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Determination of GPR55 over Expression by Quantitative Real-time PCR as a Biomarker for Skin SCC Detection

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ABSTRACT: Chronic wounds resemble the wounds arising with skin squamous cell carcinoma (SCC). Since they are cancerous, they should be treated using novel bioactive wound dressing instead of biopsy and related procedure for detection. Using a noninvasive method is necessary for early detection and proper treatment.
The aim of this study is to investigate GPR55 over expression by quantitative real-time PCR as a biomarker for early detection of skin squamous cell carcinoma (SCC).
In a clinical trial, 10 patients were randomly assigned to treatment and were compared to 10 healthy people in control group. Over expression of GPR55 gene was evaluated by quantitative real-time PCR in both groups. The expression of GPR55 gene was significantly higher in treatment group compared to the control group. The results of the study suggest that the over expression of GPR55 can be utilized as a biomarker for detection of skin SCC.

Key words: Skin squamous cell carcinoma; over expression; GPR55 expression; RT-PCR

INTRODUCTION

Chronic wounds resemble the wounds arising with skin squamous cell carcinoma (SCC). Since they are cancerous, they should be treated using novel bioactive wound dressing instead of biopsy and related procedure for detection. Using a noninvasive method is necessary for early detection and proper treatment.

Cancer can be defined as a failure within the cells' communication network (Simon, 2010). Skin cancer is currently the most common type of human cancer, and of particular concern, its incidence is increasing at an astonishing rate. Epidemiological and molecular data strongly suggest that non-melanoma skin cancers are associated with excessive exposure to the ultraviolet (UV) radiation in sunlight. Although stratospheric ozone blocks UVC(below 280 nm) radiation as well as part of UVB radiation (280-290 nm) from reaching the surface of the earth, UVB (290-315 nm) and UVA (315-400 nm)reach the surface of the earth, and cause DNA damage, inflammation and erythema, sunburn, gene mutations, post-inflammatory immune suppression, and eventually, skin cancer (Melnikova and Ananthaswamy 2005).

Cancer diagnosis and treatment are of great importance due to the high occurrence of the diseases and death rate. Survival of a cancer patient depends on early detection and thus developing technologies capable for sensitive and specific ways to detect cancer is a task for researchers. Existing cancer screening methods include: biopsy, endoscopy, CT scans, X-ray, fluorescent microscopy, ultrasound imaging and MRI (Choi *et al.* 2010). However these traditional diagnostic ways, are not powerful detection methods at very early stages. At the same time screening methods are expensive and not readily available for many patients. Therefore, investigation about methods that are reliable, specific and sensitive for detecting cancers at early stages and are easily accessible as the first-line guidance is of outmost importance (Choi *et al*. 2010).

Biomarkers and nanotechnology, two new sciences in development of diagnostic methods, are being extensively studied (Choi *et al.* 2010).

Researchers suggest some biomolecule such as G protein-coupled receptor 55 (GPR 55) as biomarker for early detection of skin squamous cell carcinoma (SCC) (Perez-Gomez *et al.* 2013). GPR55 is a seven-transmembrane G protein-coupled receptor identified in 1999 (Sawzdargo *et al.* 1999). Several groups reported that GPR55 is a possible novel type of cannabinoidreceptor (Johns *et al.* 2007; Ryberg *et al.* 2007; Lauckner *et al.* 2008).

GPR55 expression has been reported in melanoma (skin cancer), human astrocytoma, lymphoblastoid cell lines, B lymphoblastoma (Oka *et al.* 2010; Andradas *et al.* 2011),breast (Andradas *et al.* 2011; Ford *et al.* 2010), ovary, prostate (Ford, 2009), brain, skin, cervix, liver, blood, pancreas (Andradas *et al.* 2011).

Interestingly, the expression of GPR55 in human tumors correlates with their aggressiveness (Andradas *et al.* 2011). These data suggest that GPR55 expression and/or activation confers an oncogenic capability on cancer cells. Results obtained so far indicate that this capability increases cancer cells proliferative potential.

In the present study, it was shown that GPR55is expressed in SCC patients in comparison with healthy people and that it could be utilized as a possible biomarker for detection of SCC.

MATERIALS AND METHODS

Ten cases of skin SCC and ten cases of healthy people were nominated for measuring the quantity of GPR55 gene expression.

A. Sample collection and storage

Blood samples of eligible participants, both treatment and control groups, were collected in EDTA tubes by a trained pre-instructed nurse.

B. Study design

Patients in treatment group referred to Emam Khomeini Hospital, Tehran University of Medical Sciences, during the year 2014 for skin SCC were considered for eligibility in the study. The inclusion criterion was the existence of skin SCC by examining biopsy. The diagnoses of SCC and examination of biopsies were performed by dermatopathologists at the hospital, whose diagnosis was considered definitive for this study purposes. Patients with other types of skin disorders or those who were receiving other treatments at the time of admission were excluded. For control group 10 healthy people were recruited and included in the study.

C. Ethical considerations

Before being included in the study, the eligible participants signed the informed consent form. Each participant was allowed to withdraw from the study whenever they desired. All steps for collecting blood samples were provided for the participants free of charge. Finally, all of the patients' information were classified and the findings were reported anonymously.

D. RNA extraction, cDNA synthesis and gene expression analysis

Two milliliters (mL) of sterile PBS was used for diluting 2 mL EDTA-preserved fresh whole blood and carefully transferred to 4 mL Ficoll-Paque. The samples were centrifuged at 2000 rpm for 15 minutes. The buffy coat was isolated in a 1.5-mL Ependorff microtube. Total RNA was extracted by TRIZOL. The quality and concentration of the extracted RNA samples were determined using spectrophotometer by measuring optical density at 260/280 nm (Genova Life Science Analyser-DNA, Jenway, England). In order to synthesize first-strand cDNA, random hexamer primers and a commercially available kit (Revert Aid First-Strand cDNA Synthesis kit, Ferment as, Life sciences, Germany), were used. Subsequently, cDNA was amplified by PCR with GAPDH (reference gene) and GPR-55 primers (Forward primer: 5-CCTCGCATTCAAGATGGTCC-3; Reverse primer: 5-GACGCTTCCGTACATGCTGA -3) to determine the accuracy of the cDNA synthesis reaction and the PCR products were analyzed using electrophoresis on both a 2% agarose gel and acrylamide gel.

In order to determine GPR-55 expression, a quantitative real-time RT-PCR method was established. The amplification was performed using a real-time rotary analyzer (Rotor-Gene 6000, Corbett, Australia), and at the end of reactions data were analyzed by the Rotor-Gene software according to the comparative ΔΔCt method. The primers for the reference gene, exon 12 of human serum albumin were: (Forward primer: 5′TGTTGCATGAGAAAACGCCA-3′; reverse primer, 5′-GTCGCCTGTTCACCAAGGAT-3′). Amplification of cDNA was performed by Real-time RT-PCR, in a final reaction volume of 25 µL containing 3 µL target cDNA, 1 µL forward primer, 1 µL reverse primer, 10 µL DDW and 10 µL syber premix Ex TaqII (TliRNaseH Plus). The thermal cycling conditions were 10 minutes at 95°C followed by 35 cycles of denaturation at 94°C for 10 s, annealing at 65°C for 10 s and extension at 72°C for 12 s for both genes. All reactions were run in duplicates.

E. Generation of standard curve

To establish an amplification efficacy for the two segments of the target and reference DNA, standard curve was required. cDNA derived from control individual was used for generation of the calibration curve for Alb and GPR55 genes. cDNA was amplified from serial dilution with four different magnitudes of concentrations. The second derivative maximum method automatically calculated fractional cycle number, or crossing point, where the fluorescence rose above background.

RESULTS

During the year 2014, 10 patients and 10 healthy people were included in the trial. There were 8 men and 2 women in the treatment group and 1 man and 9 women in the control group with a median age of 53 years in the treatment and 28 years in the control group.

Agarose gel electrophoresis of PCR products for GAPDH (reference gene) and GPR55 for control and treatment groups, respectively, demonstrated that GPR55 gene was expressed in both control and treatment groups. The profile of electrophoresis are shown in Figures 1 (a, b) and 2 (a,b). Acrylamide gel electrophoresis of PCR products for GAPDH (reference gene) and GPR55 is illustrated for control and treatment groups, respectively (Fig. 3) and indicates that related bonds are specific and there aren't any similar bonds.

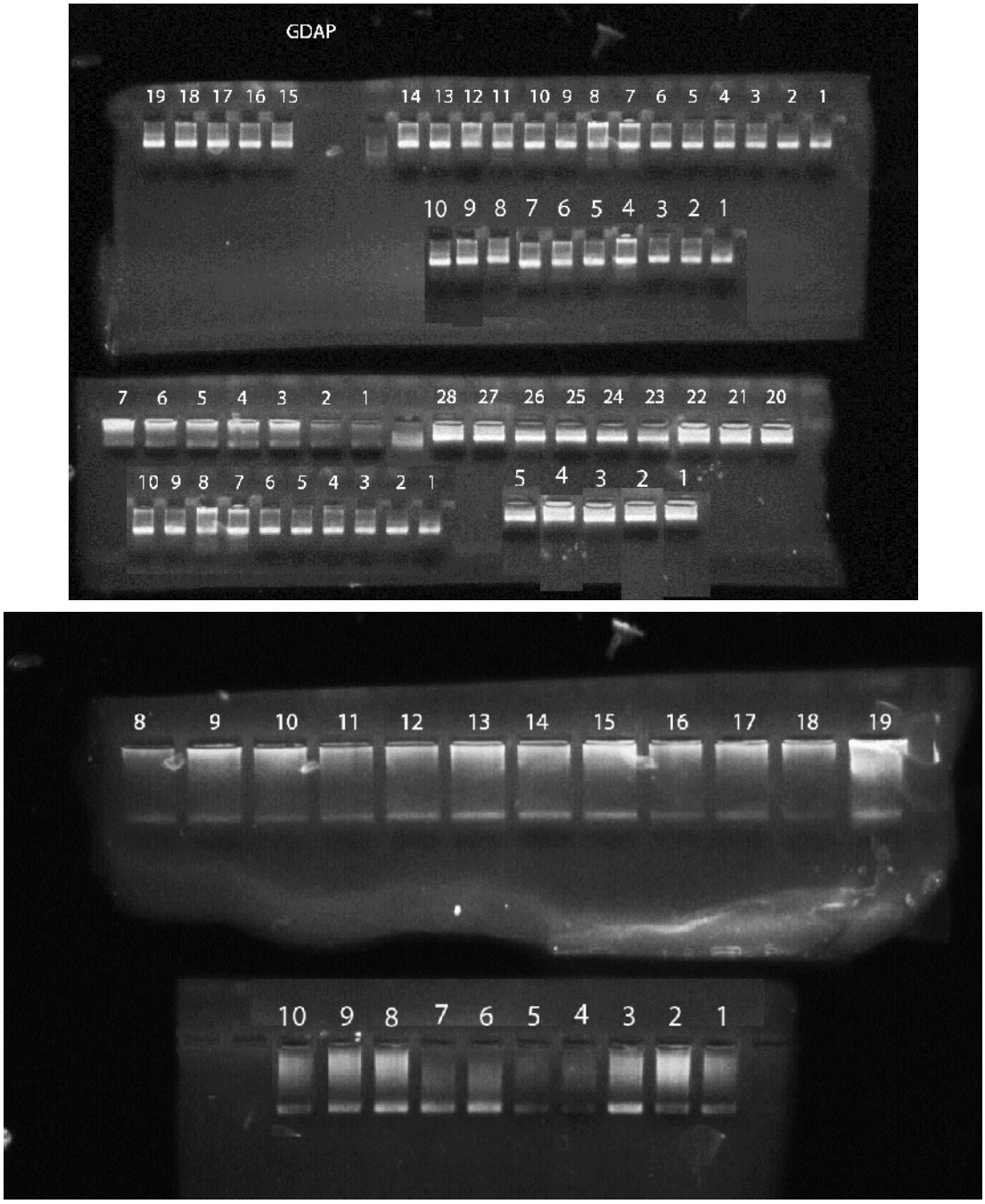

Fig. 1. Agarose gel electrophoresis of PCR products for a.GAPDH (reference gene) and b.GPR55 for control group.

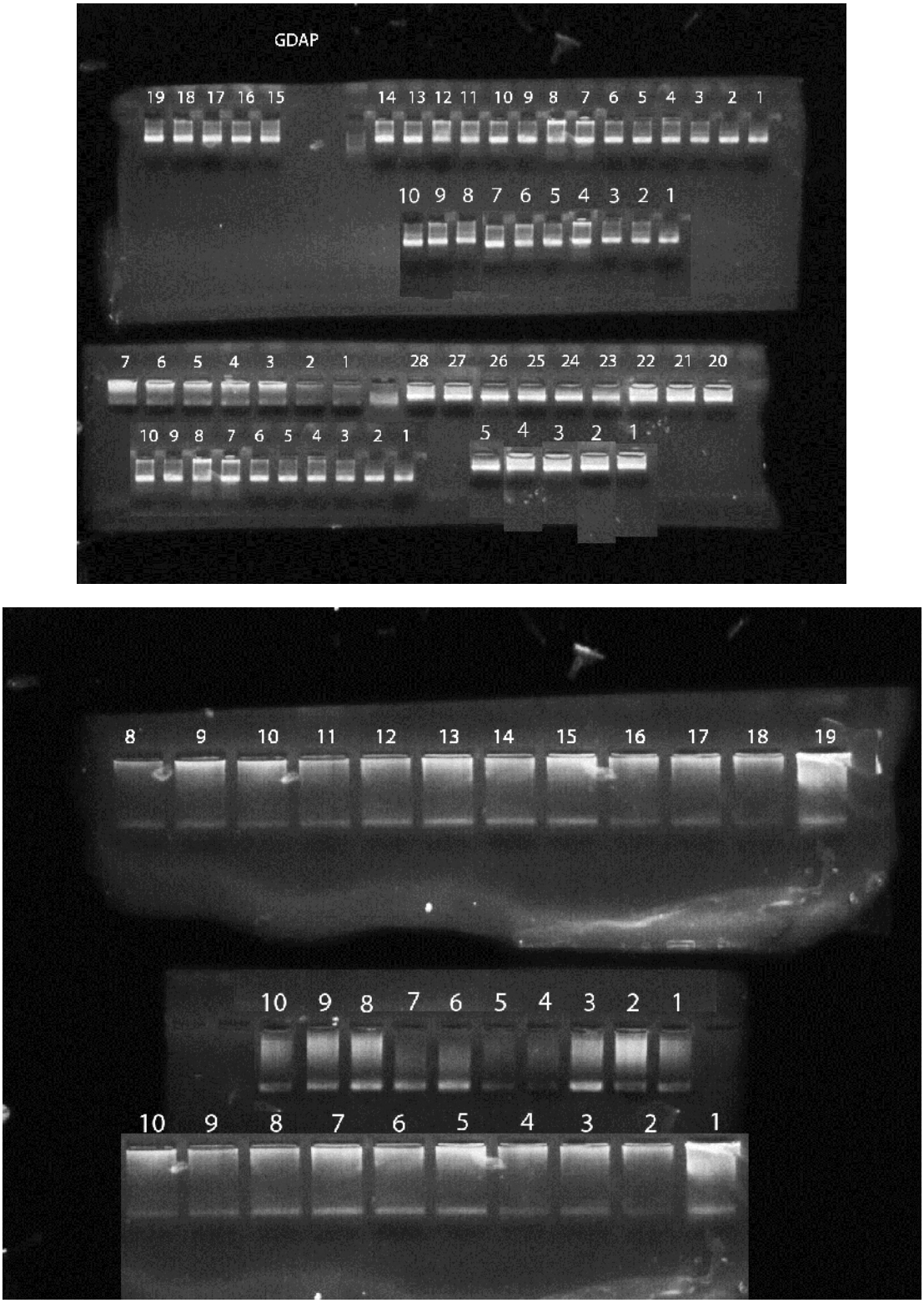

Fig. 2. Agarose gel electrophoresis of PCR products for (a). GAPDH (reference gene) and (b). GPR55 for treatment group.

Overall, the efficiency of GPR55 gene from standard curves was considered ideal (Fig. 4). The standard curve method simplifies calculations and avoids practical and theoretical problems currently associated with PCR efficiency assessment. For establishing the dosage profile of the treatment group, they were compared with control groups. The Ct value of GPR55 and albumin (reference gene) was almost identical as it can be seen on the amplification plots of a normal control group (Fig 5.a). For treatment group, the Ct values of GPR55 showed an increase in comparison to albumin (Fig. 5.b).

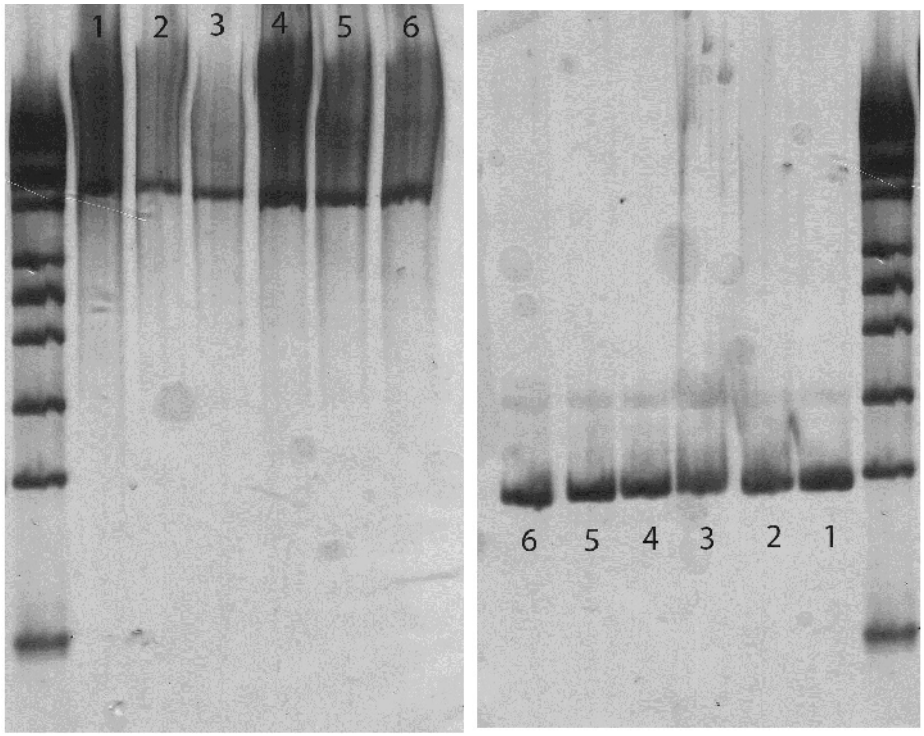

Fig. 3. Acryl amide gel electrophoresis of PCR products for (a) GAPDH (reference gene) for treatment group (number 1, 2, and 3) and control group (number 4, 5, and 6), and (b) GPR55 for treatment group (number 1, 2, and 3) and control group (number 4, 5, and 6).

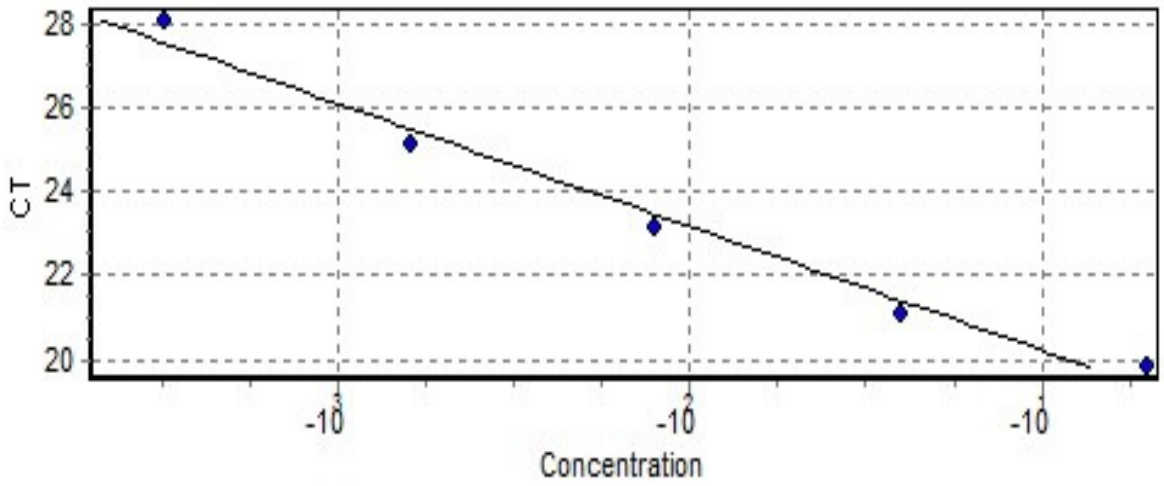

Fig. 4. Standard curve for GPR55 gene, R2= 0.9933.

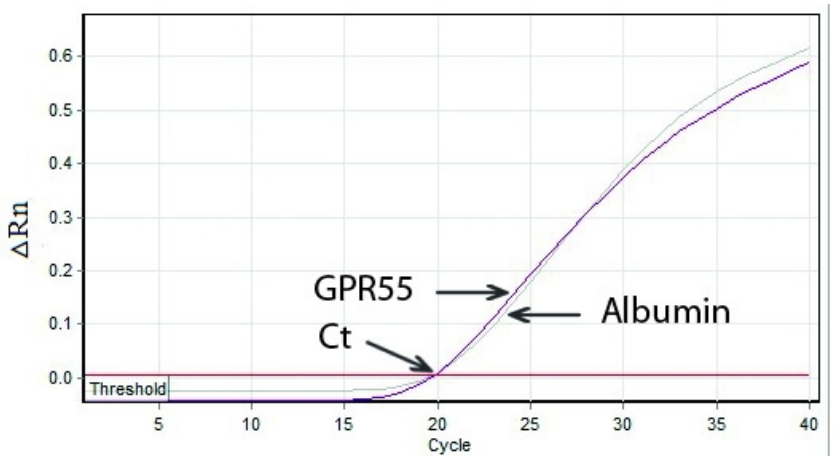

Fig. 5a. Amplification plots of GPR55 and albumin, all in triplicates, of normal control group.

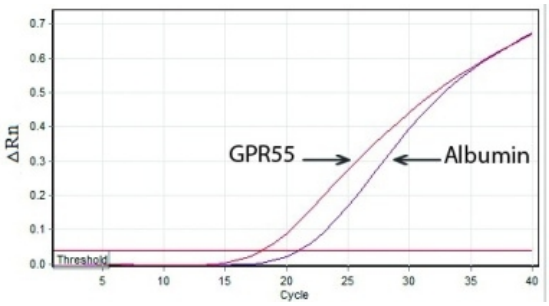

Fig. 5b. Amplification plots of GPR55 and albumin, all in triplicates, of treatment group with GPR55 duplication.

DISCUSSION

Detection of skin SCC is traditionally carried out by analyzing biopsy specimens. However taking biopsy is an invasive procedure. Possible complications including the following may occur:

-Excessive bleeding (haemorrhage)

-Infection

-Puncture damage to nearby tissue or organs

-Skin numbness around the biopsy site.

It is well known that over expression of a specific protein can be symbol of a disease. GPR55 is over expressed in human skin tumor. Recent data suggest that GPR55 may be part of the molecular controlling tumor growth. Research point that GPR55 has a pivotal role in skin tumor development, and it is suggested that this receptor may be used as a new therapeutic target and potential biomarker in skin cancer management (Perez-Gomez *et al.* 2013).

The aim of this study was to investigate the possibility of using the quantity of GPR55 expression as non-invasive biomarker for SCC diagnosis.

Using PCR demonstrated that GPR55 gene was equally expressed in both treatment and control groups. Further quantitative evaluation of GPR55 using RT-PCR determined that this gene is over expressed in treatment group.

This study was carried out based on one reference gene. The amplification efficacy of Alb and GPR55 gene were derived from the standard curve. Therefore, PCR efficiencies of both genes were approximately equal.

It is apparent that GPR55 gene is expressed by healthy people and in patients affected by skin SCC. Using RT-PCR however, has enabled the quantification of GPR55 and it is obvious that GPR55 gene is clearly over expressed in skin SCC patients when compared with healthy people.

CONCLUSION

Detection of SCC employing conclusive and non-invasive methods can be very important to early treatment of a cancer patient. It appears that the over expression of GPR55 gene can be an effective biomarker in SCC patients.

These results combined with other non-invasive early detection methods could open a new horizon for the detection and consequent treatment of SCC patients.

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