, MEG3 gene expression was shown upregulated in the hepatocytes from mice fed with high-fat diet as well as in ob/ob mice and this has been linked to increased hepatic gluconeogenesis [41].

Our study provides the first preliminary evidence that expression of the long non-coding RNAs, THRIL, and SALRNA1 were decreased in patients with type 2 diabetes and negatively correlated with hyperglycemia, senescence, and inflammation. THRIL was shown to regulate TNF-α expression through an epigenetic mechanism, and TNF-α can also reduce THRIL expression via a negative feedback action [42]. Similarly, SAL-RNA1 was earlier identified as putative age-delaying lncRNA, since its reduction with small inhibitory RNAs (siRNA) induced rapid aging changes of the fibroblasts, such as large cell morphology, positive β-galactosidase activity, and upregulation of p53 [43]. Notably, lncRNA ANRIL shown upregulated in our study was also linked to CDKN2A/B, a strong type 2 diabetes risk gene variant [44, 45].

It is interesting to note that the majority of differentially expressed lncRNAs in patients with type 2 diabetes observed in our study are involved in cell cycle regulation and senescence and their expression levels correlated to poor glycemic control, insulin resistance, accelerated senescence, and inflammation. Several lncRNAs were reported to influence the molecular processes that underlie age-associated phenotypes and play an important role in accelerated aging [4, 46]. Type 2 diabetes has been linked to cellular senescence, senescence-associated secretory phenotype (SASP), and accelerated aging [47, 48], and our lab was the first one in the world literature to report an association of increased telomere shortening in patients with type 2 diabetes [2, 3]. Earlier, we have also shown increased HDAC3 epigenetic signature in patients with type 2 diabetes [49], and in the present study, there was a positive correlation of HDAC3 mRNA expression with majority of the lncRNAs and this endorses the concerted and coordinated interactions between lncRNAs and histone modifications [50].

Our work offers an avenue for several translational applications including a role of lncRNAs in lifestyle changes. Recent findings suggest a putative role of non-coding RNAs in physical activity and several miRNAs have been identified as modulators of exercise-induced adaption at both systemic and tissue levels [51]. Contrast to miRNAs, little is known about the role of long non-coding RNAs (lncRNAs) in exercise. Identification of the role of lncRNAs in exercise will improve our understanding of exercise physiology and has the potential to enhance the application of current therapeutic approaches. In fact, a micropeptide encoded by a putative lncRNA has been shown to regulate muscle performance [52]. Although very little is known about the relationship between lncRNAs and dietary factors, it appears that dietary manipulation could also beneficially alter the expression of lncRNAs and thereby ensure health [53].

One of the limitations of our study is of its cross-sectional nature as well as small sample size, and hence, the findings of the study and its conclusions should be interpreted with caution. From this pilot study, we could not extrapolate causal link of alterations in lncRNAs with type 2 diabetes, and it needs replication and prospective follow-up studies. Secondly, considering the tissue-specific and heterogeneous actions of lncRNAs, the alterations seen in PBMCs might only mirror disease-pathology directionality. However, the altered expression profile of lncRNAs in PBMCs has been shown to reflect the pathophysiology in different disease states including multiple sclerosis [54], myocardial infarction [55], and rheumatoid arthritis [56]. In fact, a recent study of deep RNA sequencing uncovered a repertoire

of human macrophage lncRNAs modulated by macrophage activation and closely linked it to the pathophysiology of cardiometabolic diseases [57].

Conclusion

To conclude, our study is of its first kind in India to report altered lncRNA profiles linked to poor glycemic control, insulin resistance, senescence, and proinflammation in patients with type 2 diabetes. A better understanding of the mechanisms underlying the functions of lncRNAs will help us to understand the ever-expanding pathophysiology of diabetes and its complications and thereby adapt to prevention strategies as well as to develop novel therapeutic agents.

Additional files

Additional file 1: Table S1. Correlation analysis of LncRNAs with clinical and biochemical parameters. (DOCX 21 kb)

Additional file 2: Table S2. Correlation analysis of LncRNAs with molecular parameters. (DOCX 18 kb)

Abbreviations

BMI: Body mass index; GLB1: β-Galactosidase 1; HbA1C: Glycated hemoglobin; HDAC: Histone deacetylase; HOMA-IR: Homeostatic model assessment-insulin resistance; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; lncRNAs: Long non-coding RNAs; MCP-1: Monocyte chemoattractant protein 1; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; NGT: Normal glucose tolerance; P16: Cyclin-dependent kinase inhibitor 2A; P21: Cyclin-dependent kinase inhibitor 1A; P53: Tumor protein/tumor suppressor 53; PBMCs: Peripheral blood mononuclear cells; SASP: Senescence-associated secretory phenotype; Sirt1: Sirtuin (silent mating-type information regulation 2 homolog) 1; SOCS-3: Suppressor of cytokine signaling 3; T2DM: Type 2 diabetes mellitus; TNF-α: Tumor necrosis factor-α

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Availability of data and materials

The authors consent to the availability of data and materials. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MB conceived, designed, supervised, and commented on all drafts of this paper. CS and PP coordinated the clinical sample collection, conducted the overall experiments, and participated in the data collection and analysis and molecular investigations and helped in the drafts. VM and MB contributed to the data interpretation and manuscript completion. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the institutional ethics committee of the Madras Diabetes Research Foundation and conducted according to the principles of Declaration of Helsinki. Written informed consent was obtained from all the study participants prior to the start of the study.

Consent for publication

Institutional consent form is inclusive of data protection and consent for research publication.
All the authors approved the manuscript and consented for publication.

Competing interests

The authors declare that they have no competing interests.

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Research highlight

Fast forward aging link in diabetes

Researchers have shown a molecular connection between the nuclear and mitochondrial aging processes that occur in patients with type 2 diabetes[1].

The human body has a chronological age as also a biological age. The biological age is represented by the length of telomere — the DNA sequence at the end of each chromosome, like the plastic tips on shoelaces. The telomeres get shorter each time a cell divides. Short telomeres reflect accelerated ageing.

Many recently discovered genes that can be manipulated to slow the aging process also belong to pathways involved in the control of metabolism. Metabolic syndrome, in addition to being a precursor of metabolic disorders such as type 2 diabetes mellitus (T2DM) and cardiovascular disease, has been shown to be a sign of premature aging. Diabetes is a state of accelerated aging.

While telomere shortening is associated with T2DM, there is a lack of studies that explore the relationship among all the biomarkers — telomere length, oxidative stress, mitochondrial DNA (mtDNA) content, and the levels of adiponectin (a protein produced by fat cells that may play an important role in the development of obesity).

The researchers reasoned that the susceptibility to develop T2DM and cardiovascular diseases in Asian Indians could be explained by studying all these emerging biomarkers. "In a clinical setting, we have shown the existence of a molecular connection between the nuclear and mitochondrial ageing processes which occur in patients with type 2 diabetes," says lead author Muthuswamy Balasubramanyam.

Unlike chronological aging, accelerated aging can be reversed. "In other words, maintenance of appropriate mitochondrial function and telomere length either by pharmacological means or lifestyle modification will have promising therapeutic potential for Type 2 diabetes and associated vascular disorders", he adds.
The authors of this work are from: Madras Diabetes Research Foundation and Dr. Mohan's Diabetes Specialities Centre, Chennai, India.

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Research highlight

Aging fat cells signal diabetes

Researchers have found that aging fat cells could signal diabetes. In a new research, they show that aging fat cells provide a molecular link to a person's insulin resistance, a precursor for many metabolic diseases including diabetes[1].

The researchers have shown in animal models that it is possible to selectively target aging cells, eliminate them and delay or prevent age-related pathologies. This calls for further research on 'clearing away old cells' either pharmacologically or by lifestyle modifications to achieve healthy aging.

In response to a variety of stress signals, including nutrient deprivation, oxidative stress, dysfunctional telomeres and DNA damage, normally dividing cells can permanently withdraw from the typical cell cycle. These cells are then said to be in a state of 'cellular senescence', where their capacity to replicate is destroyed. Growing evidence suggests that these senescent cells contribute to aging in a variety of organisms, including mice and humans.

Senescent cells lurk in our tissues. They escape elimination due to impaired programmed cell death or altered immune surveillance. Senescent cells have been reported to behave badly, secrete chemicals that degrade surrounding tissue and harm neighboring cells. Their increasing omnipresence contributes to accelerated aging and age-related pathologies.

"In our study, fat cells subjected to oxidative stress became senescent associated secretory phenotype (SASP), started signalling proinflammation, exhibited shortened telomeres and became insulin-resistant. This is a hallmark characteristic of diabetes" says lead author Muthuswamy Balasubramanyam.

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Research highlight

Why lean diabetics could be metabolically obese

Researchers have found molecular evidence to answer why some type 2 diabetics who appear lean are actually 'metabolically obese'[1].

Overweight and obesity are known to be important risk factors for diabetes and cardiovascular diseases. In Asia, obesity rates do not directly correspond with diabetes rates. India has a low prevalence of obesity but notably high rates of type 2 diabetes.

In Asian populations, a higher risk of diabetes starts at a lower body mass index than in Europeans. Thus, one of the factors that contributes to the diabetes epidemic in Asians is the 'normal-weight metabolically obese' phenotype. Till now, the biochemical or molecular basis for 'metabolic obesity' was poorly understood.

To probe this, the researchers measured the size of fat cells (adipocytes) from human visceral and subcutaneous fats and found that lean diabetics had higher adipocyte cell size (adipocyte hypertrophy) than control subjects. Also, adipose tissue from these lean diabetics showed faster aging (senescence) as evident from their shortened telomeres. They also had a secretory profile going by the low levels of adiponectin, the protein involved in regulating glucose levels as well as fatty acid breakdown.

"When such fat cells switch to a senescent and proinflammatory phenotype, they also change their job from 'fat storage' to 'fat spillage'. This fat accumulation complicates functioning of several other organs including the heart and triggers cardiovascular disease in diabetes patients" says lead researcher Muthuswamy Balasubramanyam. The study exposes adipocyte hypertrophy and senescence as targets for new drug discoveries, he adds.

The research urges timely prevention and management to reduce adverse outcomes in all patients with type 2 diabetes, particularly in metabolically obese normal-weight patients, who may have a false sense of protection because they are not overweight or obese.

The authors of this work are from: *Madras Diabetes Research Foundation, Chennai, Dr. Mohan's Diabetes Specialities Centre, Chennai & LifeLine Multi-Speciality Hospitals, Chennai, India.*

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