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Middle-Aged Indians with Type 2 Diabetes Are More Prone to Biological Aging, particularly in terms of Serum CDKN2A

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Abstract

Objective: Indians now have a higher than average risk of developing type 2 diabetes (T2DM) due to their sedentary lifestyle and significant visceral adiposity. With particular reference to the biological ageing marker cyclin-dependent kinase inhibitor 2A (CDKN2A), we sought to examine the relationship between oxidative stress and chronic inflammatory mediators and ageing in this study of middle-aged (30–51 years old) Indian healthy and T2DM participants.

Methods: Malondialdehyde (MDA), oxidised LDL (oxLDL), interleukin-6 (IL-6), interleukin-1β (IL-1β), tumour necrosis factor α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and CDKN2A were measured in T2DM patients (n = 90) and controls (n = 90) aged 30-51 years; these subjects were further divided into G1: 30-40 years and G2: 40-50

Results: Both T2DM patients and controls demonstrated a substantial correlation between IL-6, TNF-α, MCP-1, and CDKN2A and ageing. However, the link between MCP-1 and CKDN2A and ageing was much greater in T2DM patients than in controls. In the controls, none of the mediators of oxidative stress or pro-inflammatory response was significantly correlated with CDKN2A. However, in T2DM patients, IL-6, TNF-α, and MCP-1 revealed a significant connection with CDKN2A. In comparison to the corresponding control groups, G1 and G2 T2DM patients had a higher probability of having high levels of CDKN2A.

Conclusion: According to this study, middle-aged Indians with T2DM are more likely to experience biological ageing. Middle-aged Indians are more likely to develop T2DM. T2DM may speed up the ageing process and so put Indians at an earlier age at risk for a number of age-related problems.

Keywords: T2DM and Inflammatory markers

Introduction

Diabetes prevalence has increased globally during the past ten years. The number of people with diabetes worldwide is largest in China (about 114.4 million), followed by India (more than 72.9 million), according to the International Diabetes Federation (IDF) 2017 statistics [1]. The main causes of increased risk of type 2 diabetes mellitus (T2DM) in Indians at a younger age and lower body mass index (BMI) than the western population have been recognised as rapid urbanisation, sedentary lifestyle, high-calorie diet, visceral adiposity, and high genetic predisposition [Figure 1; 2]. The average age of T2DM onset among Indians is gradually rising in the age categories under 50, according to a number of population-based studies [2, 3].

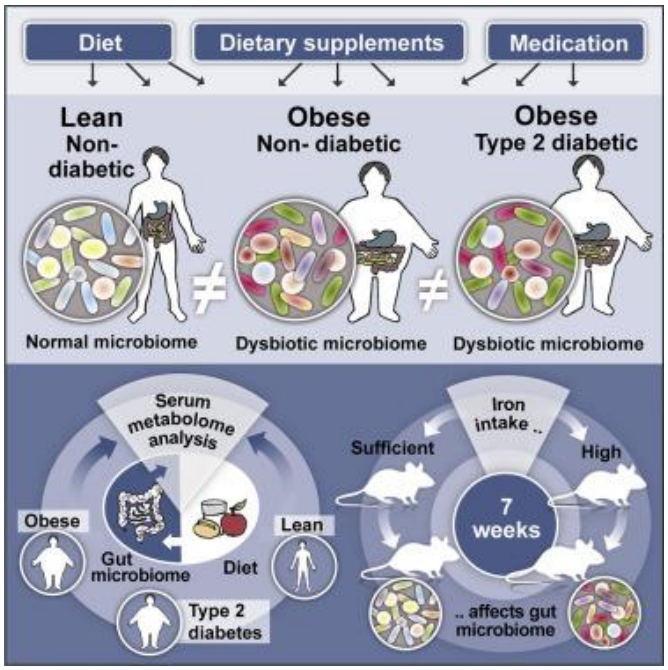

Figure 1: T2DM related to diet and BMI

Chronic hyperglycemia, dyslipidemia, and enhanced insulin resistance are the main pathological features of T2DM. These factors cause a wide range of metabolic and molecular changes that finally result in the emergence of diabetes-related vascular problems [4]. Persistent hyperglycemia increases the body's oxidative stress levels and, in turn, activates a number of stress-related pathways, including those that lead to polyol, advanced glycation end products, protein kinase C activation, and nuclear factor kappa B (NF-B) pathways [5]. According to earlier research, the lipid peroxidation indicators malondialdehyde (MDA) and oxidised LDL (oxLDL) seem to rise together with the pathogenesis of T2DM [6, 7].

The underlying persistent low-grade systemic inflammation, often referred to as meta flammation, which is brought on by metabolic changes sparked by an excess of nutrients, is a crucial factor in the pathogenesis of T2DM [8]. Islet inflammation, which contributes to beta-cell failure along with down regulation of insulin gene transcription and beta-cell apoptosis, is also brought on by persistent low-grade inflammation [9]. In the obesity-induced insulin resistance, decreased insulin sensitivity, and pathogenesis of T2DM, several studies reported that the low-grade inflammation is characterised by the overexpression of proinflammatory cytokines, such as tumour necrosis factor, interleukin-6, interleukin-1, and monocyte chemoattractant protein-1 (MCP-1) [10, 11].

Yet, the most common causes of molecular or cellular ageing are both persistent low-grade inflammation and oxidative stress [12]. A larger concentration of free radicals and ongoing inflammation produce cellular damage, and both are major contributors to the decline in tissue or organ functionality that ultimately leads to system failure [13]. The existence of persistent hyperglycemia and insulin resistance also impacts the natural ageing process and may be the cause of accelerated ageing, according to recent studies [14, 15]. Previous research has demonstrated that diabetes causes

a variety of cell types, including β-cells, endothelial cells, and cardiomyocytes, to undergo premature cellular senescence [14–16].

causes the cell cycle to irreversibly stop proliferating at the G1 phase and is a key factor in the ageing process [17]. Telomere shortening and cyclin-dependent kinase inhibitor 2A (CDKN2A) are two biomarkers of cellular senescence that represent changes in molecular or cellular processes fundamentally linked to biological ageing [18, 19]. The more dynamic senescence marker CDKN2A encodes the cell cycle inhibitors p16Ink4a and p19Arf. High amounts of p16Ink4a and p19Arf expression have been found in senescent cells, and both proteins are assumed to be essential to the senescence process [20, 21].In particular, p16Ink4a has been found to be both a marker and an effector of senescence in -cells because it inhibits the activity of cyclins and cyclin-dependent kinases (CDKs), hinders cell cycle progression [20]. The important cellular senescence marker p53, on the other hand, is stabilised by p19Arf [21]. Moreover, CDKN2A/p16 is linked to the initiation of a permanent or complete senescence process, illuminating its critical function in the ageing process [22].

Every individual's ageing process is ongoing. Population-based studies on ageing are widely available. The old and geriatric population has been the primary focus of the majority of results on ageing [7, 23]. Only a small number of studies [19] have focused on the ageing of relatively young Indians with T2DM. Yet, in the relatively young Indian population, there is a paucity of knowledge regarding the impact of T2DM-induced oxidative stress and low-grade inflammation on ageing.

Also, very few research have examined the blood levels of the biological ageing marker CDKN2A in this cohort. So, the goal was to research the relationship between oxidative stress and chronic inflammatory mediators and ageing, specifically with regard to CDKN2A in middle-aged (31–50 years) Indian healthy and T2DM individuals.

Method

Study design: A case-control research was carried out at SRS Diabetes Speciality Care

Methodology: The anthropometric measures, blood pressure, and study questionnaire were all completed by each subject [25]. Measurements of height, weight, waist circumference (WC), and hip circumference were included in the anthropometric data (HC). The biochemical studies included measurements of haemoglobin A1C (HbA1c) by the immunoturbidimetric method (Randox Laboratories), fasting plasma glucose (FPG) (glucose oxidase-peroxidase method), and lipid profile analysis, which included estimation of total serum cholesterol (TC), serum high-density lipoprotein (HDL-c), and serum triglycerides (TG) by enzymatic methods. Using Friedewald's method, low-density lipoprotein (LDL-c) was estimated [26]. The homeostatic method was used to calculate insulin resistance (HOMA-IR) [27].

Thiobarbituric acid-reacting substance technique (TBARS), as reported by Placer et al. [28], was used to assess plasma MDA. Those with hsCRP levels greater than 10 mg/L were disqualified from the trial because they were indicative of an acute inflammatory condition [29].

Sample Size: 180 participants, both males, and females in the age range of 30-51 years. Type 2 diabetic or T2DM patients (n = 90) were divided into two groups: healthy controls (n = 90), which included willing residents of Samastipur and type 1 diabetic or T1DM

patients (n = 90), who were recruited from private diabetes clinics inSamastipur.

The following age-based categories were created from the middle-aged T2DM patients and controls:

(a) Group 1 (early middle age: 30-41 years), which included 45 T2DM patients and 45 controls.

(b) Group 2 (late middle age: 41–51 years), which included 45 T2DM patients and 45 controls.

Inclusion Criteria: The American Diabetes Association's 2017 recommendations [24] were followed while determining the inclusion criteria for T2DM patients. The controls were people who were subjectively perceived to be in good health, who were not taking any drugs 1 to 2 months prior to sample collection, and who did not have diabetes or any other serious medical conditions.

Exclusion criteria: Antioxidant and anti-inflammatory medications were not being used by the patients or the controls. Exclusion criteria for the study included those with the common cold, fever, any chronic illnesses like cancer, diabetic microvascular problems, diabetic macrovascular complications, neurological diseases, pregnancy, and lactation.

Statistical Analysis: The Statistical Package for the Social Science version was used to conduct the statistical analysis. The Shapiro-Wilk test for normality and the Levene test for equality of variances were used to evaluate the assumptions of homogeneity in variances and normality of data, respectively. Interquartile range (IQR) and the median were used to display the data (Q1-Q3). The Mann-Whitney U test, Kruskal-Wallis test, Independent t-test, or Chi-square test was used to compare groups as necessary. The 75th percentile of the controls served as the cut-off value for each marker. The levels of markers were divided into low and high levels based on cut-offs. To forecast the risk of ageing in the presence of T2DM, logistic regression was used. P <0:04 was used as the statistical significance level.

Results

Table 1 displays the study individuals' initial characteristics. The BMI of G1 T2DM patients was greater (p <0.02) than that of G1 controls, whereas the central obesity rates of G2 T2DM patients were higher (WC: p <0:02; WHR: p <0:02) than those of G2 controls. When compared to their respective control groups, FPG, HbA1c, and HOMA-IR were found to be substantially different (p <0.01) in two age groups with T2DM. Only TG was substantially greater in both groups of T2DM compared to controls in the lipid profile (p <0:04). While senescence marker CDKN2A was significantly elevated in G2 T2DM patients, the cardiac risk marker hs-CRP was markedly elevated in G1 T2DM patients. Nevertheless, after controlling for FPI and HOMA-IR using the analysis of covariance, a significant difference in CDKN2A levels between T2DM patients and controls in the G1 group was discovered (p = 0:002 and p = 0:004, respectively) (ANCOVA).

Table 1: Baseline characteristics of the patients

Variables	Group- 1 (31-40)			Group 2 (41-50)		
	G1 controls (n = 45)	G1 T2DM (n = 45)	P-value	G2 controls (n = 45)	G2 T2DM (n = 45)	P-value
Age (yr)	33 (20-35)	35.4 (33.24-38)	0.023	44 (41-47)	45 (42-48.74)	0.286

Body Weight (kg)	64.94 (18.17-75.54)	71.04 (65.72-78.84)	0.053	64.24 (54.47-69.7)	66.44 (62-74)	0.010
WC	90.4 (88-93.74)	92 (83.62-102.37)	0.634	158.4 (150.24-165.74)	162 (152.87-168.74)	0.294
HC	96 (90.24-101.37)	101.1 (94-104)	0.001	27.22 (24.74-29.63)	27.22 (24.74-29.63)	0.081
WHR	0.928 (0.898-0.977)	0.914 (0.87-0.952)	0.137	0.926 (0.855-0.956)	0.955 (0.902-0.9780	0.020
FPI	13.0 (9.23-21.13)	25.25 (16.0-40.73)	<0.002	4.53 (4.22-4.7)	7.37 (6.25-10.76)	0.811
FPG	4.58 (4.24-4.96)	7.4 (5.76-9.07)	<0.002	19.45 (10.65-28.96)	16.61 (12.43-28.28)	<0.002
HOMA-IR	2.71 (1.8-3.95)	8.84 (5.16-14.58)	<0.002	4.07 (2.11-5.87)	5.21 (3.46-10.02)	0.002
TG	1.22 (0.93-1.56)	1.40 (0.96-2.36)	0.037	1.02 (0.85-1.40)	1.36 (1-1.80)	0.017
TC	4.06 (3.44-5.51)	4.15 (3.33-5.17)	0.232	4.20 (2.86-4.7)	3.75 (3.42-4.41)	0.661
HDL-c	1.04 (0.92-1.24)	0.90 (0.7-1.09)	0.003	0.95 (0.87-1.15)	0.94 (0.86-1.13)	0.865
LDL-c	2.33 (1.86-2.83)	4.15 (1.6-3.02)	0.500	2.54 (1.44-3.17)	2.17 (1.66-2.57)	0.168
CDKN2A	3.345 (2.48-4.06)	3.588 (2.40-5.7)	0.136	3.183	7.4(4.25-12.14)	<0.002

The majority of patients were taking metformin in combination with another class of hypoglycemic medications (G1: 54% and G2: 67.4%). MDA was considerably greater in both of the T2DM patient age groups (G1 and G2) compared to the corresponding control groups. On the other hand, only G1 controls and G1 T2DM patients had a significant difference in oxLDL levels. In G1 T2DM patients, proinflammatory cytokines such as IL-6, IL-1, and TNF are seen to be significantly greater, but IL-6 and MCP-1 dramatically increased in G2 T2DM patients in comparison with control.

The Chi-square test of independent association was used to examine the relationships between oxidative stress markers, proinflammatory cytokines, and senescence markers and ageing in T2DM and controls. All the markers were grouped into two levels, low and high based on their cut-off values (MDA > 7.5 μmol/L, oxLDL > 1.07 μg/mL, IL-6 > 3:03 pg/mL, TNF-α > 0:87

pg/mL, IL-1β > 1:15 pg/mL, MCP -1>77.2 pg/mL, and CDKN2A > 4.45 ng/mL.

Only oxLDL among the oxidative stress indicators shown a significant correlation with ageing in controls, whereas this correlation was not seen in T2DM patients. IL-1 and TNF- showed a highly significant association with ageing among controls ($p < 0.02$), while all proinflammatory cytokines were significantly linked with ageing. In T2DM patients, IL-6, TNF, and MCP-1 demonstrated a substantial correlation with ageing. However, compared to controls, T2DM patients' MCP-1 levels revealed a greater correlation ($p<0.02$) with ageing. T2DM patients had higher percentages of elevated CDKN2A levels than did controls. Although CDKN2A and ageing were substantially associated ($p < 0.04$ in controls), T2DM patients had a significantly stronger correlation ($p<0.02$).

Similar to this, we have looked into the relationships between the biological ageing markers CDKN2A, oxidative stress markers, and proinflammatory cytokines. In controls, all the indicators displayed insignificant correlations with the biological ageing marker CDKN2A. MCP-1, however, demonstrated a very high significant association ($p < 0.002$) while oxLDL demonstrated a marginally significant association ($p = 0:074$) with CDKN2A in T2DM patients. IL-6, TNF, and MCP-1, on the other hand, were substantially related with CDKN2A in T2DM patients.

In controls, all the indicators displayed insignificant correlations with the biological ageing marker CDKN2A. Yet among T2DM patients, IL-6, TNF-, and MCP-1 were all significantly correlated with CDKN2A, with MCP-1 having the highest significant correlation ($p < 0.002$) and oxLDL having the lowest significant correlation ($p = 0.074$). We also looked at how these markers varied depending on the length of the diabetes, and the results showed that MCP-1 ($p = 0.002$) indicated the length of the diabetes. We also studied how glycemia affected these markers, and we found that when glycemia was poorly controlled (HbA1c was greater than 7.0%), CDKN2A, a marker for senescence, dramatically increased ($p = 0.021$). A separate study of drugs was not carried out because metformin was being taken by the majority of the T2DM patients.

After that, we used logistic regression to assess the likelihood that various research groups would have elevated levels of the senescence marker CDKN2A. The variable that was dependent was CDKN2A. For G2 controls and G1 T2DM patients, G1 controls served as the reference group, whilst G2 controls served as the reference group for G2 T2DM patients. In comparison to the control groups, both groups of T2DM patients had an increased probability of having high levels of CDKN2A. Moreover, compared to G1 controls, G2 controls showed a higher probability of having high levels of CDKN2A.

Discussion

Although the onset and progression of both ageing and T2DM are particularly complex, they have a number of overlapping aetiologies in which low-grade inflammation and oxidative stress play major roles. Ageing is ultimately brought on by the accumulation of deficits over time brought on by elevated reactive oxygen species (ROS) and persistent levels of inflammatory mediators, which results in the acquisition of the senescence-associated secretory phenotype (SASP) and a progressive decline in cellular functions [13, 17]. We could not discover any conclusive links between oxidative stress markers and ageing in T2DM patients in this investigation. Both early and late middle-

aged T2DM patients had similar distributions of high MDA and oxLDL levels.

We hypothesised that diabetes may have contributed to these indicators' early emergence in T2DM patients. Previous research indicated that oxidative damage was exacerbated by high glucose levels [30]. Similar to our earlier discovery [25], we discovered that MDA, the byproduct of lipid peroxidation, was considerably higher in T2DM patients in both age groups when compared to their respective controls.

Between G1 T2DM patients and G1 controls, serum oxLDL, a well-known indicator of atherosclerosis, showed a significant difference, and between G2 T2DM patients and G2 controls, a marginally significant difference. Oxidation of LDL-c results in the formation of oxLDL. An earlier study found that having diabetes makes LDL-c more likely to be converted to oxLDL [31]. Based on the expansion of the network and remodelling theory of ageing, the chronic low-grade inflammation, also known as inflammageing, is a distinctive aspect of ageing and serves as the mechanistic foundation for many age-related diseases, including cancer, Alzheimer's disease, cardiovascular disease, and T2DM [32]. Recent research has demonstrated that inflammation and metaflammation share common pathways, and that their combined effects on T2DM patients are more severe [33]. Between G1 T2DM patients and G1 controls, we discovered substantial changes in the proinflammatory markers IL-6, IL-1, and TNF-. Significant differences between G2 T2DM patients and G2 controls were seen for IL-6 and MCP-1. According to past studies, hyperglycemia causes immune cells to respond by becoming pro-inflammatory [34].

A significant tissue- and blood-based molecular biomarker of biological ageing, CDKN2A, which encodes p16Ink4a, is generally recognised as the marker of cellular senescence and is substantially expressed in senescent cells [20, 35]. P16 expression was shown by Liu and colleagues to be related to IL-6 and to be independent of gender and BMI [35]. As a biomarker of human ageing, CDKN2A blood levels have been shown to be more favourable and dynamic than telomere length [35]. By altering fasting insulin and HOMA-IR, we discovered a substantial difference in CDKN2A concentrations between G1 T2DM patients and G1 controls. Insulin resistance has an impact on the aetiology of T2DM and is a key factor in ageing [36, 37].

The CDKN2A locus was found to significantly correlate with T2DM in earlier research [38, 39]. Moreover, we noticed a considerable rise in CDKN2A level as diabetes duration increased. We hypothesised that as diabetes is present for a longer period of time, ageing becomes worse. In T2DM patients, CDKN2A was also substantially linked to proinflammatory mediators. Oral hypoglycemic medications (OHA) may have had a positive impact on age-related oxidative stress levels. By triggering AMP-activated protein kinase and increasing the formation of the antioxidant thioredoxin, OHA, in particular metformin, has the power to control oxidative stress [40]. By inhibiting the NF-B pathway, metformin also acts as an anti-inflammatory [41]. We hypothesised, however, that the presence of persistent hyperinsulinemia and the effects of the partial correction of glycemia may be to blame for the aging-related rise in low-grade inflammation [42].

In middle-aged healthy and T2DM participants, our study offers important new understandings into the

relationships between oxidative stress and low-grade inflammation and biological ageing. The results of the current investigation show that T2DM considerably increases the risk of early ageing and age-related problems. With their high levels of visceral adiposity, higher fat-to-lean mass ratios, sedentary lifestyles, and genetic susceptibility, Indians are more likely than other ethnic groups to have cardiometabolic disorders at a younger age [43].

Conclusion

The results support the idea that oxidative stress and chronic inflammation brought on by T2DM may hasten biological aging in middle-aged Indians by accelerating the natural aging process. Future prospective studies will build on this research to determine whether altering a patient's lifestyle will prevent premature aging and age-related problems.

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