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Serological and oncoinformatic analysis of HbA1c as a prognostic biomarker in screening the risks of different cancers among the male T2D patients of Bangladesh

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ABSTRACT

Hemoglobin A1C (HBA1c) represents the average serological sugar status of the T2D patients of the past three months, considered a clinically standard method of studying sugar metabolism. Overexpressing HbA1 can metabolically forecast the risk of different cancers among the T2D patients. Based on which, the study aimed to analyze the impact of sugar metabolism in cancer development considering the overexpression of HbA1 as the prognostic biomarker of screening the risks of eight different cancers among the chronic male T2D patients of Bangladesh. Serological analysis of the concentrations of FBS, THABF, creatinine, SC, STGs, HDLC, and LDLC of the T2D patients were conducted in response to their individual HbA1c concentration. Afterward, HbA1 overexpression and promotor-methylation responsible for BLCA, BRCA, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD cancers in the male T2D patients were profiled as the oncoinformatic screening, where the sample types used, individual cancer stages, racial-footprints, gender, age, nodal metastasis, p53-methylations, pancreatitis, diabetes status, smoking behaviors, and survivability status were studied. Finally, the genetic involvement of a group of genes responsible for genetic co-expression of HbA1, endophytic vesicle regulation, antioxidant regulation, and reactive oxygen species based-metabolic regulation in T2D males were identified and comprehensively discussed. The research revealed, significant correlation between BMI and FBS in both the patient and the control groups ($p<0.0001$). Besides, FBS, THABF, and creatinine were found significantly regulated with their respective HbA1c concentrations ($p<0.0001$) for each group. The SC, STGs, HDLC, and LDLC regulated ardently and equally for both groups ($p<0.0001$), while HbA1c ranged from 3.8-5.8% and 5.11-15.8%, for the controls and patients respectively. HbA1 was found interactive with diversified cancer-causing genes, while HbA1 was mostly downregulating with the progressing metastasis. To receive maximum benefits from using HbA1c in clinically profiling of cancer-risks among the chronic-male T2D patients in minimal time and expense further studies can be needed with larger sample size.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by an inadequate supply of insulin or inappropriate utilization of insulin. Among the classification of diabetes mellitus, more than 95% of people are affected with type 2 diabetes (T2D) [1]. People with T2D are at elevated risk for suffering different types of cancer, cardiovascular comorbidities, and intravascular complications, including nephropathy, retinopathy, and neuropathy, due to hyperglycemia, which sometimes results in

insulin resistance (metabolic) syndrome [2]. Various lifestyle variables are linked to the growth of T2D, such as a sedentary lifestyle, physical inactivity, food habit, stress, and excessive alcohol use [3]. Globally, diabetes mellitus impacted 285 million adults in 2021 and is expected to affect 683 million by 2030 [4]. Diabetes affects 79% of T2D, especially in low and middle-income countries [5], and Bangladesh is second among the top five Southeast Asian nations in terms of individuals (aged 20-79 years) with T2D [4]. Diabetes incidence experienced rapid growth in Bangladesh, and a scoping assessment (1994–2013) indicated that T2D prevalence ranged from 4.5% to 35.0% throughout the nation, which is very alarming [6]. However, in patients with T2D, the cells in the liver become insulin-resistant, resulting in decreased glucose metabolism in the circulation, and the outcome is hyperglycemia [7]. T2D is diagnosed using a variety of serological markers, the most prevalent of which is glycated hemoglobin (HbA1c), an estimated average of three months' blood glucose concentrations [8]. Dyslipidemia occurs due to lipid profile abnormalities such as increased serum triglyceride, low-density lipoprotein- cholesterol, and decreased high-density lipoprotein cholesterol. Dyslipidemia is also linked to T2D, as insulin shortage and insulin resistance lead to impaired lipid metabolic pathways [9]. Changes in total cholesterol are associated with an increased risk of developing T2D, regardless of whether or not anti-hyperlipidemic medications are used [10]. An increase in the BMI over the normal weight range can also increase the risk of T2D. Men risk these problems more than women for BMI issues [11]. Diabetes is also diagnosed with higher fasting blood glucose levels and impaired oral glucose tolerance testing [12]. The concentration of HbA1c often randomly fluctuates, and many research findings have established a link between diabetes and multiple carcinomas in terms of HbA1c concentration [13].

According to the International Diabetes Federation, diabetes affects 17.7 million more males than women worldwide [4]. In Bangladesh, the number of death and suffering from diabetes were higher in males than in females [14]. Chronic hyperglycemia caused by abnormalities in insulin secretion from the pancreatic beta cells and insulin release in target tissues such as skeletal muscle, adipose tissue, and liver characterizes type 2 diabetes, a multifactorial disease [15]. According to the studies, epigenetic variables, such as DNA methylation and histone changes, may impact the etiology of type 2 diabetes [16]. Higher levels of methylation were linked to lower levels of the corresponding gene's mRNA expression in diabetic islets and higher levels of glycated hemoglobin A1c (HbA1c, a marker for long-term plasma glucose levels), suggesting a potential role for β-cell disruption in T2D. Promoter hypermethylation inhibits insulin expression in islets in people with type 2 diabetes. According to many epidemiological studies, diabetes individuals are more likely to develop several cancers such as BLCA (Bladder urothelial carcinoma), BRCA (Breast invasive carcinoma), CHOL (cholangiocarcinoma tumor), COAD (colon adenocarcinoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), PAAD (pancreatic adenocarcinoma) and PRAD (prostate adenocarcinoma) [17-19]. Some other studies reported HbA1c as a biomarker for chronic hyperglycemia. Except for prostate cancer, persistent hyperglycemia is associated with a higher risk of developing a lot of malignancies. Additionally, there is proof that the risk of various malignancies is already rising in the pre-diabetic and normal ranges [20].

In light of the facts mentioned above, the present research aimed to figure out the correlation of HbA1c with the risk of eight common types (BLCA, BRCA, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD) of cancers. In addition, the oncoinformatic analysis of the HbA1 gene was conducted considering factors such as tumor specificity, hypermethylation, and overexpression in case of selective cancers formation, so that

HbA1c can be utilized as a functional biomarker for the cancer-risk profiling of the T2D male patients in Bangladesh.

MATERIALS AND METHODS

Selection of patients for categorizing

In this research, 300 male T2D patients of different stages were serologically tested to compare with 60 clinically normal individuals used as control. The study protocol was approved under the project of 'Category C3; ID. #13-2021/22' by the 'Committee of Ethical Issues in Clinical Research' of Jashore Medical College (Bangladesh Medical and Dental Council) as dated November 2021. The endorsement of the patients and the criteria of ethical clearances directly follow the Declaration of Helsinki and the Ministry of Health of Bangladesh. The sample size of the patients was calculated following the standard methodology of clinical selection [21]. All the patients who participated in this study were from Jashore Medial College and all were regular patients of some senior physicians there.

Serological assays

To assess the status of type 2 diabetes in the patients, several specific quantitative serological profiling was conducted, considering- HbA1c (%); fasting blood sugar (mmol/L); two hours after breakfast (mmol/L); serum creatinine (mg/dL); cholesterol of serum (mg/dL); serum triglycerides; HDLC-LDLC (mg/dL); and BMI (kg/m2) [22]. Depending on the concentration of the HbA1c biomarker, all the other parameters were correlated with it individually. The measurement of the serum HbA1c concentration was taken using the fingers tick blood collection method, where the D-10 HPLC analyzer system was operated with the recommended protocol [23]. The fasting blood glucose (FBS) and two hours after breakfast (THABF) were tested using a continuous glucose monitoring system (CGMS®) (Dexcom G7 Dexcom, Inc., USA, LBL-1001419 Rev003) [24]. The serum creatinine, cholesterol, and triglycerides were assayed using- 'Creatinine Assay Kit,' Sigma Aldrich® (Product No. MAK 080) (Gillingham, Dorset, SP8 4XT, UK); 'Cholesterol/Cholesteryl Ester Kit II,' Sigma Aldrich® (Product No. MAK 396) (Gillingham, Dorset, SP8 4XT, UK); and 'High Sensitivity Triglyceride Fluorometric Assay Kit,' Sigma Aldrich® (Product No. MAK 264) respectively. Besides, HDLC and LDLC were quantified by 'HDL and LDL/VLDL Quantitation Kit' Sigma Aldrich® (Product No. MAK045) (Gillingham, Dorset, SP8 4XT, UK). Each of the samples from the T2D patients and the control group was handled carefully to ensure the most accurate results from the assays.

Oncoinformatic analysis of HbA1

HbA1c is a significant biomarker of both the type 2 diabetes mellitus and different carcinomas in male patients, the comprehensive oncoinformatic parameters of the HbA1 gene (Gene ID: 3039) encoding HbA1c (Accession: NP_000549.1), were analyzed to establish a functional correlation between T2D and differential cancers. The clinical and genomic impact of the HbA1c was initially characterized using the 'GEO Interface-NCBI' and the 'GEO2R Interface- NCBI' (https://www.ncbi.nlm.nih.gov/geo/geo2r/), respectively. The log2 expression of the HbA1 transcript per million for the cancers-BLCA, BRCA, CHOL, COAD, LUSC, LUAD, PAAD, and PRAD was analyzed in men through 'GEPIA 2' (http://gepia2.cancer-pku.cn/#index). The profiling of the HbA1

gene expression and its promoter methylation for cancer formation was conducted based on sample types, cancer stages; geographical footprints; gender specificity; aging; tumor grade; p53 mutation frequency; nodal metastasis; diabetes status; and smoking behavior using ULCAN' (http://ualcan.path.uab.edu/index.html) [25]. The molecular string networking of the gene-gene interaction networks regulated by the HbA1 gene was characterized considering the involvements in the aspect of genetic co-expression, endophytic vesicle regulation; antioxidant regulation; oxygen species metabolic regulation; and gene-mediated response to the reactive oxygen molecules. In that case, Cytoscape 3.9.1 (https://cytoscape.org/) [26] was used, which functions scripting on JAVA (https://www.java.com/en/download/manual.jsp). The clustering of the HbA1c protein due to the activity of the HbA1 gene was generated depending on the factors including- experimentally determined interaction (EI); database annotation (DA); combined score (CS); co-expression (CE); phylogenetic co-occurrence (PC); neighborhood on the chromosome (NC); gene fusion (GF); homology (H); and the automated text mining (AT), where Heatmapper and Morpheus tools (https://software.broadinstitute.org/morpheus/) were used [25,26].

Statistical analysis

The biostatistical analysis and representation of the data resulting from the serological assays and oncoinformatic interfaces were performed using computational 'R Programming Scripts' (version R-4.0.2, for Linux) [27-29]. 'GraphPad Prism' (version 8.1.2, for Mac OS) premium software packages were used to assess data analysis using two-way ANOVA, followed by Tukey's multiple comparison tests [30-32] and Dunnet's multiple comparison tests. A significant value was considered ($p < 0.0001$) on the scale of significance $\alpha = 0.05$ [33-35].

RESULTS

Fasting blood sugar (FBS) regulation with body mass index (BMI)

In the current study, a strong correlation was observed between the FBS (mmol/L) and BMI (Kg/m2) for both the T2D patients ($p < 0.0001$) and the control ($p < 0.0001$), in the scale of significance $\alpha = 0.05$ (Figure 1). The BMI and FBS were observed ranging from 18.4 to 37.1 (Kg/m2) and 4.2 to 15.41 (mmol/L) respectively (Figure 1A), where the values of the same respective parameters were 18 to 24 (Kg/m2) and 3.9 to 6.9 (mmol/L) in the control group (Figure 1B).

Assessment of FBS, THABF, and creatinine in response to HbA1c

According to the findings, the range of HbA1c for the T2D patients ranged between 5.11 to 15.8% concerning 4.2-15.41 mmol/L FBS (Figure 2A), 4.58-21.97 mmol/L THABF (Figure 2B) and 0.14-1.98 mg/dL creatinine (Figure 2C) was observed. In contrast, the limits of HbA1c for the control individuals were 3.8 to 5.8%, while the FBS, THABF, and creatinine concentrations were 3.9-6.9 mmol/L (Figure 2D), 4.04-6.99 mmol/L (Figure 2E), and 0.6-1.4 mg/dL (Figure 2F) respectively. In response to the concentration of HbA1c, the resulting individual correlation of FBS, THABF, and serum creatinine was significant ($p < 0.0001$) for both the patients (Figure 2A-C) and control groups (Figure 2D-F) simultaneously.

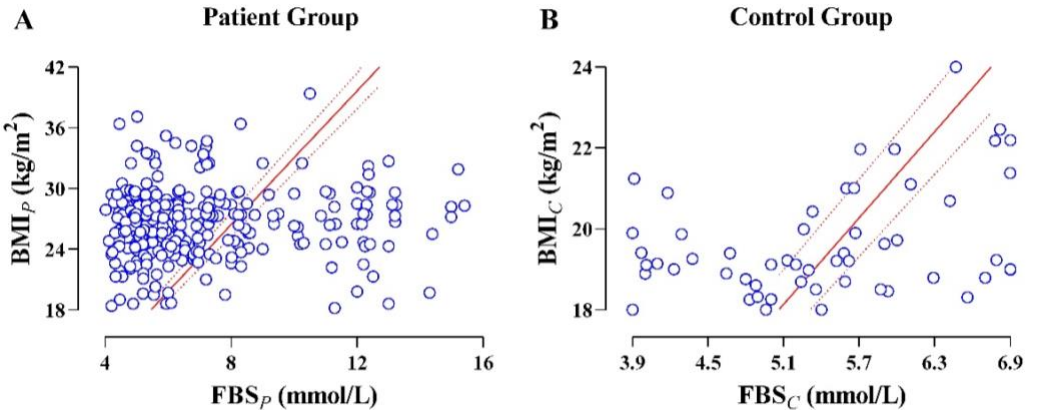

Figure 1. The correlation between BMI (Kg/m2) and FBG (mmol/L) among the patients (A) and control group (B). In all the cases, a strong correlation was found for both groups ($p < 0.0001$) on the scale of significance of $\alpha = 0.05$. The linear regression line validates the ranges of BMI and FBS for each group precisely. In these figures, p indicates patients and C indicates the control group. The value of standard error for T2-DM Patients was calculated at 0.07467 and for the control group at 0.08381. The 95% Confident Interval for T2-DM patients were between 3.160-3.454 and that of the control group was between 3.389-3.724.

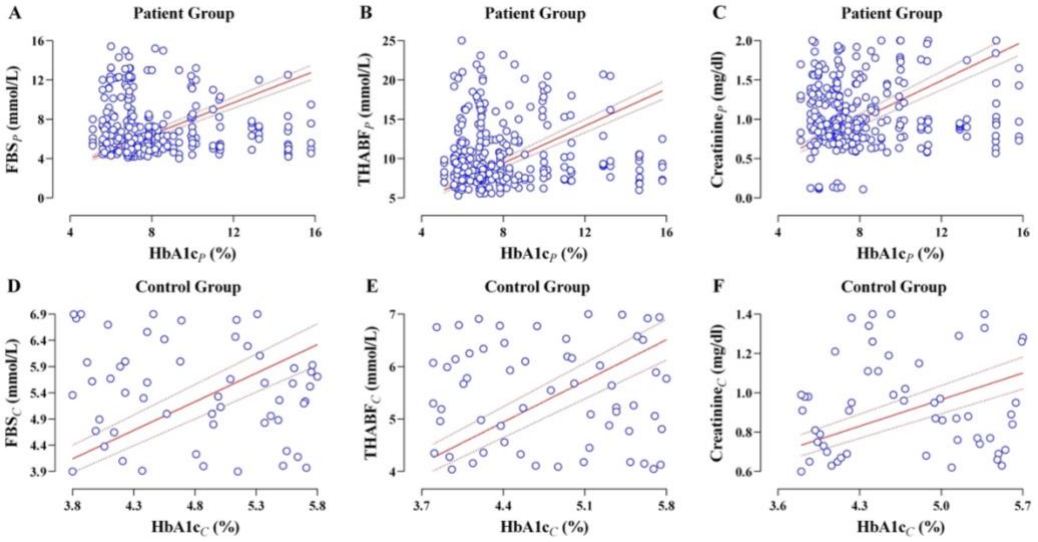

Figure 2. The correlations of HbA1c (%) with the patients' FBS (mmol/L) (A); THABF (mmol/L) (B) and creatinine (mg/dL) (C) in comparison to their corresponding control groups on the same parameters (D-F). In all the individual parameters of the patients and controls, significant correlations were observed ($p < 0.0001$). THABF indicated Two Hours After Breakfast. For the patient group, the Standard Error (SE) of HbA1c Vs FBS was calculated at 0.02380, and the 95% Confidence Interval (CI) value was between 0.7601 and 0.8538. SE of HbA1c vs THABF was 0.03560 whereas the 95% CI was between 1.109 and 0.1339. Lastly, the SE of HbA1c vs Creatinine was 0.004939 and the value of 95% CI was between 0.1145 and 0.1339. On the other hand, in the case of the control group, the SE value for HbA1c VS FBS was 0.03408 and the 95% CI value was between 1.021 and 1.158. SE value for HbA1c VS THABF in the control group was 0.03333 and the 95% CI value was between 1.056 and 1.189. Finally, the SE for HbA1c Vs Creatinine in the control group was 0.007136 and 95% CI was between 0.1788 and 0.2074. In all these cases, the scale of significance was $\alpha = 0.05$, and the p-value was < .0001 on this scale.

Lipid profiling considering the levels of HbA1c

The value of the patient's serum cholesterol (SC) was found to be 150.01-249.3 mg/dL (Figure 3A) following the concentration of HbA1c within 5.11-15.8% (Figure 3B) when the serum triglycerides (STGs), HDLC and LDLC were 120-332 mg/dL (Figure 3B), 92-180 mg/dL and 32-42 mg/dL respectively (Figure 3C). Based on HbA1c concentration,

the STGs, HDLC, and LDLC were highly significant ($p < 0.0001$). Similar significant relationships were also found for the control group ($p < 0.0001$), in which the concentrations encompassed 3.8-5.8% for HbA1c, 149.1-199.3 mg/dL for SC (Figure 3D), 10-197 mg/dL for STGs (Figure 3E); 46-127 mg/dL for HDLC (Figure 3F) and 23-149 mg/dL for LDLC (Figure 3F).

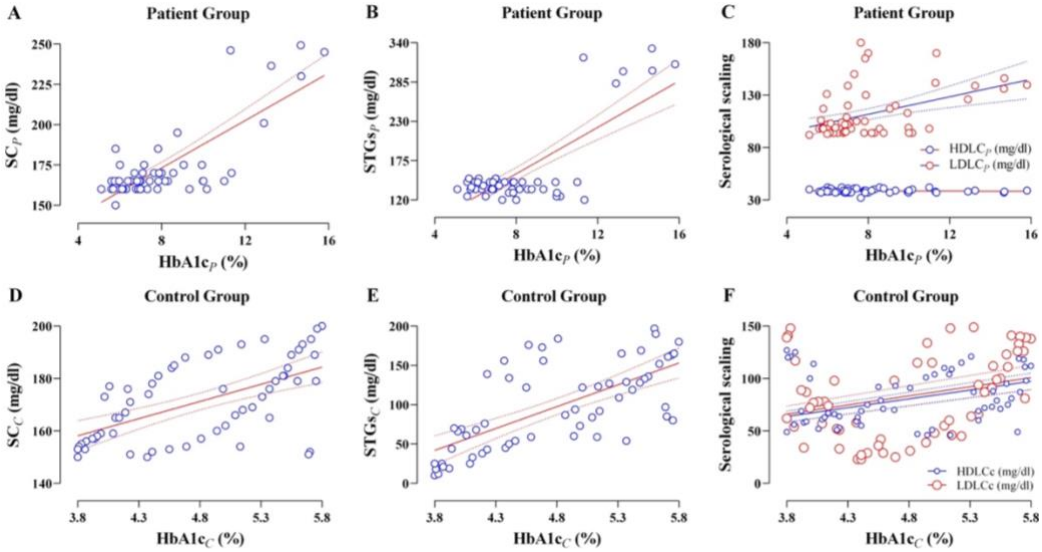

Figure 3. Comparative analysis of different serological parameters in response to the individuals' HbA1c concentration. The correlations of individual SC (mg/dl), STGs (mg/dl), HDLC (mg/dl), and LDLC (mg/dl) in response to the HbA1c (%) for the T2D patients (A-C) and the control individuals (D-F) have been illustrated, where the R square values were determined in addition to the SE and 95% CI values for all. The scale of significance was considered $\alpha = 0.05$.

Effects of HbA1 gene overexpression using oncoinformatic analysis

The HbA1 activity is regulated by diversified genes present in both the normal and tumor cell types (Figure 4). It has resulted in the HbA1 gene being upregulated with selective BLCA tumor (Figure 4A). In contrast, the HbA1 gene downregulates in BRCA, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD tumor types. The upregulation and downregulation of the HbA1 gene over different tumor types are also represented along with the PAAD (Figure 4B).

In this study, the number of HbA1 transcripts per million in the host was calculated, through which the overexpression status was profiled (Figure 5). In primary tumors, the HbA1 gene dramatically downregulates more than that of the normal cells and tissue types (Figure 5A). The surprising fact was that the HbA1 gene expression downregulates with the progression of cancer stages which means that stage-1 cancer patients have higher HbA1 expression than all the other cancer stages. At the same time, normal individuals contain a strong level of HbA1 hyperexpression (Figure 5B), as shown in this research. Caucasian patients showed relative overexpression of HbA1 more than African-American and Asian people (Figure 5C). As regards the sex of the cancer patients, in both males and females, HbA1 is downregulated in contrast to average persons (Figure 5D). For the aged population (81-100) years, HbA1 expression is prominently higher in them than in the younger population (Figure 5E). HbA1 gene expression is downregulated with the progression of nodal metastasis in cancer patients (Figure 5F). Clinically, p53 mutated patients showed the least HbA1 gene expression than the patients with non-mutated p53 and normal subjects (Figure 5G). T2D patients with chronic pancreatitis possess comparative overexpression to normal

individuals and T2D patients without any chronic pancreatitis (Figure 5H). Likewise, type 2 diabetes patients experience significant downregulation of HbA1 more than normal individuals and even patients free from T2D (Figure 5I).

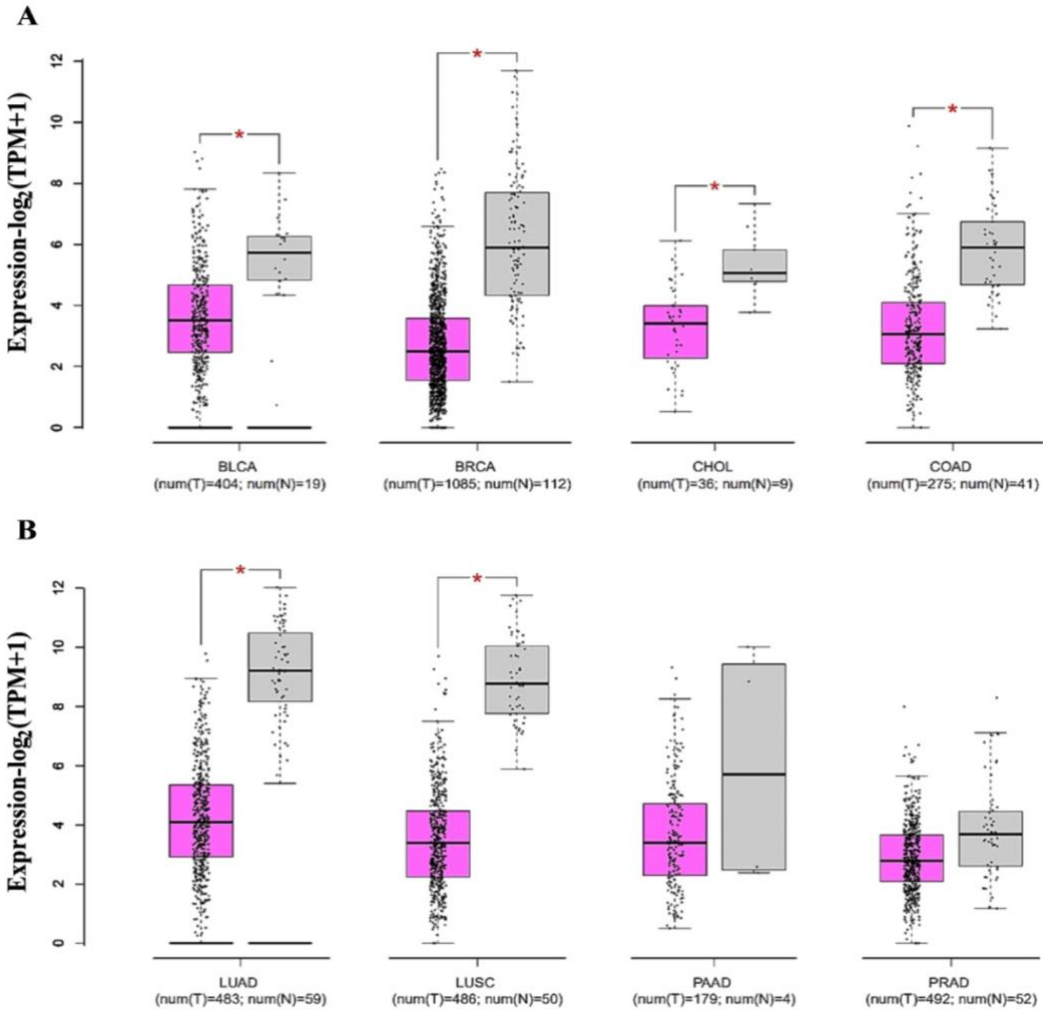

Figure 4. Profiling of the HbA1 gene expression among cancer patients in comparison to the normal individuals. Five different cancer types were considered, where among the breast cancers- bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA); colon cancers- cholangiocarcinoma tumor (CHOL), colon adenocarcinoma (COAD); lung cancers- lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC); pancreatic cancer- pancreatic adenocarcinoma (PAAD); and prostate cancer- prostate adenocarcinoma (PRAD) were considered.

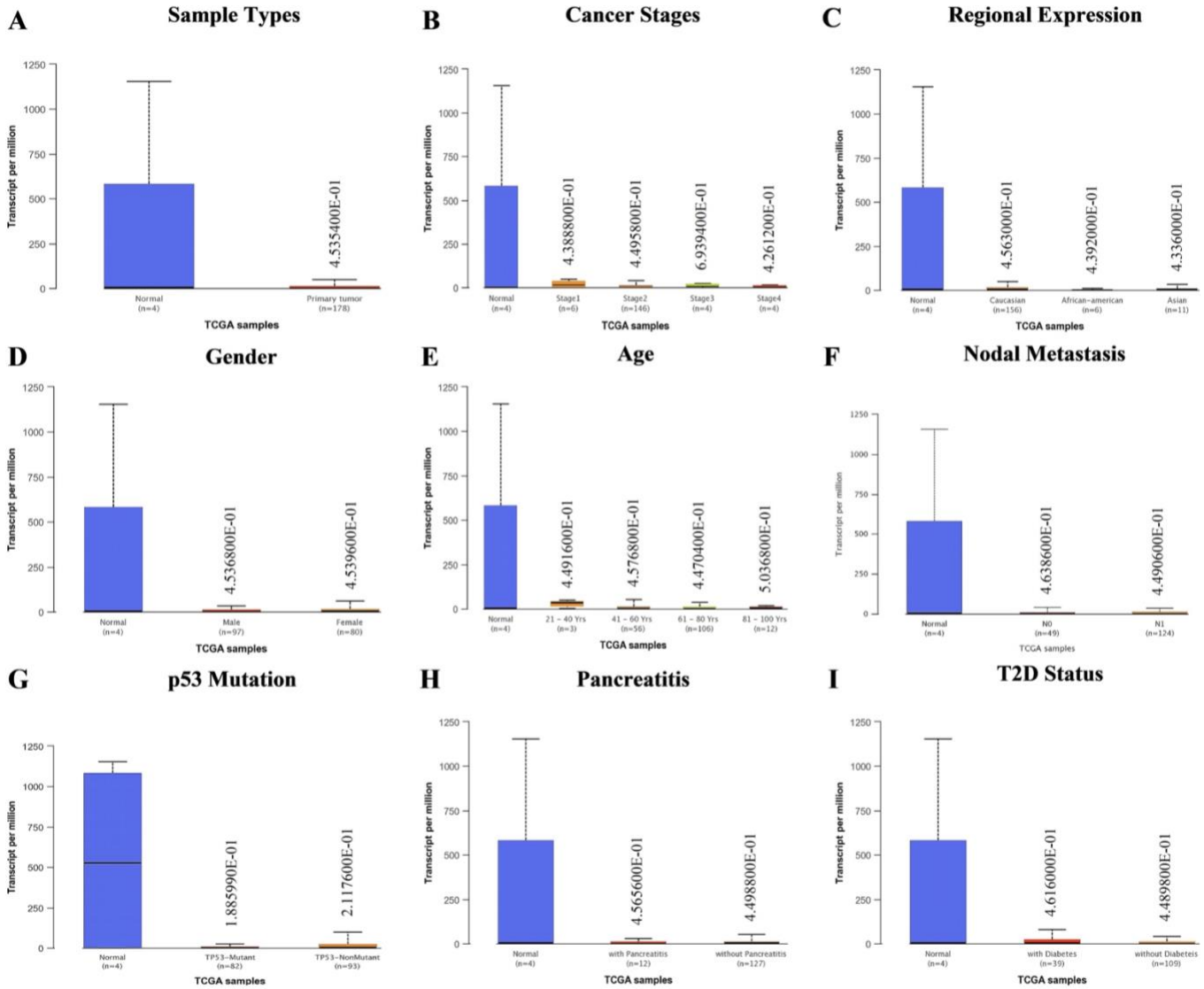

Figure 5. Multifactorial influence in profiling the expression of HbA1 among the individuals. In that case, factors like sample types (A), individual cancer stages of patients (B), regional expression (C), gender specificity (D), age factor (E), nodal metastasis (F), level of the p53 mutation (G), pancreatitis status of the diabetes patients (H), and the diabetes status (T2D) (I) were considered.

Hypermethylation of HbA1 promoter for T2D and cancers

The hypermethylation of the HbA1 promoter regulates primary tumor formation in normal conditions (Figure 6A). With the development of cancers, the methylation frequency drops down gradually among the patients (Figure 6B). Geographically, Asian people, especially male T2D and cancer patients, recorded a very stable range of HbA1 promoter methylation incidences, which is almost similar to Caucasians but less than the African-American (Figure 6C). The male subjects are more prone to have HbA1 promoter hypermethylation than the women subject with T2D and cancers according to this assessment (Figure 6D). For the aged population, the rate of HbA1 promoter methylation is always significantly more prominent than in the younger population (Figure 6E). The methylation process is downregulated in advanced tumor grades compared to the first among the patients (Figure 6F). In the case of the non-mutated p53 subjects, the methylation is much more elevated (Figure 6G). Similarly, at the beginning of nodal metastasis, the methylation is comparatively higher than in the progressed stages (Figure 6H). Finally, concerning smoking habits, smokers expressed a much higher methylation rate than non-smokers (Figure 6I).

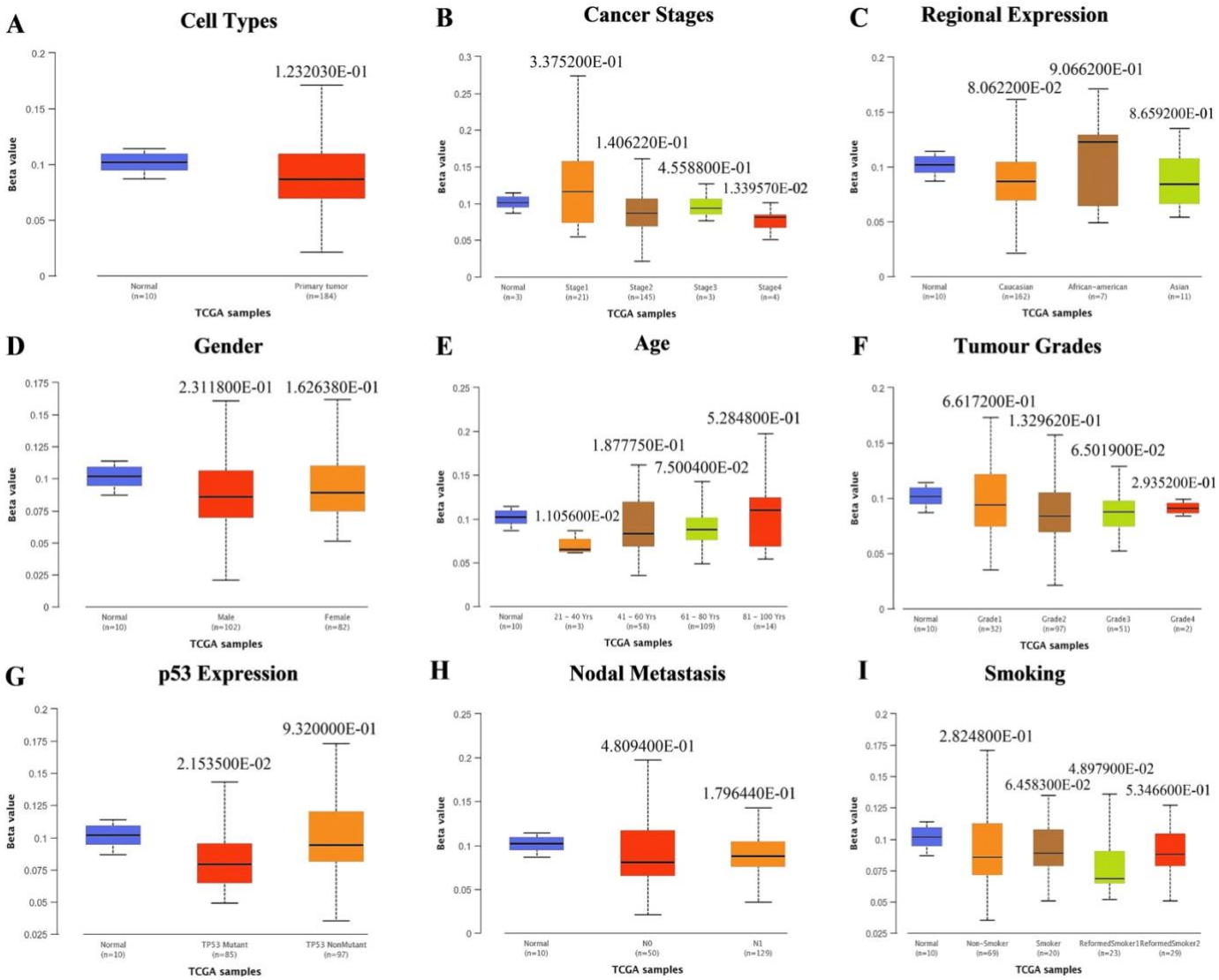

Figure 6. The profiling of the HbA1 –promotor methylation (HbA1P*) in risk-profiling of different cancers among the T2D patients studied. To analyze comprehensively some parameters such as cell types (A), cancer stages (B), regional expression (C), gender specificity (D), age factor (E), tumor grades (F), p53 expression (G), nodal metastasis (H), and smoking habits (I) were preferred. The Cancer Genome Atlas (TCGA) referred that the HbA1 gene expression level (beta-value) remains static within the range of 0.081 to 0.121 for normal individuals whereas the level fluctuates dynamically as compared to normal persons depending on the aforementioned parameters.

HbA1-mediated genetic interactions involved in cancer development among the diabetic patients

According to this research, the HbA1 gene interacts as a cluster or operon with the genes, namely Alpha-hemoglobin-stabilizing protein (AHSP); Apolipoprotein A1 (APOA1), Aquaporin-1 (AQP1), Carbonic anhydrase 1 (CA1), Carbonic anhydrase 2 (CA2), Calumenin (CALU), Scavenger receptor cysteine-rich type 1 protein M130 (CD163), Cytochrome b5 reductase 3 (CYB5R3), Hemoglobin subunit beta (HBB), Hemoglobin subunit delta (HBD), Hemoglobin subunit mu (HBM), Hemoglobin subunit zeta (HBZ), Hemoglobin subunit alpha (HBA1), Hemoglobin subunit alpha 2 (HBA2), Hemoglobin Subunit Gamma 1 (HBG1), Hemoglobin subunit gamma-2 (HBG2), haptoglobin (HP), Haptoglobin-related protein (HPR), Hemopexin (HPX), and Rh-associated glycoprotein (RHAG) in the male human body (Figure 7A). These genes are involved in co-expression with the HbA1 gene in terms of diabetes and cancer (Figure 7B). Among the aforementioned genes, the HbA1 gene only regulates the

APOA1, CD163, HbB, HP, and HPX genes that are accountable to form the vesicle in the human biological system (Figure 7C). On the other hand, CYB5R3, HbB, HbD, HbM, HbZ, HbG1, and HP genes are involved in antioxidant regulation and expression with the help of the HbA1 gene (Figure 7D). Similarly, genes AQP1, HbA1, HbB, HP (Figure 7E) and HbA1, HbB, HbD, HbZ, HbM, HbG1, and HbG2 (Figure 7F) are involved in oxygen species' metabolic regulation and gene-mediated response to the reactive oxygen molecules in the metabolic pathway respectively.

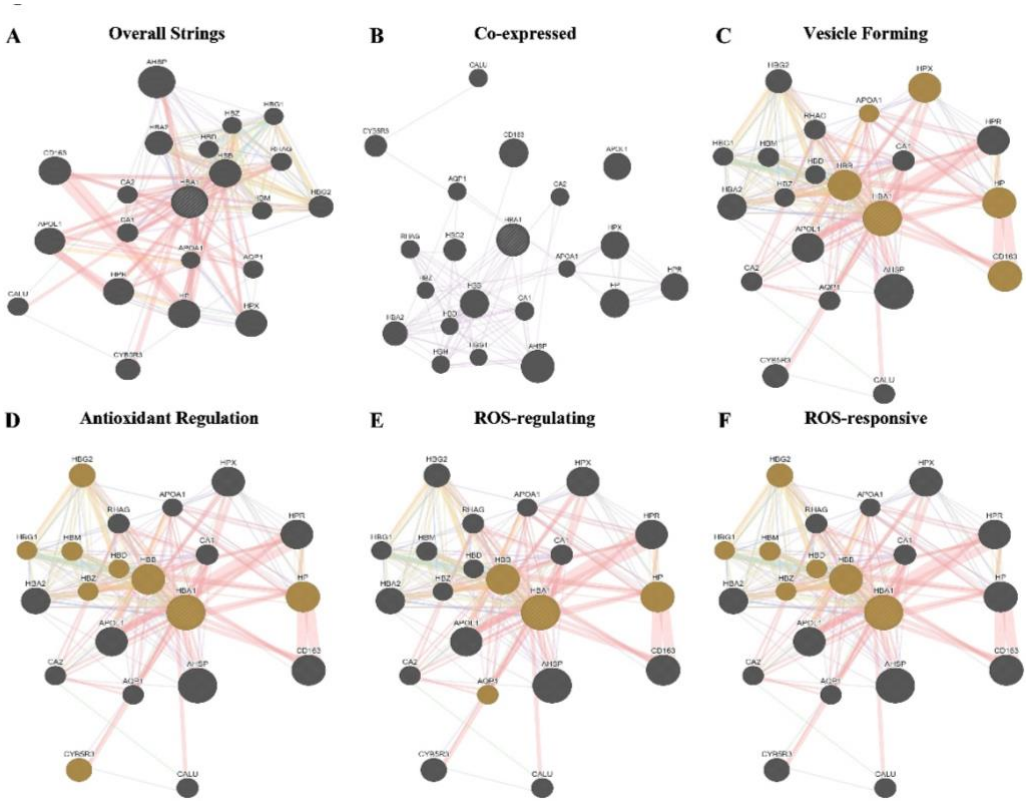

Figure 7. Analysis of the HBA1-mediated gene-gene interaction (GGI) inside the complicated cellular systems in human. Parameters like overall GGI regulated by the HbA1 gene (A); HbA1 genetic co-expression with diabetes and cancer gene (B); gene involved in vesicle formation (C); Antioxidant regulation (D); oxygen species metabolic regulation (E); and gene-mediated response to the reactive oxygen molecules (F) were considered for a comprehensive study. Different protein-coding genes are represented including Alpha-hemoglobin-stabilizing protein (AHSP); Apolipoprotein A1 (APOA1), Aquaporin-1 (AQP1), Carbonic anhydrase 1 (CA1), Carbonic anhydrase 2 (CA2), Calumenin (CALU), Scavenger receptor cysteine-rich type 1 protein M130 (CD163), Cytochrome b5 reductase 3 (CYB5R3), Hemoglobin subunit beta (HBB), Hemoglobin subunit delta (HBD), Hemoglobin subunit mu (HBM), Hemoglobin subunit zeta (HBZ), Hemoglobin subunit alpha (HBA1), Hemoglobin subunit alpha 2 (HBA2), Hemoglobin Subunit Gamma 1 (HBG1), Hemoglobin subunit gamma-2 (HBG2), haptoglobin (HP), Haptoglobin-related protein (HPR), Hemopexin (HPX), and Rh-associated glycoprotein (RHAG) coding genes.

HbA1-mediated clustering of operons involved in cancer

It's notified that different genes are involved with varying modes of action which help in triggering the HbA1 gene overexpression (Figure 7). These genes prepare nine proteins, namely AHSP, HbA1, HbA2, HbB, HbD, HbE1, HbG2, RPS12, and RPS19 in human nature. According to the findings, the HbA1 gene is strongly interconnected with AHSP and HbA2 genes based on the relying factors- database annotation (DA) and combined score (CS) than other factors such as experimentally determined interaction (EI); co-expression (CE); phylogenetic co-occurrence (PC); neighborhood on

the chromosome (NC); gene fusion (GF); homology (H); and the automated text mining (AT) resulted in this study.

DISCUSSION

The findings in this research indicate that sustaining a normal blood glucose level requires a modest BMI. In comparison to the values of different parameters between the T2DM patients and control groups, it becomes even more statistically significant and precise as seen in previous studies [18]. Increased BMI has been associated with several common and uncommon cancers in adults [36]. Multiple forms of cancer, including oral, esophageal, colorectal, biliary, hepatic, lung, breast, and thyroid, were strongly associated with FBS, BMI, and type 2 diabetes mellitus [21].

Depending on the HbA1c values, the contribution of a certain FBS and THABF number in diabetic patients varies. After serological profiling in this study, it can be seen that a typical level of HbA1c correlates to atypical levels of FBS and THABF in patients with T2D as opposed to normal individuals. Moreover, there is a significant correlation between serum creatinine and HbA1c in both the patient and control groups. Hyperglycemia causes protein glycation, which can lead to the development of damaging advanced glycation end products and oxidative stress in T2D patients [37]. Thus, it can be concluded that all three parameters shown are substantially influenced by the HbA1c percentage directly.

In this current research, 5.11-15.8% concentration of HbA1c in patients and 3.8-5.8% concentration of HbA1c in controls, the values of patient's serum cholesterol were found to be in a slightly elevated range (150.01-249.03 mg/dl) than that of the controls (158-183 mg/dL). However, it is not statistically significant, supporting the findings of Alzahrani and his team [38]. But when STG, LDLC, and HDLC of the patients and the controls were compared respectively, all three of them possessed higher significance ($p < .0001$) based on HbA1c concentration in both groups with 10-197 mg/dL for STGs; 46-127 mg/dL for HDLC and 23-149 mg/dL for LDLC. This positive association is similar to a previous study that demonstrated the significant association of TG, LDLC, and HDLC in comparison to HbA1c [39].

Based on the findings, there is a significant correlation between where the HbA1 gene is upregulated with selective tumor types (Figure 4A), such as BLCA tumors. In contrast, the HbA1 gene downregulates in BRCA, PAAD, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD tumor types (Figure 4B). The downregulation of HbA1c in BRCA is corroborated by previous studies [40].

According to our findings, the HbA1 transcript per million (tpm) value for four normal individuals was considered and found to be approximately 600. In contrast, in the case of 178 patients suffering from the primary tumor of selective cancers, the gene showed downregulation than the healthy persons (Figure 5A) with only a value of around 20 TPM. While the tumor was in stage 1, it exhibited good prognostic results, but it deteriorated as the cancer progressed in subsequent stages (Figure 5B), which were also reported by others as well [41]. Our study revealed that the HbA1 gene continues downregulating as the cancer stage progresses compared to normal individuals. So, all stages of cancer were found to have apparent downregulation. In this research, a regional expression of the HbA1 transcript per million (TPM) value was considered for the Caucasian, African-American, and Asian people. In the case of 156 Caucasians, the value was around 20 TPM. At the same time, it was even less in the 6 African-American and the 11 Asians (Figure 5C). Furthermore, gender specifically we observed in this

analysis that both 97 males and 80 females with HbA1 (TPM) were downregulated contrary to the normal individuals (Figure 5D). It revealed that in the four healthy subjects, the HbA1 transcript per million was around 600. But in patients with cancers in all age groups, it was downregulated. Though in the case of the three younger people aged (21-40) years TPM was around 35, while in older people (81-100), it was much less (Figure 5E). Similarly, surveying nodal metastasis, it was found that, the HbA1 value decreased as nodal stages (N0, N1, and so on) advanced (Figure 5F). Mutations in the p53 gene and its isoforms have resulted in more aggressive cancers of all types with an earlier onset. Research revealed p53 to be mutated in 75% of pancreatic adenocarcinoma patients [42]. While considering this, the value of HbA1 in disease-free individuals resulted to be approximately 1050 TPM, about ten TPM in patients with mutated p53, and about 30 TPM in patients with pancreatic cancer with no mutated p53 gene (Figure 5G). Thus, it was evident that the p53 mutated patients showed the lowest expression of the HbA1 gene compared to the other two groups. Besides, the analysis revealed that, in the case of 12 patients with T2D and chronic pancreatitis, the HbA1 gene showed significant downregulation than the remaining 131 individuals consisting of both normal subjects and patients with T2D without chronic pancreatitis (Figure 5H).

Considering the T2D status of the patients, HbA1 showed drastic overexpression in patients suffering from both T2DM and breast, lung, pancreatic and colorectal cancers (about 35 TPM) rather than that in normal people or patients suffering from cancers without T2DM (Figure 5I). Hence, after evaluating the influence of all these factors on the HbA1 gene in different types of cancer patients, it can be concluded that the gene was downregulated in all these circumstances and exhibits higher viable expression in healthy subjects (Figure 5I). Other studies also supported the association of HbA1c with breast and colorectal cancer [11].

The oncoinformatic analysis based on sample types (Figure 6A) revealed that the presence of methylated HbA1 promoter (p) decreases with the increase in the stages of cancer (stage-1 to stage-4), which means the preliminary stage of cancer can be detected by hypermethylation of the HbA1 promoter (Figure 6B), also obtained from many previous studies [43]. Asian people, including the Bangladeshi population, are prone to cancers in terms of HbA1 TPM frequency in their genome (Figure 6C). Regarding gender specificity, the amount of methylated promoter (p) was comparatively lower in female patients than in normal patients due to hormonal factors (Figure 6D), which is supported by previous studies [44]. Although the methylated HbA1 promoter (p) was found in all groups of ages, it was present in a lower amount in younger age groups (21-40 years) than in older ones (Figure 6E). It has been demonstrated in this research that methylated HbA1 promoter (p) plays a key role in both diabetes and eight types of cancers (BLCA, BRCA, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD) that are present in the early stage. It decreases with an increase in the metastatic stages of cancer. This methylation range decreased with the proliferation of tumor grades (Figure 6 F), which was also reported in other studies accordingly [25,27]. The hypermethylated HbA1 promoter was lower in the amount of the p53 mutated gene than the p53 non-mutated gene (Figure 6G) resulting in this research. Similarly, the effect of hypermethylation of the HbA1 promoter (p) was seen in nodal metastasis patients at a progressed stage than normal conditions (Figure 6H). Finally, the presence of hypermethylation of HbA1 promoter (p) was recorded at a higher amount among smokers than in non-smokers (Figure 6I). Similar findings regarding the relation of hypermethylation with the p53 mutated gene, nodal metastasis, and smokers were also reported by other researchers [45].

Considering the survivability status of the infected patients depending on the stages of cancer and reactivation of different oncogenes presented inside simultaneously, it can be reported that HbA1 expression has a significant influence on the survival of the patients. Patients with breast cancer and colon cancer showed HbA1 downregulation rapidly over a while compared with the disease-free patient. The present study revealed that patients with PAAD, LUAD, LUSC, PAAD, and PRAD have a low survivability rate in contrast to BLCA, BRCA, CHOL, and COAD patients. This association of HbA1 with PAAD has also been validated by several established previous studies [18].

According to the results, HbA1 is involved in expressing some genes, such as APOA1, AQP1, CA1, CA2, CD163, etc., involved in diabetes and certain cancers (Figure 7A-B). According to the researchers, a high concentration of APOA1 was found in vesicle formation among T2D patients and different cancers (Figure 7C) such as colorectal, lung, breast, and cancer patients [46]. According to the Cytoscape analysis, CYB5R3, HBB, HBD, HBM, HBZ, HBG1, and HP genes are involved in antioxidant activity (Figure 7D). In the presence of the HbA1 gene, the hemoglobin-binding ability of the antioxidant protein haptoglobin (HP) is altered, resulting in oxidative damage through the CD163 macrophage scavenger receptor (Figure 7E-F). Additionally, clinical research revealed that the HP gene polymorphism is a distinct risk factor for the development of diabetes [47]. As a biomarker for ovarian cancer with undetectable metastases, the Hemoglobin Subunits HbA and HbB with increased expression were found by other researchers [48].

Besides AHSP, HbB, HbD, HbA1, HbA2, HbG2, and HbE1, it has been discovered that ribosomal proteins, including RPS12 and RPS-19, regulate the onset of malignant transformation primarily via interaction with p53. This study revealed HbA1 is an important triggering factor that is involved directly and indirectly with diabetes and eight different carcinomas due to the modification of the genes mentioned above in our findings. It's reported to have compromised immune response and opsonization to the pathogenic interferences on progressed metabolic anomalies among T2D patients [49-50]. Different beneficial microbial interventions in the metabolic cycles can significantly regulate the HbA1c and insulin issues simultaneously as reported [51,52].

Since many advanced techniques and treatments are used for the detection and prevention of cancer, there is still lacking recognition of the specific biomarker which is the risk factor for the development of both type-2 diabetes and cancer [49]. According to our findings, the HbA1 gene can help to reduce the risk of different cancers in T2D patients which aids in the clinical understanding of cancer stages as well as serological parameters in T2D patients. A clinical physician can easily identify and dissect cancer risk screening in male chronic T2D patients. Besides, reactive oxygen species (ROS) mediated cancer formation and consecutive progress amid T2D patients have become a matter of greater concern among researchers globally where a range of metabolic parameters are considered to profile the cancer status [53].

CONCLUSION

This study comprehensively exposed a relationship between the serum levels of glycated hemoglobin (HbA1c) of male type 2 diabetes (T2D) patients and their potential risk of developing BLCA, BRCA, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD cancer. Oncoinformatic analysis was also carried out to elucidate the role (if any) of HbA1 gene regulation on the development of specific carcinoma. BMI and FBS were significantly correlated in both the control and patient groups. In response to the

concentrations of HbA1c, each of the selective serological parameters mean- FBS, THABF, creatinine, cholesterol, STGs, HDLC, and LDLC resulted in ardent correlations individually. Besides, the downregulation of the HbA1 gene and the increased level of promoter methylation were observed among the male T2D patients. The results also revealed that the oncoinformatic parameters could strongly be provoked by excessive alcohol intake and smoking to generate diversified tumors. Considering all the facts, the HbA1 gene and HbA1c protein can be introduced as genetic and serological biomarkers for profiling the risks of different cancers in men T2D patients.

The authors faced some issues throughout the study, such as sample size was supposed to be more than the number of participants. In addition, there were some control group individuals whose FBS was tested following morning walk and/or, even fasting times. Consequently, some FBS concentrations fluctuated more randomly than usual, though clinically and circumstantially the conditions were perfect. Many patients were found to have different pre-existed and chronic disorders whose serological and oncoinformatic parameters fluctuated more randomly than usual. At the same time, taking samples from some recently-antibiotic-taking individuals among a few T2D patients was a dilemma for the authors at sampling and testing.

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AUTHOR CONTRIBUTIONS

Supervision: SA. Co-supervision: MARM. Conceptualization: MAH, HMIA, MARM. Administration and methodology: SS. Resources: SI, AM, SS. Data curation: SR, AAK, FHR. Original draft preparation: ASP, PB, HRS, FHR, RNZ, MS. Virtual screening: PB. Scrutinization: MARM, MUH. Software/tools: MS, MUH. Statistical Data Analysis and Consultation: MUH. Correspondence: SA.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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