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Comparing the Expression Levels of GCG and FBN-1 in the Plasma of Patients with Basal Cell Carcinoma (BCC) and Healthy Individuals

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ABSTRACT

Introduction: One of the most common types of skin cancer is basal cell carcinoma (BCC), which puts a big burden on the healthcare system. Direct and dermoscopic examinations are used to diagnose basal cell carcinoma. GCG is a protein-coding gene expressed in various cells throughout the body, including the small intestine, brain, and skin. Fibrillin-1 is an extracellular protein found in many body tissues. In this study, we compare the expression of GCG and FBN1 genes in the blood of patients with BCC and a healthy control group.

Materials and Methods: 1. Selection of patients and sampling. 2. Blood sampling of BCC patients and the control group. 3. Isolation of RNA from blood using an extraction kit. 4. Measurement of RNA concentration and purity. 5. cDNA synthesis and real-time PCR using a specific miRNA cDNA synthesis kit. 6. Statistical analysis

Results: The GCG biomarker was positive in 9 out of 15 patients in the group of patients with basal cell carcinoma (BCC). The rate of positivity for this biomarker in the group of healthy individuals was 4 out of 15, indicating a statistically significant difference between the two groups (P-value<0.001). The FBN1 biomarker was positive in 11 out of 15 patients with basal cell carcinoma (BCC). The rate of positivity for this biomarker in the group of healthy individuals was 5 out of 15 people, indicating a statistically significant difference between the two studied groups. (P-value<0.001).

Conclusion: The expression of GCG and FBN1 is significantly higher in patients with BCC compared to healthy individuals. Further studies can be done to ensure the role of these genes in the diagnosis of skin cancers.

Keywords: Basal cell carcinoma; Glucagon gene (GCC); Fibrillin-1 (FBN-1); MicroRNA.

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Introduction

Basal cell carcinoma (BCC), as one of the most prevalent types of skin cancer, is a global concern. BCC, along with squamous cell carcinoma (SCC), are parts of a bigger group of malignancies called non-melanoma skin cancer (NMSC) [1]. BCC appears in individuals of various age groups and primarily affects the sun-exposed parts of the body. BCC can drastically change patients' quality of life, although it rarely results in metastasis [2]. Considering the rising incidence of this disease, it is essential to consider the significant burden BCC places on economic factors and healthcare providers [1]. Many studies indicate that the Hedgehog (HH) pathway plays a crucial role in the pathogenesis of BC [2-5]. The HH pathway is mostly inactive in adults but can be activated in certain situations, such as tissue repair [6]. This pathway is also responsible for tissue patterning and managing skin stem cells [7]. The activation of the HH signaling pathway can also contribute to carcinogenesis in other malignancies—for example, prostatic and gastric cancers [8]. The inactivation of this pathway has therapeutic value in managing BCCs [4,5].

BCC diagnosis can be made using direct and dermoscopic examinations. However, a histopathological examination of the skin lesion is necessary to confirm the results. Distinct histologic features differentiate the various types of BCC, including superficial, morpheaform, infiltrative, fibroepithelial, and pigmented. As previously mentioned, BCCs seldom cause metastasis; therefore, properly managing the primary lesion will likely lead to a complete cure. The most effective therapy is surgical excision of the lesion with tumor-free margins. Radiation therapy is another method of use, but tumors that recur following radiation therapy are more likely to show aggressive behaviors [2].

MicroRNAs (miRNAs) are non-coding fragments of RNA that are composed of 17 to 23 nucleotides and play a prominent role in gene expression and carcinogenesis [9]. In 1993, Lee et al. discovered the first miRNA, lin-4. Lid-4 miRNA regulated the production of the related protein, lid-4 [10]. The role of miRNAs in the pathogenesis of cancer has been noticed since. In 2002, a study demonstrated that miRNAs are involved in the pathogenesis of chronic lymphocytic leukemia [11]. GCG is a protein-coding gene expressed in various cells throughout the body, including the small intestine, brain, and skin. The expression of GCG is increased in liver cell carcinoma and decreased in colorectal cancer [12,13].

Fibrillin-1 is an extracellular protein found in many body tissues. This protein creates a scaffold that gives the skin and artery walls elastic properties. Studies have investigated the role of FBN-1 in different tumors, such as liver cell carcinoma and thyroid carcinoma [14-17]. In this study, we measure the expression of GCG and FBN1 in the blood plasma of BCC patients compared to a control group to determine the future of diagnostic and therapeutic approaches.

Materials and Methods

1. Selection of patients and sampling

Sampling will be done from both men and women in the 30-80 age range. Patients with a history of receiving treatments such as chemotherapy, radiotherapy, or any adjuvant treatment will be excluded from the study. This original study received approval from the local ethics committee (ethical code: IR.TUMS.AMIRALAM.REC.1403.004). All participants provided informed consent.

2. Blood sampling of BCC patients and the control group

After sampling, the samples are immediately transferred to the pathology laboratory on ice.

3. Isolation of RNA from blood using an extraction kit

MicroRNA extraction will be done using the GENE ALL kit. Due to the sensitivity of the RNA extraction steps and the high resistance of the RNase enzyme, all RNA extraction steps will be performed in RNase-free conditions. For this purpose, RNase-free microtubes and head samplers are used.

4. Measurement of RNA concentration and purity

To read the concentration of the extracted RNA, it will be nano-dropped from the spectrophotometer. The Nanodrop Spectrophotometer can determine all wavelengths in the desired spectrum in a short time without the need for a cuvette and using only 1-2 microliters of the sample.

5. cDNA synthesis and real-time PCR using a specific miRNA cDNA synthesis kit

First, cDNA is synthesized from all the isolated miRNAs. In the next step, using GCG and FBN1-specific primers and a real-time PCR technique, the expression level of GCG and FBN1 in comparison with a housekeeping gene (in this study, the Housekeeping gene is used to normalize the expression level) is measured

quantitatively in each of the samples.

Statistical Analysis

The results are analyzed using SPSS software, the ANOVA test, and a subsequent LSD test to check the existence of correlation. The gene expression correlation of the data will be checked using the Spearman correlation coefficient. Real-time PCR results analysis will be used using the delta CT method.

Results

The studied population includes 15 samples of patients with basal cell carcinoma (BCC) with a mean age of 35±12.12 and 15 samples of healthy people with a mean age of 32±12.24. These 2 groups were matched in terms of age variables. The groups were compared using the T-test in terms of average age and did not show any significant difference in terms of average age, so it can be concluded that the age factor does not cause problems in the studied groups. Real-time RT-PCR reaction was performed. The results were interpreted according to the melting curve. The GCG biomarker was positive in 9 out of 15 patients in the group of patients with basal cell carcinoma (BCC). The rate of positivity of this biomarker in the group of healthy people was 4 out of 15. The statistical comparison of the rate of positivity of this biomarker in two groups was done using the two-sample binomial test, which shows a statistically significant difference between these two groups (P-value<0.001). The FBN1 biomarker was positive in 11 out of 15 patients with basal cell carcinoma (BCC). The rate of positivity of this biomarker in the group of healthy people was 5 out of 15 people. The statistical comparison of the rate of positivity of this biomarker in two groups was done using the two-sample binomial test, which indicated a statistically significant difference between the two studied groups. (P-value<0.001). To calculate the folding change, the Ct of each sample was determined first. The relative difference in the expression of markers in the two groups was calculated using the formula $\Delta\Delta$ Ct -2. In this way: The level of FBN1 mRNA expression in sick people was 1.89 times that of healthy people. Also, the level of GCG mRNA expression in sick people was 1.06 times that of healthy people.

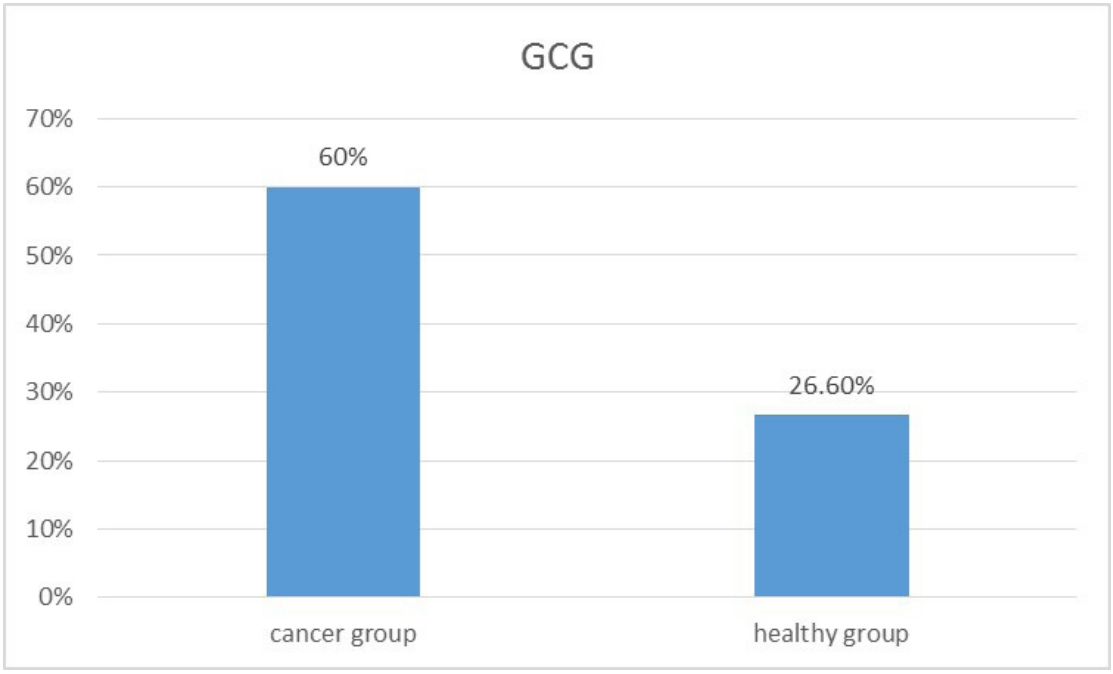

Figure 1. GCG mRNA positivity percentage in peripheral blood of patients with basal cell carcinoma (BCC) and healthy individuals.

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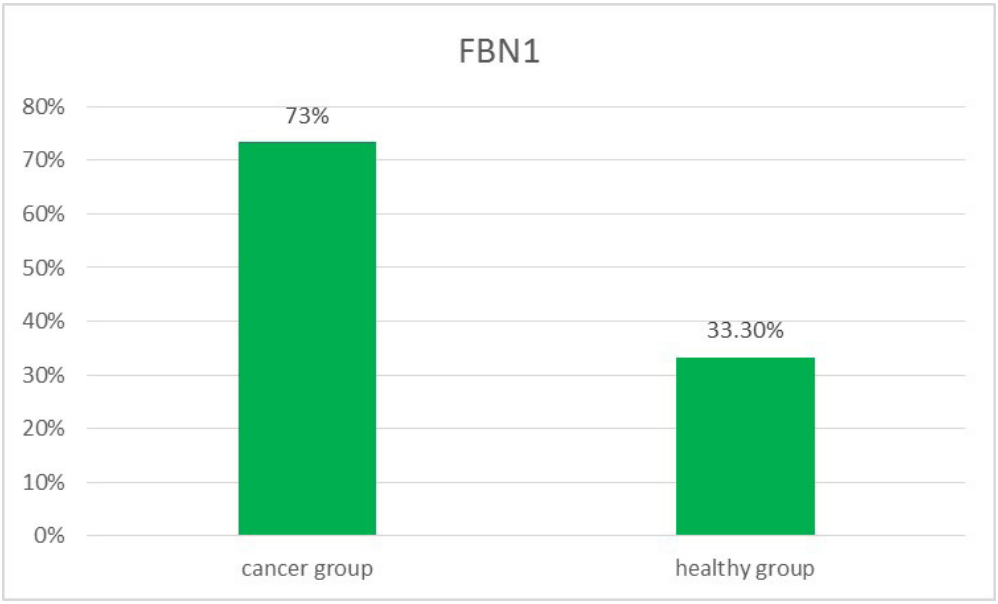

Figure 2. Percentage of FBN1 mRNA positivity in peripheral blood of patients with basal cell carcinoma (BCC) and healthy individuals

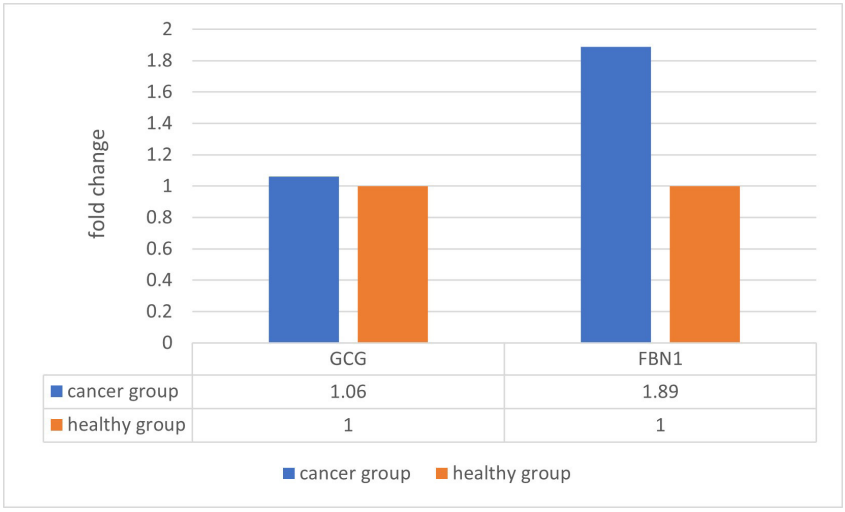

Figure 3. The difference in the expression of FBN1 and GCG genes in patients with basal cell carcinoma (BCC) and healthy subjects.

Discussion

In this study, we show that the expression of the GCG and FBN1 genes is significantly higher in BCC patients compared to the control group. MicroRNAs are important regulatory agents that affect the post-transcriptional phase of gene expression [18]. Calin et al. presented the first evidence of miRNAs role in cancer in the year 2002. This study showed that nucleotide deletions occurred with a higher frequency on 13q14. Two miRNA genes, miR15 and miR16, are located on this chromosome, and in the majority of chronic lymphocytic leukemia patients, these genes are down-regulated or deleted [11]. Since then, numerous studies have investigated the role of miRNAs in various types of cancer. In 2023, Tamas et al. conducted a study regarding non-melanoma skin cancers. They showed that miRNA-221 is an essential agent in forming non-melanotic skin cancers. In their study of 38 patients, the miRNA expression in the tumor lesion increased significantly in SCC patients19. Previously, miRNA-221 was known as a potential diagnostic factor for SCC20. A 2018 study by Al-Eryani et al. studied miRNA expression in skin lesions caused by arsenic as a carcinogenic factor. There were miRNAs with an increased expression rate. BCC and SCC samples showed increased amounts of miR-425-5p and miR-433, but there was no significant difference between the two groups. In BCC's pathologic samples, the amounts of miR-590-5p, miR-494, miR-487b, miR-452, miR-381, and miR-29c were significantly suppressed [21]. San et al. (2012) introduced 16 miRNAs with significantly el-

evated expression in biopsy samples of BCC and identified ten miRNAs with a significant decrease in BCC samples. These miRNAs were related to many essential pathways in BCC's pathogenesis, such as the Hedgehog signaling pathway [22]. Sonkoly et al. worked on the deregulation of miRNAs in BCC. They showed that miR-203 was significantly decreased in the BCC samples. MiR-203 is mainly expressed in the skin's epidermis and regulates the expression of many genes. Based on this information, miR-203 might be a tumor suppressor agent [23].

In 2017, Vand-Rajabpour et al. investigated the expression of BMI1, TWIST1, and SNAI2/SLUG mRNA in 170 patients with skin cancers compared to healthy samples. The results of these studies showed a decrease in BMI1 mRNA expression in BCC, SCC, and melanoma in comparison to healthy samples, a decrease in TWIST1 mRNA only in BCC, and an increase in SNAI2/SLUG mRNA expression in melanoma and SCC24. Sand et al. investigated the expression level of miR-17-92 and miR-143-145 clusters in BCC and SCC. MiR-17-92 has been known as an oncogenic agent. The results of these studies showed that members of the miR-17-92 family, including miR-17-5p, miR-18a-5p, miR-19a-3p, miR-19b-3p, and miR-143-5p, are increased in SCC. The expression of miR-145-5p was significantly downregulated in BCC [25].

In 2018, Sun et al. investigated the expression level of MicroRNA-451a as a tumor suppressor in human and mouse BCC samples. The results of this study showed an escalation in the expression of this miRNA in both samples and showed its essential role in BCC tumor formation [26]. In 2023, Gürsel Ürün et al. investigated the expression level of SMAD4 in non-melanotic skin cancers (cSCC, BCC, and BSC). These studies showed that the level of SMAD4 mRNA expression decreases in all three types of these cancers and is directly related to their pathogenesis [27].

These results all point to the prominent role of biomarkers in cancer pathogenesis. Following this pattern, our study shows that the expressions of both GCG and FBN1 were significantly increased in patients with BCC. In 2024, Wang and colleagues conducted a study on the effect of GLP-1 receptor agonists, which are approved as diabetes treatment drugs by the FDA, on the risk of pancreatic cancer in individuals with type 2 diabetes. GLP-1 is a glucagon-like protein produced by the breakdown of proglucagon, encoded by the GCG gene. In this study, the group using GLP-1 agonists had a lower risk of developing pancreatic cancer [28]. Additionally, a meta-analysis by Piccoli and colleagues showed that the use of GLP-1 receptor agonists does not affect the increased risk of breast cancer [29]. In this study, we examined the expression of GCG in individuals with BCC (Basal Cell Carcinoma) and found a significant contrast in the expression of GCG between individuals with BCC and healthy controls. This suggests that GCG expression could be a diagnostic factor in patients with BCC. Drugs derived from glucagon or drugs that target these proteins or their receptors may be effective in treating patients with BCC, and further research in this area would be beneficial.

Compared to GCG, FBN1 showed a greater difference in expression between BCC patients and healthy individuals, with the relative expression of FBN1 being 1.89 times higher in BCC patients. This significant increase in FBN1 in BCC patients is particularly important because fibrillin-1, a protein encoded by FBN1, is a crucial part of the extracellular matrix (ECM) and is essential for maintaining tissue integrity and regulating signaling pathways such as TGF-β signaling [30]. Many studies have explored the role of FBN1 in various cancers. In a study conducted by Wang and colleagues, it was shown that FBN1 plays a role in the metastasis of ovarian cancer through the p53 and SLUG signaling pathways [31].

Zhang and colleagues, in another study, investigated the role of FBN1 in gastric cancer. This study showed that the expression of FBN1 increases in gastric cancer and has a direct correlation with the depth of tumor invasion [32]. These findings align with the results of the present study and highlight the significant role of FBN1 in the formation and progression of cancer. Significant differences in the expression of GCG and FBN1 between patients with BCC and healthy individuals suggest that these biomarkers could be valuable tools for diagnosing BCC or monitoring disease progression.

Conclusion

The expression of GCG and FBN1 is significantly higher in patients with BCC compared to healthy individuals. Further studies can be done to ensure the role of these genes in the diagnosis of skin cancers.

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Conflict of Interest

There is no conflict of interest to declare.

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