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REG Iα Levels in Type 1 and Type 2 Diabetics With and Without Complications Compared to Controls

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Abstract

***Background*:** Diabetes and its complications are major global health challenges, prompting the search for early biomarkers. The REG Iα protein plays a role in the repair and restoration of damaged β-cells.

Objective: To evaluate circulating REG Iα levels in diabetes patients (type 1 and 2) and assess their association with metabolic factors and disease complications.

***Methods*:** It is a cross sectional study that include 20 healthy controls (age- and sex-matched) and 70 patients (10 T1D and 60 T2D). REG Iα levels in serum were determined using ELISA, and clinical parameters such as Fasting plasma glucose (FPG) and HbA1c were analyzed. Statistical correlations were evaluated using SPSS.

***Results*:** Circulating REG Iα levels were significantly elevated in both T1D and T2D patients versus controls. T2D patients demonstrated an inverse correlation between REG Iα levels and disease duration, whereas T1D patients showed elevated levels with advancing age. Patients with diabetic complications exhibited higher REG Iα levels than those without.

***Conclusion*:** Elevated REG Iα levels in diabetics suggest their potential as biomarkers for β-cell regeneration and disease progression. Their correlation with complications highlights the need for further exploration into their diagnostic and prognostic utility.

Keywords: Gastrointestinal, invasive mucormycosis, immunocompetent patients.

1. INTRODUCTION

"Diabetes develops as a result of multiple pathogenic processes. These can include abnormalities that result in insulin resistance as well as islet β cells damage due to immune mediation leading to a secondary deficiency in insulin. Type 1 & type 2 diabetes (T1D & T2D) are two broad categories that comprise the majority of instances of diabetes. Only 5-10% of individuals with diabetes have T1D, which is also referred to as insulin-dependent diabetes mellitus (IDDM) or juvenile onset diabetes" [1]. T1D is an inflammatory chronic illness, an autoimmune condition which is caused by immune cells that invade the pancreatic islets of Langerhans, kill the β cells that secrete insulin, and then cause overt T1D [2] which eventually causes a lifelong reliance on exogenous insulin [3].

"Non-insulin dependent diabetes mellitus" (NIDDM), commonly referred to as "adult-onset diabetes," or T2D, encompasses a spectrum of conditions characterized by insulin resistance, abnormalities in insulin secretion, and hyperglycemia. This condition accounts for the overwhelming majority of diabetes cases, comprising approximately 90-95% of the total. Amongst these patients, persistent insulin resistance imposes an ever-increasing demand upon the insulin secreted by the islets, ultimately culminating in the demise of the islet β-cells and a consequent deficiency of circulating insulin [4-6].

Beyond sociodemographic determinants, factors such as physical inactivity, obesity, mental health, and other lifestyle choices have been strongly associated with an elevated risk of diabetes [7, 8]. Insulin therapy becomes necessary for a substantial proportion of individuals with T2D as the disease progresses, to effectively manage hyperglycemia.

Of note, owing to the rising prevalence of obesity and sedentary lifestyles, T2D is now being identified with increasing frequency amongst adolescents, surpassing the incidence of T1D in this age group—a condition which itself affects an estimated 5-10% of adults [9, 10]. Thus, the historical distinction based on the age at which these two forms of diabetes manifest has, in effect, become obsolete.

The C-Type Lectins superfamily, so named "CTLs" owing to their frequent calcium-dependent ligand binding (the "C" in CTL denoting calcium) [11], encompasses a diverse array

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of proteins. Amongst this superfamily, the Reg family constitutes but a modest subset. Lectins, by their nature, are proteins that exhibit an affinity for binding carbohydrates, while C-Type Lectins are found variously within serum, the extracellular matrix, and cellular membranes. These proteins are typically composed of 120 amino acids forming what is termed a carbohydrate-recognition domain (CRD).

Reg proteins, in contrast, are diminutive in size, weighing approximately 16 kDa, and, unlike other CRD-containing proteins, they are not observed to bind carbohydrates [12, 13]. Notably, the Reg family proteins are highly conserved, both within their own group and between species such as humans and rodents, as evidenced by their amino acid sequences. Universally, members of the Reg family possess a putative signal peptide comprising 21 to 25 amino acids, an N-terminal structure, and a single CRD. These proteins are secretory in nature, soluble in form, and serve a multitude of physiological purposes [14].

Previous studies highlight that Reg Iα plays an important role in β-cell proliferation and helps improve experimentally induced diabetes [15]. Based on these findings, the current hypothesis states that in both T1D and T2D diabetics, the production of REG Iα protein is elevated, as a means of regenerating islet β-cells that have been damaged by autoimmunity, glucolipotoxicity, and increased metabolic demand. This study aims to measure circulating levels of REG Iα in individuals with T1D and T2D with/ without problems in comparison to controls.

2. METHODOLOGY

This investigation was of a cross-sectional design, undertaken at the Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, as well as the Centre for Research in Experimental and Applied Medicine (CREAM), National University of Science and Technology (NUST), Islamabad. Ethical approval for the protocol was duly obtained from the Ethical Committee of Army Medical College, and all participants provided their written informed consent. The study was conducted in strict accordance with established ethical standards, the Declaration of Helsinki.

The research encompassed 10 patients diagnosed with type 1 diabetes (T1D), 60 individuals suffering from type 2 diabetes (T2D), and 20 healthy controls, all carefully matched for age and sex. The participants were drawn from the Medical Outpatient Department (OPD) of PNS Shifa Hospital, while the molecular work was executed at the Molecular Biology Research Lab, Ziauddin University, Karachi. Eligibility was determined in conformity with the diagnostic criteria set forth by the American Diabetes Association. Adults aged between 18 and 60 years were included in the study, whilst those with autoimmune disorders, malignancies, recent infections, or those who were pregnant, were excluded. Healthy controls were rigorously confirmed to possess no previous history of diabetes or related metabolic disorders.

Blood samples (10 mL) were drawn after an overnight fast into clot activator tubes. Samples were allowed to clot for 30 minutes at room temperature, followed by centrifugation at 2,500×g for 30 minutes at 2–8°C. Serum was then separated, aliquoted, and stored at −20°C until further analysis. Biochemical parameters, including fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), and triglycerides (TG), were assessed using standard protocols.

Blood samples, each of 10 mL, were drawn after an overnight fast into clot activator tubes. These samples were permitted to clot for a duration of 30 minutes at ambient temperature, after which they were subjected to centrifugation at 2,500×g for a further 30 minutes within the temperature range of 2–8°C. The resultant serum was then carefully separated, aliquoted, and stored at a temperature of −20°C until it would be required for further analysis.

Biochemical parameters, namely fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), and triglycerides (TG), were assessed in accordance with established and widely accepted protocols.

The concentration of circulating REG Iα was measured employing a Human REG Iα BioAssay ELISA Kit (USBiological, Life Science), in strict accordance with the protocol provided by the manufacturer. Serum samples were diluted at a ratio of 1:500 using phosphate-buffered saline (PBS) through a two-step procedure. Initially, 20 µL of serum was combined with 180 µL of PBS, achieving a dilution of 1:10. To this, 10 µL of the resultant solution was added to 490 µL of PBS, thus obtaining the final dilution. At each stage, the mixture was thoroughly agitated to ensure uniformity and precision.

The ELISA procedure was conducted utilizing microtiter plates pre-adorned with an antibody of singular specificity against REG Iα. Into each of the duplicate wells, precisely 100 µL of standards, blanks, and samples' dilutions were dispensed. The plates were thereafter permitted to repose at 25°C for a duration of two hours. Subsequently, 100 µL of biotin-conjugated Detection Reagent A was introduced, followed by incubation at 37°C for the span of one hour.

The wells were then subjected to a regimen of three washings with an appropriate cleansing solution, after which 100 µL of avidin-horseradish peroxidase (Detection Reagent B) was administered. A further incubation of 30 minutes at 37°C ensued, culminating in five additional washes to ensure the utmost precision.

Thereupon, 90 µL of tetramethylbenzidine (TMB) substrate solution was carefully added, and the enzymatic reaction

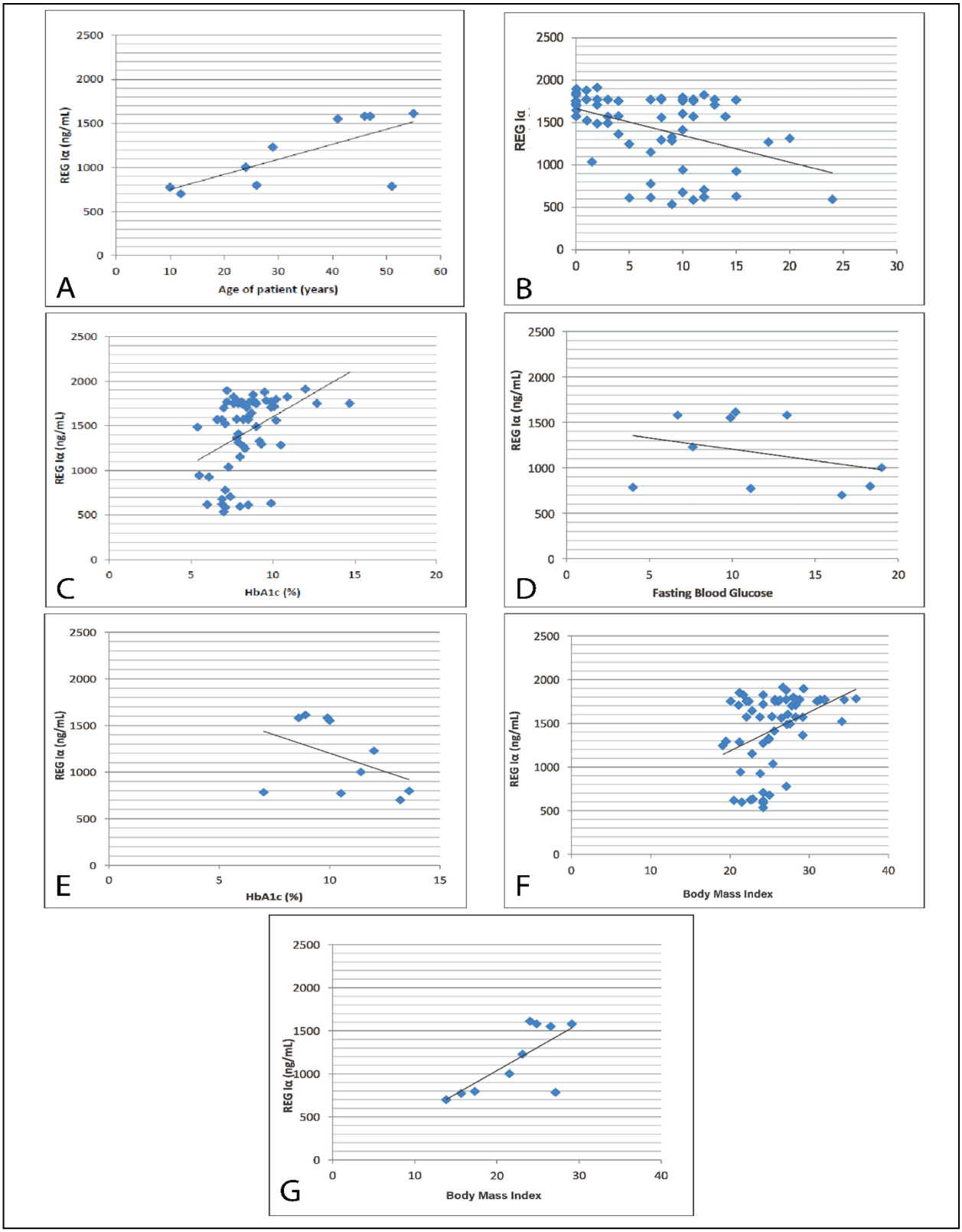

Figure 1: The associations of REG Iα protein levels with various parameters: (**A**) Age and REG Iα levels in T1D. (**B**) REG Iα levels and disease duration in T2D. (**C**) REG Iα protein and HbA1c in T2D. (**D**) FBG and REG Iα levels in T1D. (**E**) HbA1c and REG Iα levels in T1D. (**F**) BMI and REG Iα concentrations in T2D. (**G**) BMI and REG Iα levels in T1D.

Table 1: The correlation coefficients (r) and their corresponding p-values for clinical parameters in TD1 and TD2.

Groups	Variable	BMI	FBG	HbA1c	Disease Duration	Age
I	r	0.612	-0.224	-0.467	0.255	0.709
	p	0.06	0.533	0.174	0.476	*0.022
II	r	0.411	0.407	0.444	-0.355	-0.309
	p	*0.001	*0.001	**0.000	*0.005	*0.019

*Significant correlations are indicated by asterisks, with ** denoting high significance (*p < 0.01, p < 0.001).*

was allowed to proceed for a quarter of an hour. To arrest this reaction, 50 µL of Stop Solution was delicately introduced. The optical density of the resultant solution was ascertained at a wavelength of 450 nm, employing a microplate spectrophotometer of reliable pedigree.

All statistical computations were executed with the aid of SPSS software (version 16). The conformity of the data to normal distribution was ascertained employing the Shapiro–Wilk test. Descriptive measures were rendered as mean ± standard deviation (SD) for data exhibiting normal distribution, whereas the median accompanied by the interquartile range was presented for data deviating from normality.

To compare two groups, the Mann–Whitney U test was applied, while for comparisons involving three or more groups, the Kruskal–Wallis test was employed. Correlations were examined utilizing Spearman's rank test. A threshold of statistical significance was firmly established at $p < 0.05$.

3. RESULTS

This study evaluated the relationship between circulating REG Iα levels and clinical as well as biochemical variables in T1D and T2D patients. In T1D, REG Iα levels exhibited a significant positive correlation with age ($p = 0.022$, Spearman $r = 0.709$) (Fig. **1A**). Conversely, in T2D, an inverse correlation was observed between REG Iα levels and disease duration ($p < 0.005$, Spearman $r = -0.355$) (Fig. **1B**). Additionally, REG Iα levels in T2D demonstrated a strong positive association with HbA1c ($p < 0.001$, Spearman $r = 0.444$) (Fig. **1C**).

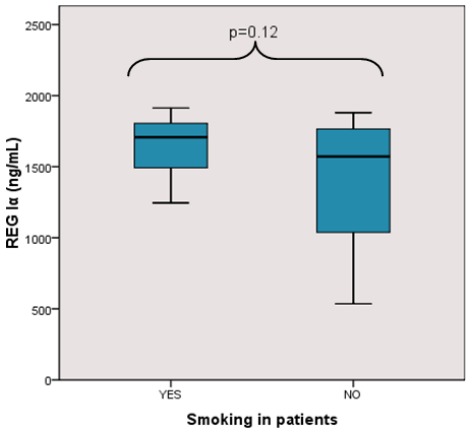

Figure 2: Comparison of Smoking with REG Iα concentration and in Individuals with T2D.

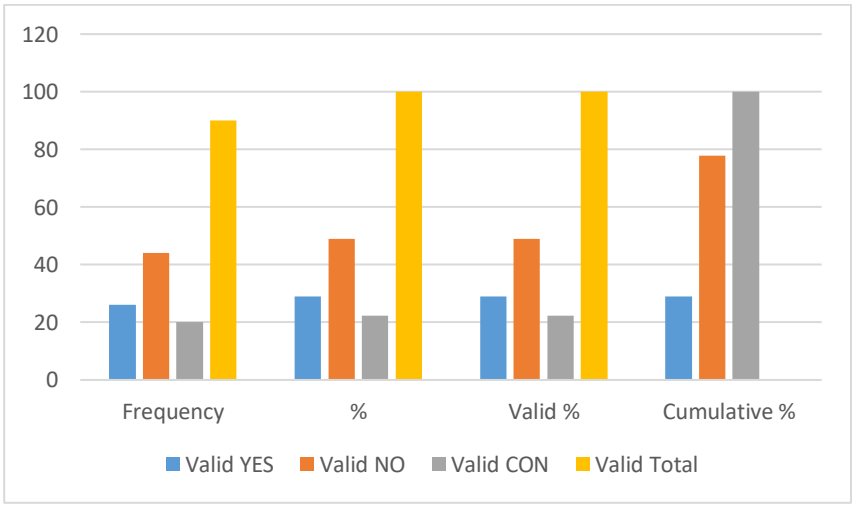

Figure 3: Comparison between diabetics (with and without complications) and controls.

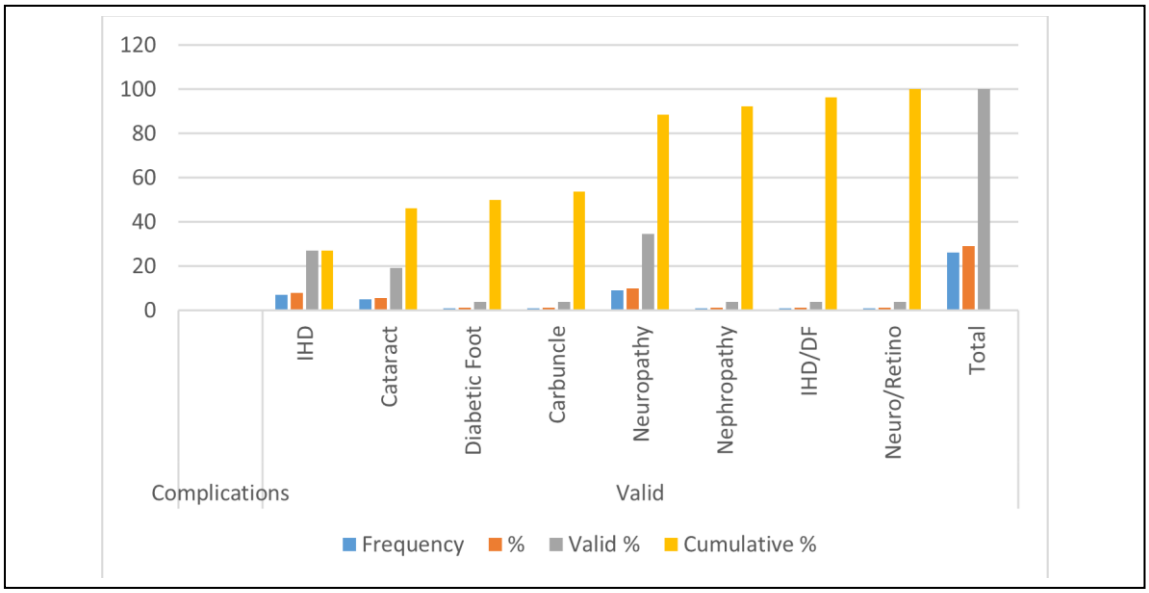

Figure 4: Types of complications in diabetics.

In T1D, minimal correlations were noted between REG Iα levels and fasting blood glucose (FBG) (Fig. **1D**) or HbA1c (Fig. **1E**). No significant associations were identified between REG Iα levels and total cholesterol (TC) or triglycerides (TG) in either group. Detailed comparisons of clinical and biochemical parameters are summarized in Table **1**.

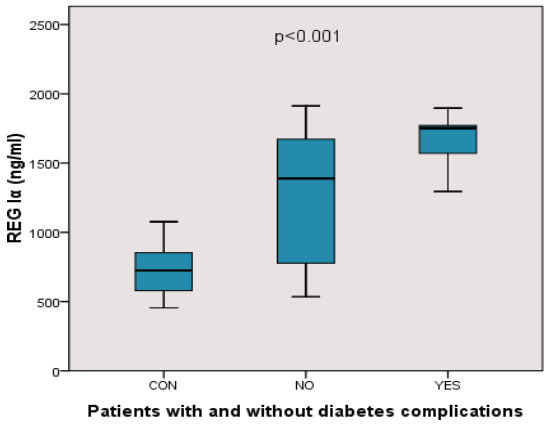

Figure 5: Serum levels of REG Iα in controls, as well as in T1D and T2D with and without complications.

The influence of lifestyle factors, such as smoking and body mass index (BMI), on serum REG Iα levels was also assessed. Among T2D, smokers exhibited higher REG Iα levels *vs* non-smokers, although this difference was statistically insignificant ($p = 0.12$) (Fig. **2**). BMI, however, was significantly associated with REG Iα levels in T2D ($p = 0.001$, Spearman $r = 0.411$) (Fig. **1F**), while a positive but non-significant association was observed in T2D (Fig. **1G**). Circulating REG Iα were also evaluated in relation to diabetes-associated complications (Fig. **3**-**4**). Diabetics with complications exhibited significantly higher REG Iα levels (mean: 48.32 ng/mL) compared to diabetics without complications (mean: 30.90 ng/mL, $p < 0.001$) and healthy controls (mean: 15.26 ng/mL).

A comparative analysis of serum REG Iα levels across controls, T1D, and T2D, both with and without complications, revealed significant differences between all groups ($p < 0.001$). These findings are illustrated in Fig. (**5**), highlighting the distinct profiles of REG Iα levels in the studied populations.

4. DISCUSSION

Compared to normal patients, it was observed that both T1D and T2D diabetics had significantly higher REG Iα levels. T2D had levels that were more up-regulated than the other diabetics. Patients with T2D who had just developed the disease and, to a lesser extent, those who had had it longer also had elevated levels. Increased levels of circulatory REG Iα have been documented in individuals with T1D and T2D diabetes, as well as in patients with MODY (maturity-onset diabetes of the young) starting in their third decade [16-18]. This study, however, is the first of its type in our nation, where the number of people afflicted with this crippling and potentially fatal illness is alarmingly on the rise. Additionally, this finding validates the extensive prior research conducted in β-cell regeneration and diabetes experimental models [15].

In T1D, there is an increased expression of the Reg I gene following the death of β-cells as a result of local immune cell infiltration [19]. Additionally, it has been shown that in mice models of high-lipid diets, its expression is elevated from an early stage during the transition from obesity to T2D [20]. Numerous research has discussed the significance of the inflammatory process in T2D and obesity [21, 22]. "Increased Reg I expression was associated with immune cell infiltration around the islets and elevated levels IL-6 and IL-22 as well as other cytokines/chemokines, in the islets in animal studies of T2D [23, 24]. In human β-cell lines, treatment with Dexamethasone (Dx) and IL-6 combined enhanced expression of REG I" [25]. The Reg I gene promoter region has been shown to have an IL-6 response element, and elevated local IL-6 levels are essential for upregulating Reg gene expression [26]. A connection between β-cell death and regeneration has been shown to occur when apoptosis occur in β-cells, they trigger expression of REG I gene in the surrounding cells, which aids in regeneration of β-cells, enhancing their capacity to secrete insulin [27].

Insulin secretion and Reg gene expression are enhanced by high extracellular glucose concentration [27]. Furthermore, elevated serum 'REG Iα' levels in both the T1D and T2D diabetes patients in this study support the theory that increased expression of REG Iα in regenerating β-cells and acinar cells of the exocrine pancreas is a result of reduced β-cell mass due to heightened damage from increased metabolic demand and inflammation.

In T2D, there was a noteworthy inverse relationship between the duration of the disease and levels of 'REG Iα'. Patients with shorter disease duration had higher amounts of protein. Animal models of T2D and obesity both showed elevated expression of Reg I in the initial stages of the disease [20]. As the illness worsens, the metabolic demands placed on the insulin-producing cells rise. At the same time, humans' ability to regenerate these cells declines as a result of their reduced ability to reproduce [28]. Furthermore, it has been noted that the ageing process and age-related islet β-cell dysfunction are associated with a decrease in REG I gene expression [29].

5. CONCLUSION

In T1D and T2D patients, elevated levels of biomarkers (REG Iα) show their potential for β-cell regeneration and apoptosis. They may also indicate the risk of complications in these conditions. Understanding the protective mechanisms of REG Iα, such as its role in reducing AIF-dependent apoptotic signaling, could improve diabetes management and prevention strategies. Furthermore, the g.209T variant of the REG Iα gene is found to be significantly associated with an elevated risk of type 2 diabetes (T2D), with minor variations being linked to the habit of smoking.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for the protocol was duly obtained from the Ethical Committee of Army Medical College and all participants provided their written informed consent.

HUMAN AND ANIMAL RIGHTS

The study was carried out in compliance with the Declaration of Helsinki (WMA, 2000) and Good Clinical Practices as authorized by the FDA in 1996.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

FUNDING

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Declared none.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study with the corresponding author and will be provided upon reasonable request.

AUTHOR'S CONTRIBUTION

SU: Proposed the research question and design of the study.

FA: Performed the data entry and analyzed the results.

AS: Drafting of the manuscript.

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