doi:10.20944/preprints202208.0042.v1

Article

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: This research aimed to figure out the applications of HbA1c protein and HbA1 gene as the prognostic biomarkers for assessing the risks of different cancers among male T2D patients in Bangladesh considering their serological and oncoinformatic parameters. Depending on the concentrations of HbA1c (%) of the T2D patients (n=300), their individual FBS (mmol/L); THABF (mmol/L); creatinine (mg/dl); SC (mg/dl); STGs (mg/dl); HDLC (mg/dl); and LDLC (mg/dl) were estimated. The values of the patients were compared with the control (n=60) group as the serological analysis. Besides, HbA1 gene (encoding hBA1c protein) 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 parameters based on the sample types; caner stages; racial footprints; gender; age; nodal metastasis; p53 methylations; pancreatitis; diabetes status; smoking behaviors; and overall/disease-free survivability. Finally, the 'HbA1 gene strings' responsible for genetic coexpression; endophytic vesicle regulation; antioxidant regulation; oxygen species metabolic regulation; and gene-mediated response to the reactive oxygen molecules were studied comprehensively. A strong correlation between BMI and FBS was observed in both the patients and the control ($P<0.0001$). Similarly, the values of FBS, THABF, and creatinine resulted in equal significance ($P<0.0001$) as compared to the HbA1c concentrations of all the T2D and control individuals. The SC, STGs, HDLC, and LDLC concentrations regulated ardently in both the control ($P<0.0001$), and patients group ($P<0.0001$), while HbA1c ranged from 3.8-5.8%, and 5.11-15.8% respectively. HbA1 gene is found downregulating with cancer progressed in most of the oncoinformatic parameters. According to the DA, CS, EI, CE, PC, NC, GF, H, and AT profiles; the HbA1 gene interacts with 8 other genes responsible for creating a protein cluster comprising- AHSP, HBA1, HBA2, HBB, HBD, HBE1, HBG2, RPS12, and RPS19 proteins for cancer formation. To recapitulate, HbA1c protein and HbA1 gene can be used as the prognostic serological and molecular biomarkers respectively for determining the risks of cancers among male chronic T2D patients.

Keywords: HbA1c biomarker; type 2 diabetes; cancer risk profiling; serological diagnosis; oncoinformatic screening; genetic overexpression; promoter methylation

1. 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]. A variety of lifestyle variables have been 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 adult people 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]. Surprisingly, a high level of HbA1c in diabetes patients are correlated with an impaired fasting glucose level that was observed in pancreatic carcinoma patients [7]. It has been clinically reported to have T2D and elevated FBS present in around 85% of patients with pancreatic cancer at diagnosis [8]. Another researcher also reported that increased HbA1c levels are connected with the occurrence of pancreatic cysts [9].

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 [10]. T2D is diagnosed using a variety of serological markers, the most prevalent of which is glycated hemoglobin (HbA1c), which is an estimated average of three months' blood glucose concentrations [11]. Dyslipidemia occurs due to abnormalities of lipid profile such as increased serum triglyceride, low-density lipoprotein- cholesterol, and decreased high-density lipoprotein cholesterol. Dyslipidemia is also linked to T2D as both insulin shortage and insulin resistance lead to impaired lipid metabolic pathways [12]. Changes in total cholesterol are associated with an increased risk of developing T2D, regardless of whether or not anti-hyperlipidemic medications are used [13]. Research suggests that lower levels of adiponectin are linked to insulin resistance, which influences the development of hyperlipidemia in T2D, and adiponectin is positively linked to triglycerides [14]. Renal function is also affected due to abnormal blood creatinine levels which are again linked to an increased risk in T2D patients [15]. An increase in the BMI over the normal weight range can also increase the risk of T2D. Men possess a larger risk of these problems than women for BMI issues [16]. Diabetes is also diagnosed with higher fasting blood glucose levels and impaired oral glucose tolerance testing [17]. The concentration of HbA1c often randomly fluctuates, and many research findings have established a link between diabetes and pancreatic adenocarcinoma in terms of HbA1c concentration [18]. 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 both were higher in males than in females [19].

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 characterize type 2 diabetes, which is a multifactorial disease [20]. Epigenetic variables, such as DNA methylation and histone changes, may have an impact on the etiology of type 2 diabetes, according to the studies [21]. 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. Patients with T2D face a risk of experiencing serious, potentially fatal consequences, which would entail more frequent medical attention and lower quality of life. It is necessary to take steps to find undiagnosed diabetes and prevent it in people who are at high risk. More recent research indicates that changes in DNA methylation may even more significantly contribute to the risk of T2D than genetic variability and may be a useful indicator of T2D risk [22]. In many human cancers, including bladder cancer, abnormal promoter methylation (also known as hypermethylation) is a key mechanism for suppressing tumor suppressor genes and other cancer-associated genes. DNA is an effective noninvasive strategy for identifying bladder cancer [23]. Hope et al. (2016) carried out a systematic review to assess the relationship between HbA1c and cancer in people with or without diabetes [24]. A total of 19 studies from 1006 met the inclusion criteria, of which 5 were nested case-control studies and 14 were cohort studies. 8 studies examined the results of all cancer locations, and according to 4 of these studies, all cancers had greater incidence and/or mortality rates when HbA1c levels were higher. One study concluded that there is a U-shaped correlation between cancer incidence & death and HbA1c. The risk of developing colorectal, pancreatic, pulmonary, and female genital tract malignancies increased with rising HbA1c levels. In the case of gastrointestinal, urological, or breast cancers, no higher risk was seen.

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) [2,25-27]. Some other studies reported HbA1c as a biomarker for chronic hyperglycemia and if the association of cancer risks elevates with Hba1c independent of diabetes, people with diabetes have higher chances of developing numerous different cancer forms. Common features of the two disorders include inflammation, changed hormone concentrations, hyperglycemia, hyperinsulinemia, and hyperglycemia itself. The information shows that, 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 [28]. In their findings, the results vary depending on the risk measure, the chance of developing colorectal, gastric, pancreatic, and respiratory malignancies is already higher in the pre-diabetic and normal ranges. Cancer incidence is increased for colorectal, gastric, pancreatic, breast, and liver cancers when HbA1c levels are in the diabetic range.

In light of the aforementioned facts, the present research aimed to figure out the correlation of HbA1c with the risk of common eight 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 our selective cancers formation, so that HbA1c can be utilized as a functional biomarker for the risk profiling of both the T2D and different types of carcinoma of the male patients in Bangladesh.

2. Materials and methods

2.1. Selection of patients for categorizing

In this research, a total of 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 [29].

2.2. Serological assays

To assess the status of type 2 diabetes in the patients, several specific quantitative serological profiling was conducted, considering- hemoglobin A1c (%) [30]; fasting blood sugar (mmol/L) [31]; two hours after breakfast (mmol/L) [32]; serum creatinine (mg/dL) [33]; cholesterol of serum (mg/dL) [34]; serum triglycerides [35]; HDLC-LDLC (mg/dL) [36]; and BMI (kg/m^2) [37]. Depending on the concentration of the HbA1c biomarker [38], all the other parameters were correlated with it individually. The measurement of the serum HbA1c concentration was accomplished following the finger stick blood collection process, where the sophisticated D-10 HPLC analyzer system was operated with the recommended protocol [39]. The fasting blood glucose (FBS) and two hours after breakfast (THABF) were tested using a continuous glucose monitoring system (CGMS®) [40]. The serum creatinine, cholesterol, and triglycerides were assayed using- 'Creatinine Assay Kit', Sigma Aldrich® (Product No. MAK 080); 'Cholesterol/Cholesteryl Ester Kit II', Sigma Aldrich® (Product No. MAK 396); 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). Each of the samples of both the T2D patients and the control individuals was handled very carefully to ensure the most accurate results from the assays.

2.3. 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 [24]. 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 were 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 UL-CAN' (http://ualcan.path.uab.edu/index.html) [41]. The survivability assessment of the aforementioned cancer patients considering the expression of the HbA1 gene was established through 'GEPIA 2' (http://gepia2.cancer-pku.cn/#index), where both the overall and disease-free survivability were analyzed simultaneously. In this case, the survivability (%) of the aforementioned cancer patients was estimated following the HbA1 gene expression profiles from very low to very high scales in terms of times (months). 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 coexpression; 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/) [42] was used which functions scripting on JAVA (https://www.java.com/en/download/manual.jsp). The clustering of HbA1c protein due to the activity of the HbA1 gene was generated relying on the factors including- experimentally determined interaction (EI); database annotation (DA); combined score 9CS); co-expression (CE); phylogenetic co-occurrence (PC); neighborhood on the chromosome (NC); gene fusion 9GF); homology (H); and the automated textmining (AT), where Heatmapper and Morpheus tools (https://software.broadinstitute.org/morpheus/) were used [43].

 doi:10.20944/preprints202208.0042.v1

2.4. Software packages for data 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) [44-47]; and 'GraphPad Prism' (version 8.1.2, for Mac OS) premium software packages [48-54].

3. Results

3.1. The serological analysis

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 3.11 to 6.9 (mmol/L) in the control group (Figure 1B). Following the standard clinical range of the normal male individuals' BMI (18.5-24.9 Kg/m²) and FBS (3.9-6.9 mmol/L), the correlation between the two parameters is found significant for both groups ($p < 0.0001$ for both groups).

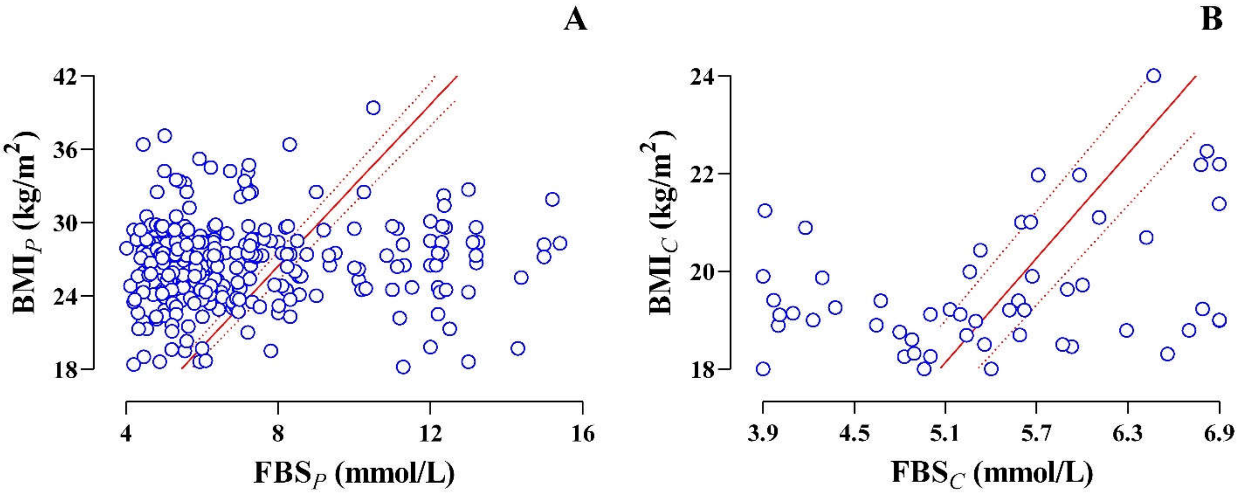

Figure 1. The correlation between BMI (Kg/m2) and FBG (mmol/L) among the patients' (A) and control group (B). In all the cases, a strong correlation was found for both the groups (p < 0.0001) on the scale of significance of α= 0.05. The linear regression line validates the ranges of BMI and FBS for each 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 was between 3.160-3.454 and that of the control group was between 3.389-3.724.

In response to the concentration of HbA1c (%), the resulted individual correlation of FBS (mmol/L), THABF (mmol/L), and serum creatinine (mg/dL) were phenomenally significant ($p < 0.0001$) for both the patients (Figure 2 A-C) and control groups (Figure 2 D-F) simultaneously. The range of HbA1c for the T2D patients ranged between 5.11 to 15.8% in relation to 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) were observed. In contrast, the limits of HbA1c for the control individuals was 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. The serological values are all significant in the scale of significance $\alpha = 0.05$.

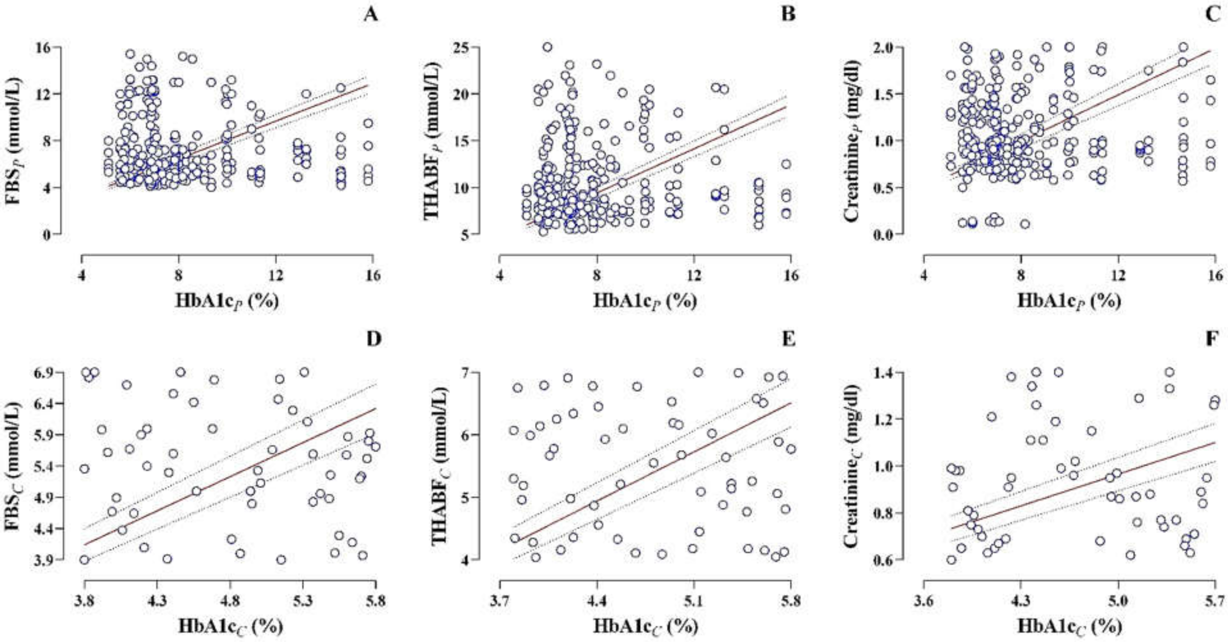

Figure 2. The correlations of HbA1c (%) with the patients' FBS (mmol/L) (A); THABF (mmol/L) (B) and creatinine (mg/dL) (C) in comparison to their corresponding control groups on the same parameters (D-F). In all the individual parameters of the patients and controls, significant correlations were observed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

The value of the patients' serum cholesterol (SC) was found 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 are all highly significant ($p < 0.0001$). Similar significant relationships were also found for the control group as ($p < 0.0001$), in which the concentrations encompassed 3.8-5.8% for HbA1c, 149.1-199.3 mg/dL for SC; 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).

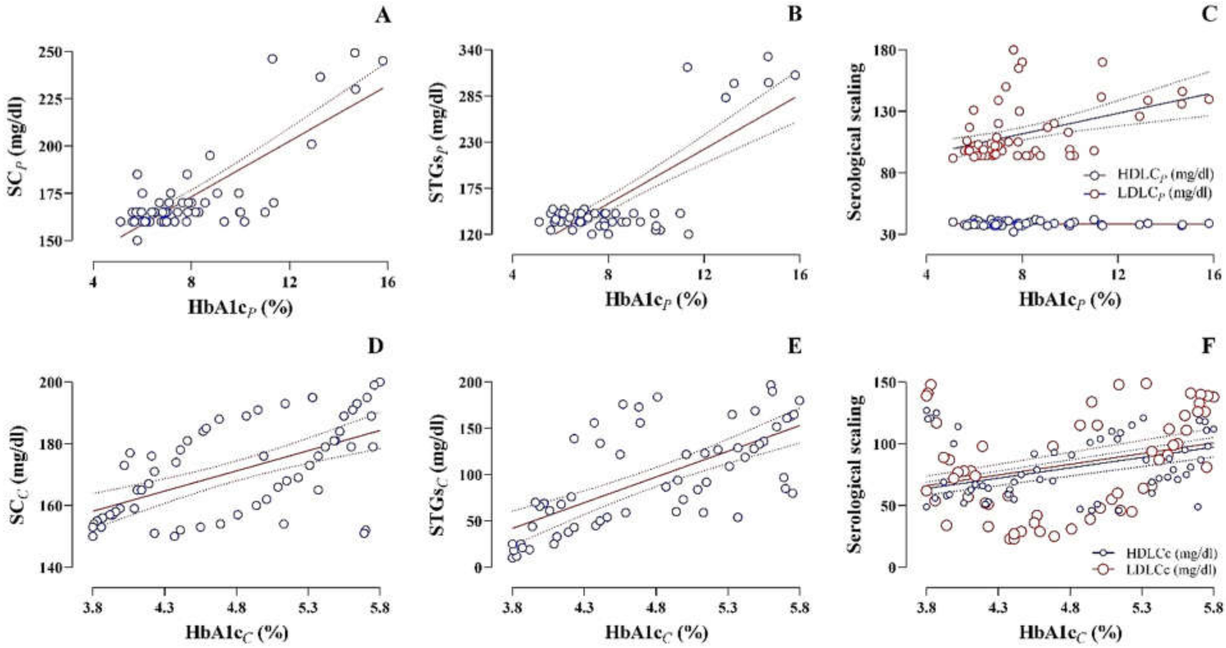

Figure 3. Diagrammatic presentation of the correlations of SC, STGs, HDLC, and LDLC in response to the HbA1c (%) individually for the T2D patients (A-C) and the control individuals (D-F), where the R square values were determined in addition to the SE and 95% CI values for all. The scale of significance was considered $\alpha = 0.05$.

4. The oncoinformatic analysis

4.1. Multifactorial overexpression of HbA1

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

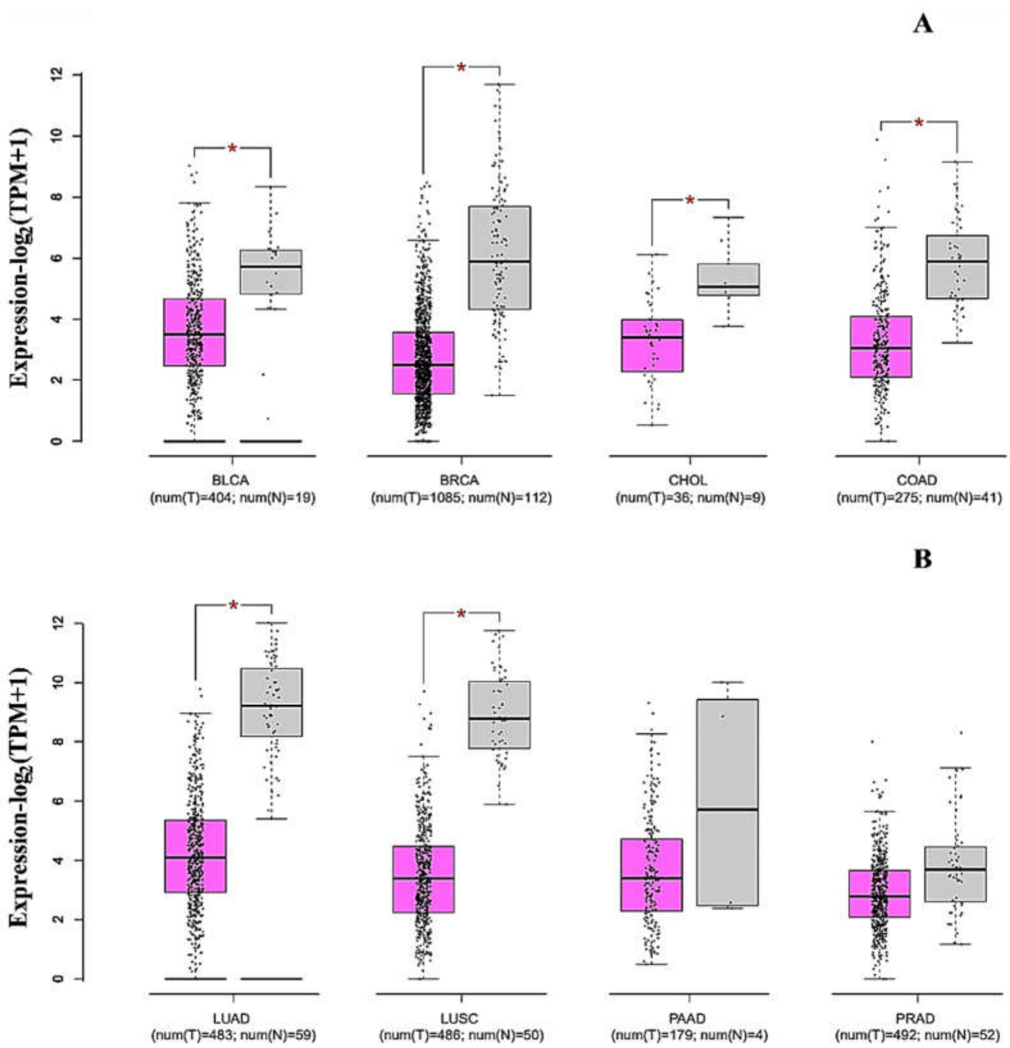

Figure 4. Comparative expression of the HbA1 gene in different cancer patients and normal individuals, where BLCA (Bladder urothelial carcinoma), BRCA (Breast invasive carcinoma), CHOL (cholangiocarcinoma tumor), COAD (colon adenocarcinoma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), PAAD (pancreatic adenocarcinoma), and PRAD (prostate adenocarcinoma) were considered.

In this study, the number of HbA1 transcripts per million into the host was calculated, through which the overexpression status was profiled. 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 normal 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).

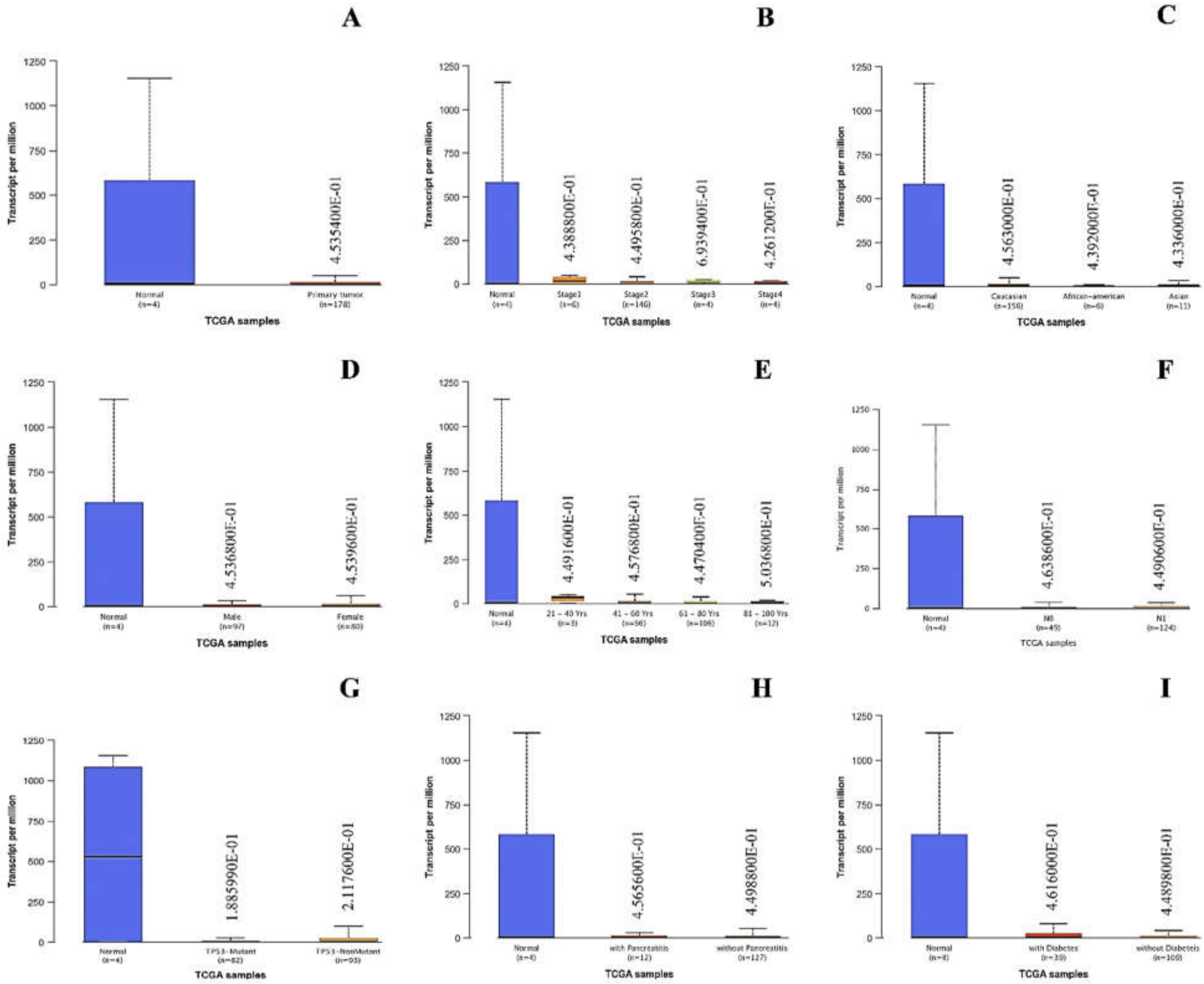

Figure 5. The genetic expression profiling of the HbA1 considers factors such as sample types used (A), individual cancer stages of patients (B), regional expression (C), gender specificity (D), age factor (E), nodal metastasis (F), level of the P53 mutation (G), pancreatitis status of the diabetes patients (H) and the diabetes status (T2D) (I).

4.2. Hypermethylation of HbA1 promoter for T2D and cancers

In the case of type 2 diabetes and mentioned cancers strong hypermethylation of the promoter region of the HbA1 gene has been observed in the present study (Figure 6). Primary tumor formation is regulated by the hypermethylation of the HbA1 promoter to normal conditions (Figure 6A). With the development of cancers, the methylation frequency drops down gradually among the patients (Figure 6B) as evaluated. 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 Afro-Americans (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 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 nonmutated 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). Last but not least, concerning smoking habits, smokers expressed a much higher methylation rate than non-smokers (Figure 6I).

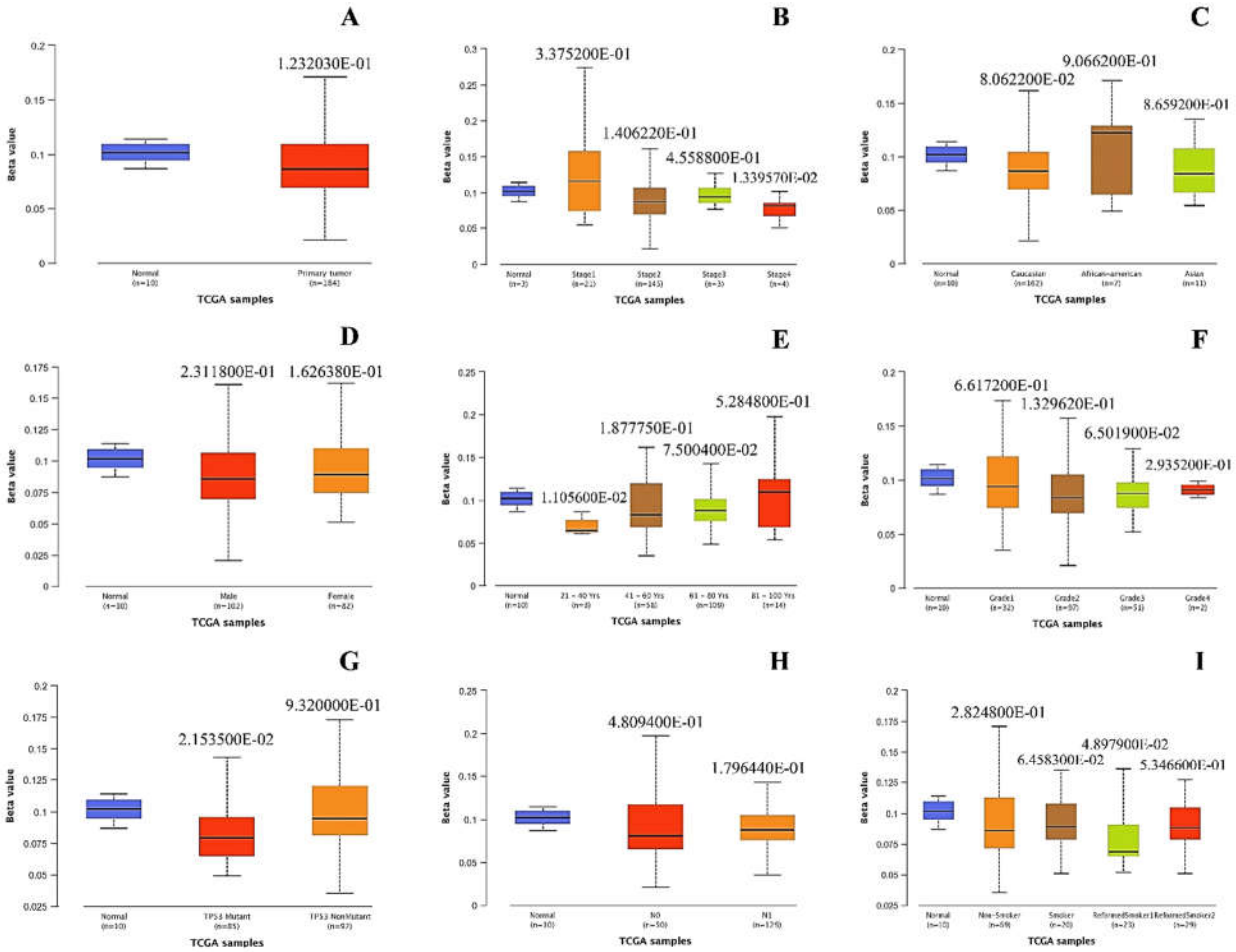

Figure 6. The promoter methylation profiles of the HbA1 gene (HbA1P*) in risk profiling the cancers among the T2D patients based on- cell types (A), cancer stages (B), regional expression (C), gender specificity (D), age factor (E), tumor grades (F), P53 expression (G), nodal metastasis (H) and smoking habits (I).

The percentage survival of disease-free patients and patients with BLCA, BRCA, CHOL, and COAD cancer have been illustrated (Figure 7A). Initially, the percentage survivability decreased in the patient compared to the disease-free patients, and over time when cancer exponentially grows, the percentage of low and high HBA1 down-regulates rapidly. On the other hand, patients having lung and pancreatic cancer (LUAD, LUSC, PAAD, and PRAD) showed more down-regulation of HBA1 compared with normal individuals (Figure 7B). From the results, it is evident that patients with pancreatic adenocarcinoma and lung cancer have the lowest survival rate compared to other types of carcinoma due to low HBA1 down-regulation.

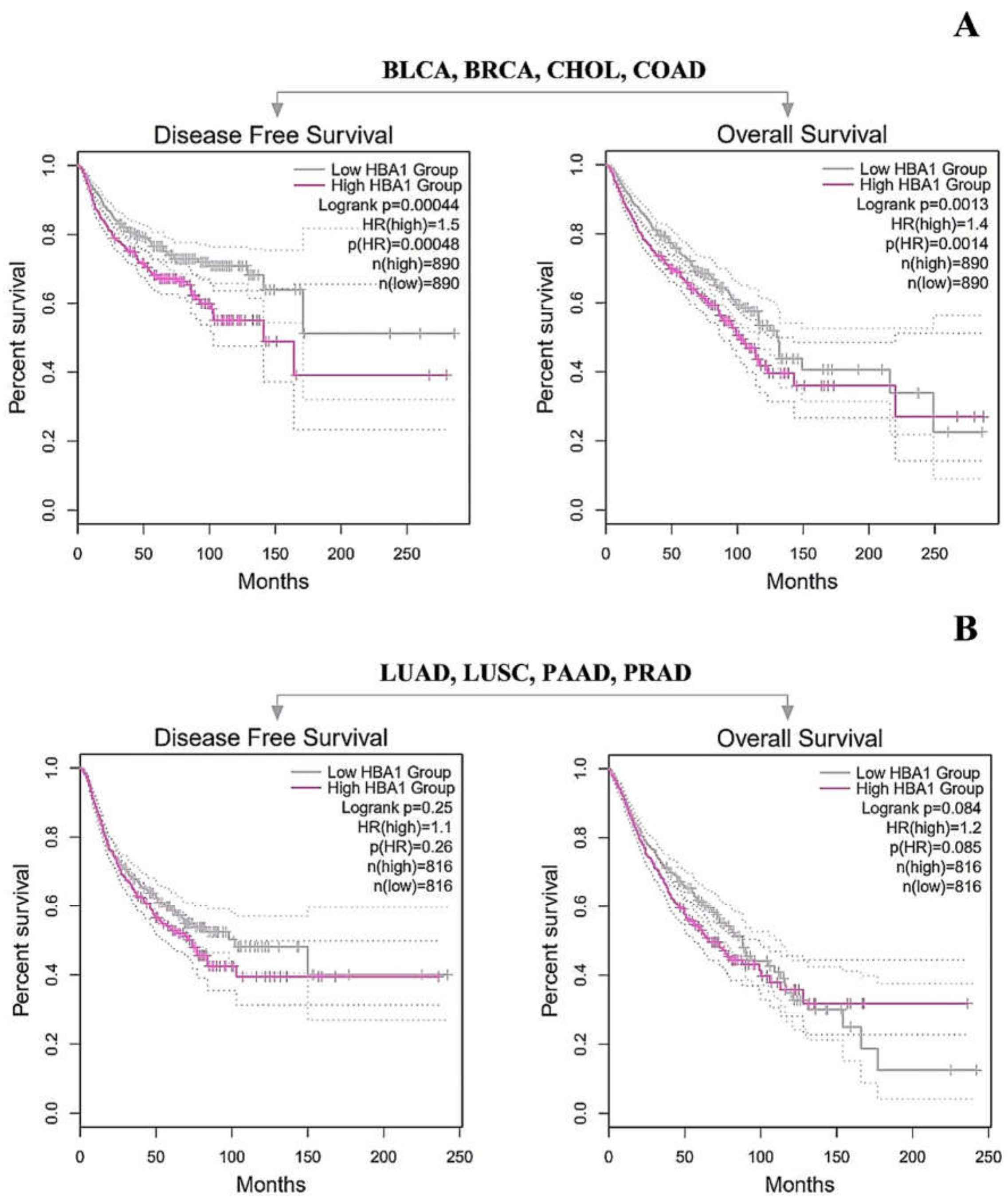

Figure 7. The HbA1 gene overexpression in the stimulation of the survival probability of the disease-free survival with overall survival of different cancers.

According to this research, The HbA1 gene is interact as a cluster or operon with the genes namely AHSP, APOA1, APOL1, AQP1, CA1, CA2, CALU, CD163, CYB5R3, HBB, HBD, HBM, HBZ, HBA2, HBG1, HBG2, HP, HPR, HPX and RHAG in the male human body (Figure 8A). These genes are involved in co-expression with the HBA1 gene in terms of diabetes and cancer (Figure 8B). 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 8C). 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. Similarly, genes AQP1, HBA1, HBB, HP (Figure 8E) and HBA1, HBB, HBD, HBZ, HBM, HBG1, and HBG2 (Figure 8F) are involved in oxygen species' metabolic regulation and gene-mediated response to the reactive oxygen molecules in the metabolic pathway respectively.

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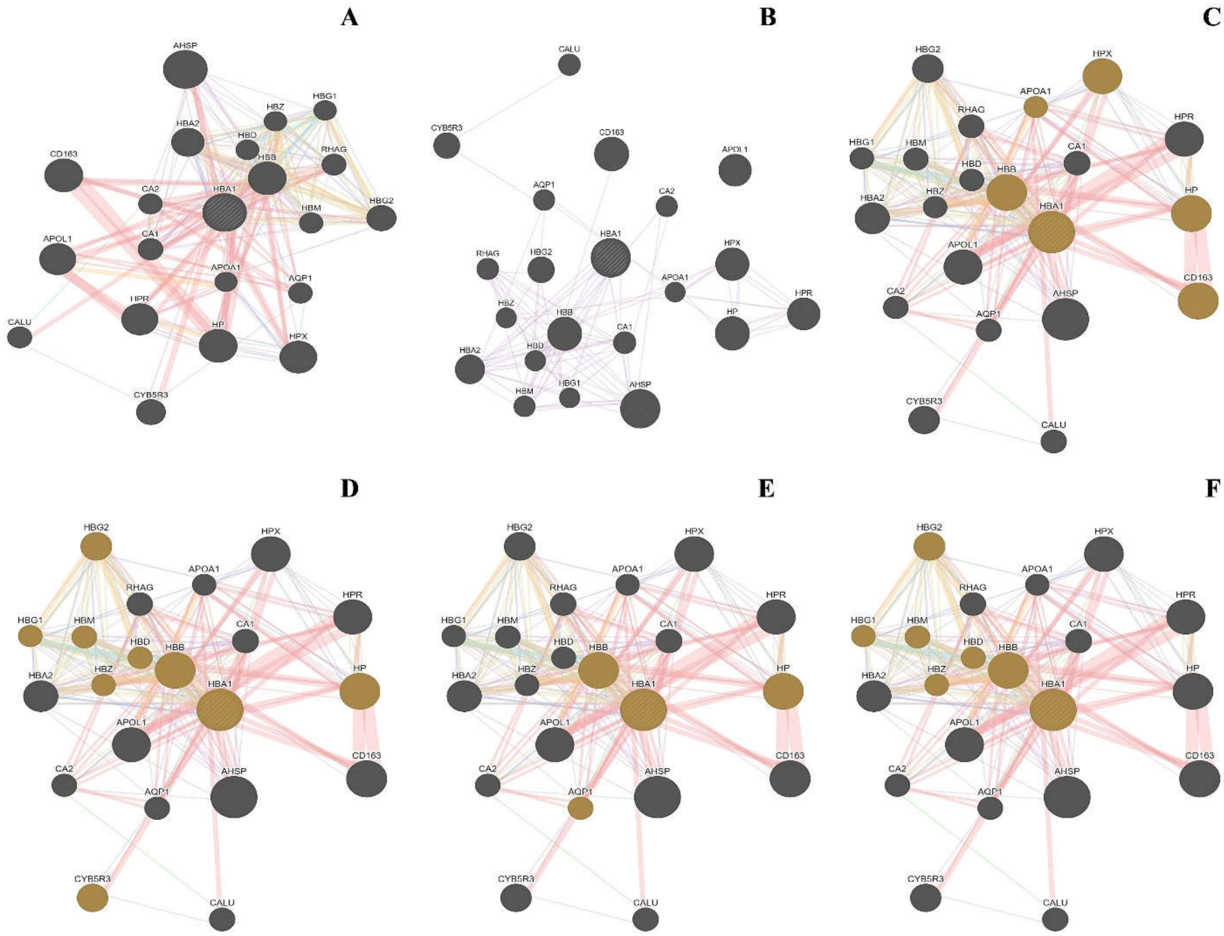

Figure 8. Illustration of the overall gene-gene interaction networks regulated by the HbA1 gene (A); HbA1 genetic co-expression with diabetes and cancer gene (B); gene involved in vesicle formation (C); Antioxidant regulation (D); oxygen species metabolic regulation (E); gene-mediated response to the reactive oxygen molecules (F). 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.

It's notified that the different genes are involved with different modes of action with help of triggering the HBA1 gene (Figure 8). These genes prepared nine proteins namely AHSP, HBA1, HBA2, HBB, HBD, HBE1, HBG2, RPS12, and RPS19 in human nature (Figure 9). 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 textmining (AT).

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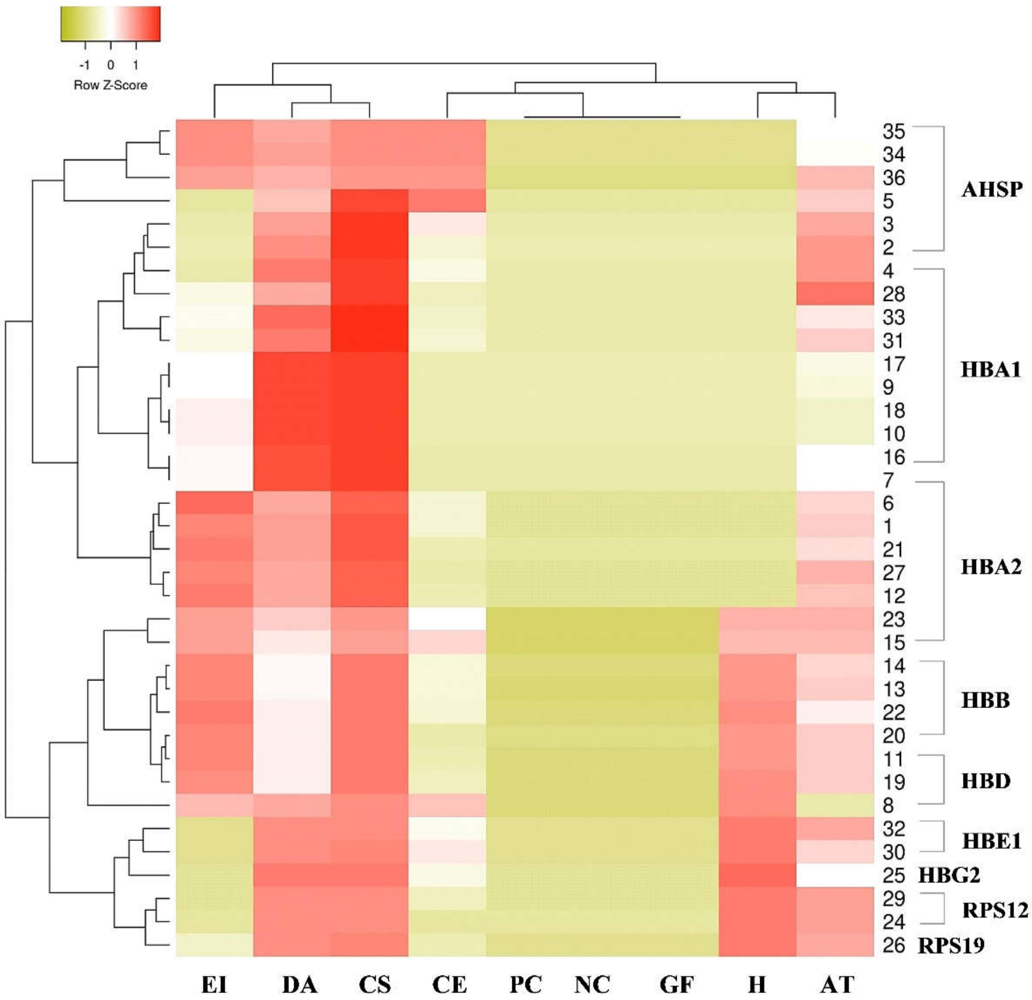

Figure 9. Heatmap analysis of 9 proteins that are regulated by the HbA1 gene.

5. Discussion

5.1. Analysis of the serological parameters

Regarding the levels of fasting blood sugar (FBS), there was a direct correlation between FBS and BMI which is confirmed once again in the present study. It can be concluded from the results that a low BMI is vital for maintaining a normal blood glucose level (Figure 1). In comparison to the values of different parameters between the T2DM patients and control groups, it becomes even more statistically significant and clear as seen in previous studies [18]. Increased BMI has been associated with several common and uncommon cancers in adults [55]. 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 [56]. FBS (Fasting blood sugar) has been the most common and known parameter when diagnosed with type 2 diabetes (T2D) and has effects on HbA1c levels in both patients (Figure 2A), and the control (Figure 2D). According to a study, the link between HbA1c and FBS is quite substantial, especially in diabetics [57]. Additionally, the THABF test is done along with FBS to indicate the management of diabetes. The correlation of fasting and postprandial hyperglycemia with HbA1c in patients with T2D was investigated among Japanese patients, resulting in a strong postprandial hyperglycemia correlation with HbA1c [58]. 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 atypical levels of HbA1c correlate to atypical levels of FBS and THABF in patients with T2D (Figure 2B) as opposed to normal individuals (Figure 2E). Similarly, there is a significant correlation between serum creatinine and HbA1c (Figure 2) in both the patient (Figure 2C) and control groups (Figure 2F). This indicates that urine microalbumin excretion rises with the increase of HbA1c. Hyperglycemia causes protein glycation, which can lead to the development of damaging advanced glycation end products and oxidative stress, which might be linked to early kidney function impairment and a higher urine albumin-to-creatinine ratio in T2D patients [59]. Levels of serum creatinine are also elevated along with the percent of HbA1c in patients (Figure 2C) in contrast to the control in (Figure 2F). Thus, it can be concluded that all three parameters are shown (Figure 2) are substantially influenced by the HbA1c percentage directly.

In regards to serum lipid profiles such as STG, LDLC, and HDLC, there was a positive correlation between HBA1C and these serum lipid profiles (Figure 3). With 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), though it is not statistically significant (Figure 3A and 3D), supporting the findings of [60]. 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 (Figure 3D); 46-127 mg/dL for HDLC (Figure 3C) and 23-149 mg/dL for LDLC (Figure 3F). This positive association is similar to a previous study that demonstrated the significant association of TG, LDLC, and HDLC in comparison to HbA1c [61].

5.2. Analysis of the oncoinformatic parameters

There was also a strong relationship between HbA1c, and pancreatic adenocarcinoma due to HbA1c's correlation with a group of genes such as HBA2, C14orf139, HBB, REM1, C20orf160, ECEL1, C1orf54, CCL3, IGF1, PTGDS, GNG11, C1QTNF1, GRASP, LMO2, IGFBP4, FOSB, MUSTN1, RAMP2, CTSG, RPL17, PLAC9, RBP5, MADCAM1, MGC29506, HSPC157, COPZ2, OSR1, DUSP1, GYPC, GMFG, PI16, and COX7A1, which are directly connected in forming PAAD in different extents. There is also a significant correlation between where the HbA1 gene is upregulated with selective tumor types, such as BLCA tumor. In contrast, the HbA1 gene downregulates in BRCA, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD tumor types (Figure 4). The downregulation of HbA1c in BRCA is corroborated by previous studies [62]. The association of HbA1c with PAAD has also been validated by several established previous studies [18].

The present study considered the effects of several factors such as the presence of primary tumors, individual cancer stages of patients, regional expression, gender specificity, age factor, nodal metastasis, level of the P^{53} mutation, pancreatitis status of the diabetes patients, and the diabetes status (T2D) in the regulation of the HbA1 gene among the patients suffering from eight different types of cancers (BLCA, BRCA, CHOL, COAD, LUAD, LUSC, PAAD, and PRAD) using the 'TCGA Gene Analysis Interface- ULCAN' for comprehensive data analysis. Firstly, 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 with only a value of around 20 *tpm* (Figure 5A). In any case of cancer, staging, and grading were done to see the tumor's extent, progression, and prognosis. While the tumor was in stage 1, it exhibited good prognostic results but it deteriorates as cancer progresses in subsequent stages which were also reported by others as well [63]. Our study revealed that the HbA1 gene continues to downregulate as the cancer stage progresses when compared to normal individuals. So, all stages of cancer were found apparent downregulation like another researcher's findings [24]. The highest HbA1 transcript per million (tpm) values were present in stage 1 rather than in stage 4 (Figure 5B). In this research, a regional expression of the HbA1 transcript per million (*tpm*) value was considered for the Caucasian, African-American, and Asian people. The value for four normal individuals was found to be around 600 (*tpm*). In the case of 156 Caucasians, the value was around 20 *tpm* while it was even less in the 6 African-Americans and the 11 Asians (Figure 5C). Furthermore, gender specifically we observed in this analysis that both in 97 males and 80 females HbA1 (*tpm)* was downregulated contrary to the normal individuals (Figure 5D). When taking the notice of the age factor, our study found similar instances as above and revealed that in the four healthy subjects 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 3 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, we found that the HbA1 value decreases as nodal stages (N0, N1, and so on) advance, but it's higher in people without the disease (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 [64]. While considering this, our analysis revealed the value of HbA1 in disease-free individuals to be approximately 1050 *tpm*; about 10 *tpm* in patients with mutated p53, and about 30 *tpm* in patients with pancreatic cancer with no mutated p53 gene. Thus, it was evident that the P53 mutated patients showed the least expression of the HbA1 gene when compared to the other two groups (Figure 5G). Since a meta-analysis of chronic pancreatitis was identified as a risk factor for pancreatic cancer [65], this study took patients suffering from T2DM and pancreatitis into consideration as a factor to see the effect of these diseases on the HbA1 gene. 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. Other studies also supported the association of HbA1c with breast and colorectal cancer [62,66].

According to the global data analysis reports from 'TCGA gene analysis interface-ULCAN' (Figure 6), the presence of hypermethylation of HbA1 promoter (*p*) was observed at a lower amount in a primary tumor cell than in a normal cell (Figure 6A). According to oncoinformatic reports, 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 [67]. Asian people including the Bangladeshi population are prone to cancers in terms of HbA1 transcript per million (*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 [68]. 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 presents in the early stage and it decreases with an increase in the metastatic stages of cancer. This methylation range decreased with the proliferation of tumor grades (Figure 6F), which was also reported in other studies accordingly [2,25,27,69]. It was observed in this current study that the hypermethylated HbA1 promoter was lower in the amount of the P53 mutated gene than the P53 non-mutated gene (Figure 6G). 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

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the P53 mutated gene, nodal metastasis, and smokers were also reported by other researchers [70-72].

T2D is often found in people who have pancreatic cancer, it is thought to be a risk factor for the disease [73]. A strong correlation was observed between the HbA1 macromolecule and various types of cancers (Figure 7). The analysis revealed that patients with breast cancer and colon cancer (BLCA, BRCA, CHOL, and COAD) showed HBA1 downregulation rapidly over a while compared with the disease-free patient (Figure 7A). An increased level of HbA1 can be identified as a risk factor to aid in the diagnosis of pancreatic cancer in such T2D patients [74]. It is observed that patients with lung and pancreatic cancer (LUAD, LUSC, PAAD, PRAD) also down-regulated the HBA1 than the healthy patient but the down-regulation of low HBA1 is more than the down-regulation of high HBA1 (Figure 7B). The present study also showed that patients with 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]. HBA1 gene continues to downregulate as the cancer stage progresses as in comparison to normal individuals, PAAD in all stages shows apparent down-regulation. Hence, after evaluating the influence of all these factors on the HBA1 gene in PAAD patients, it can be concluded that the gene is downregulated in all these circumstances.

According to figure 8, HBA1 is involved to express some genes such as APOA1, AQP1, CA1, CA2, CD163, etc. that are involved in diabetes and certain cancers. According to the researchers, a high concentration of APOA1 was found in T2D patients and different cancers such as colorectal, lung, breast, and cancer patients [75-77]. Another gene CD163 was also found in cancer and diabetes patients when somehow increased the expression of this gene [78,79]. Some genes are prime responsible for oxidative stress that play a role to induce diabetes and cancer. A higher expression of HBA1 and HBB genes was found in cervical cancer than in normal healthy patients using the qRT-PCR assay [80]. According to the Cytoscape analysis, CYB5R3, HBB, HBD, HBM, HBZ, HBG1, and HP genes are involved in antioxidant activity. 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. Additionally, clinical research revealed that the HP gene polymorphism is a distinct risk factor for the development of diabetes [81]. As a biomarker for ovarian cancer with undetectable metastases, the Hemoglobin Subunits HBA and HBB with increased expression were found by other researchers [82]. Based on figure-9, 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 [83,84]. In our research, it was found that HBA1 is an important triggering factor that is involved directly and indirectly with diabetes and eight different carcinomas due to the modification of the aforementioned genes in our findings.

6. Conclusion

This study tried to establish 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 found to be significantly correlated in both the control and patient group. 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 along with the increased level of promoter methylation was observed among the male T2D patients. The results also revealed that the oncoinformatic parameters can strongly be provoked by excessive alcohol intake and smoking to generate diversified tumors. Considering all the facts, the HbA1 gene and HbA1c protein

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can be introduced as genetic and serological biomarkers respectively for profiling the risks of T2D patients for different cancers in men.

Research ethics: The total research work was conducted under the Ethical Guidelines and Monitoring of Jashore Medical College (JMC), Bangladesh Medical and Dental Council (BMDC) in collaboration with the RPG Authority (Govt. Registration ID: 05-060-06021) under the Project 'Category C3; ID. #13-2021/22'. The endorsement of the patients and Ethical Committee criteria directly follows the Declaration of Helsinki and the Ministry of Health, Bangladesh.

Consent for publication: The authors are very cordial to publishing this manuscript and the consent is clear.

Availability of data and materials: All the necessary documents (individual patients' details; ethical approval certificates; project registration details; individual author's details; and so on) are carefully conserved by the authors. The documents will be shared to the journal authorities upon reasonable request by the Corresponding Author.

Competing interests : The authors are free from any types of competing intentions with others.

Funding information: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2020R1I1A2066868), the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (No. 2020R1A5A2019413).

Authors' contribution: Supervision: Bonglee Kim. Co-supervision: Md. Ataur Rahman. Conceptualization: Md. Al Hasibuzzaman, Salauddin Al Azad, Md. Abdur Rashid Mia. Administration and methodology: Salauddin Al Azad. Resources: Samarth Islam, Afsana Masud. Data curation: Salauddin Al Azad, Samiur Rahman, Arysha Alif Khan, Fahmida Hoque Rimti. Original draft preparation: Salauddin Al Azad, Ashrak Shad Pyash, Partha Biswas, Homayra Rahman Shoshi, Fariha Hoque Rimu, Ramiza Nausheen Zaman. Virtual screening: Partha Biswas. Scrutinization: Md. Abdur Rashid Mia. Software/tools: Salauddin Al Azad. Correspondence: Bonglee Kim

Acknowledgments: The authors are grateful to the 'RPG Organization' and the RPG Interface Lab Authorities for providing all types of technical and logistic support needed during this study. In addition, the contribution of Mr. Parag Kumar Paul (Dept. of Electrical & Electronic Engineering, United International University, Bangladesh) for the formatting, grammatical checking, and plagiarism checking of this manuscript was very effective.

List of abbreviations:

HbA1c (glycated hemoglobin); SC (serum cholesterol); FBS (fasting blood sugar); BMI (body mass index); THABF (two hours after breakfast); STGs (serum trgiglycerides); HDLC (high density lipoprotein cholesterol); LDLC (low density lipoprotein cholesterol); BLCA (bladder carcinoma); BRCA (breast cancer gene); CHOL (cholangiocarcinoma); COAD (colon adenocarcinoma); LUAD (lung adenocarcinoma); LUSC (lung squamous cell carcinoma); PAAD (pancreatic adenocarcinoma); PRAD (prostate adenocarcinoma); AHSP (Alpha Hemoglobin Stabilizing Protein); HBB (beta-globin); HBD (haemoglobin subunit delta); RPS (rhibosomal proteins)

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