Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

# Allele frequency of CTLA-4 rs231775 (+49 A>G) gene polymorphism and its association with cancer susceptibility in the Saudi Arabian population and other ethnic groups

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**Article Info**

**Article history:**

**Received:** June 01, 2025
**Accepted:** October 26, 2025
**Published:** November 30, 2025

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**Abstract**

Single-nucleotide polymorphisms (SNPs) of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) gene found in exon 1 are directly associated with the progression and onset of autoimmune disease and various human cancers. These SNPs are commonly known prognostic biomarkers for the prediction and early onset of cancer risk. The variant frequency of CTLA-4 rs231775 (+49 A>G) polymorphisms may affect the various ethnic groups differently. This study assessed the allelic frequency distribution of rs231775 (+49 A>G) polymorphisms in the Saudi Arabian population and compared it with other world populations. The data were extracted from case-control studies in several ethnic groups using PubMed (Medline) and similar web databases. The frequency of CTLA-4 rs231775 (+49 A>G) variant allele (G) was observed at 33.0% and different frequencies were found significant for Pakistan (p=0.02), China (p=0.05), China (p= 0.00), Iran (p=0.00), Poland (p=0.00), the USA (p=0.00) and Turkey (p=0.02) when the prevalence of Saudi Arabian population is compared to that of other populations. The observed finding reveals a distinct pattern of CTLA-4 rs231775 (+49 A>G) polymorphism variant allele in the populations of Saudi Arabia, maybe because of the differences in ethnicity. The observed findings can help assess the risk for the population harboring the risk allele of the rs231775 (+49 A>G) SNP and their subsequent susceptibility to cancer.

**Keywords:** CTLA-4, rs231775, +49A >G, Single nucleotide polymorphism, Cancer, Immune system.

## 1. Introduction

Cancer is a complex genetic disorder and is an important factor in death worldwide [1], with approximately 10 million deaths related to cancer reported in the year 2020. Worldwide, it is a major health problem and the second leading cause of death reported in the United States [2]. The rising incidence and mortality of cancer are currently it is regarded as the major causes of death in the globe.

The incidence of cancer variation globally is caused mainly by genetic as well as environmental factors like ionizing radiation, which causes damage to DNA and genomic loss of integrity associated with defects in DNA repair systems, which increases the risk of cancers.

The impact of genetic changes on the repair system of host DNA contributes significantly to the susceptibility to cancer. SNPs are the changes of a single base found in at least 1% of populations, consisting of effects of minor individuals, although multiple SNPs' additive impact on the onset and progression of cancer makes them important targets of studies. The SNP reduces the DNA repair capacity (DRC), which renders the host increasingly susceptible to cancers in comparison to other populations.

There are several causative factors of cancers, some external factors (like tobacco, chemicals, some infectious agents, and radiation) and various internal factors (like mutations, hormones, and immune conditions). These factors interplay simultaneously, leading to the initiation or progression of carcinogenesis. The chemical substances

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**Doi:** http://dx.doi.org/10.14715/cmb/2025.71.11.4

found in the environment that have a carcinogenic nature play a direct or indirect role in the cytoplasmic and nuclear regions of cells and cause genetic disorders. Other factors that cause cancers are viruses, bacteria, and exposure to radiation comprise approximately 7% of all types of cancers. Cancer is one of the important risk factors for mortality, and it is estimated that in the year 2012, about 14.1 million people were diagnosed with cancer, also reported that worldwide approximately 8.2 million deaths occurred. In 2018, the global incidence of new cancer cases was approximately 18.1 million, with cancer-related fatalities reaching around 9.6 million. These figures highlight the substantial burden of cancer worldwide and underscore the critical need for effective prevention, early detection, and treatment strategies to reduce morbidity and mortality associated with the disease.

The cancer patient's survival rate, having unresectable tumors, can be increased by using chemotherapy or targeted radiation therapy, although their harmful effects and resistance towards drugs are considered challenging, because of the heterogeneous properties of tumors [3]. Several studies have reported that the intricate relationship between cancer and the immune system forms the foundation for the development and application of immunotherapy as a treatment strategy.

The CTLA-4 gene, known as CD152, is a costimulatory molecule gene that shows an important function in immune responses. Transiently CTLA-4 expression-activated T cells inhibit the production of cytokine T cells. The binding affinity of CTLA-4 has costimulatory receptors B7-1 (CD80) and B7-2 (CD86) over the antigen-presenting cells (APC), which produces an inhibition of signals that suppresses the activation of T cells. The immune response regulation by co-stimulation and co-inhibition of signals plays an important role in cancer progression control. CTLA-4 is a widely studied checkpoint molecule in the progression of cancer [4]. The CTLA-4 expression by different cell tumor types reduces antitumor immunity via T-cell-mediated activity.

The gene CTLA-4 comprises 4 exons, situated at chromosome 2 (2q33.2). This region has several genes associated with immune regulation [5]. In breast cancer progression, the antitumor role played by activated T-cells can be attenuated by over-expression levels of CTLA-4, although the antitumor properties can be stimulated by lowering CTLA-4 actions [6]. The highly polymorphic CTLA-4 gene and several SNPs of this gene affect protein activities and gene expression. However, several studies reported that this gene polymorphism has a direct correlation with many diseases in diverse populations.

Evident from research has shown that earlier studies have reported that the genetic variants of the CTLA-4 gene have an association with several cancers, such as colorectal cancer (CRC), hepatocellular carcinoma (HCC), and head and neck cancer (HNC) [5]. CTLA-4 inhibits the immune response and reduces tumor-killing activity. Over 100 SNPs have been identified in the CTLA-4 gene; among these, the A>G polymorphism at position +49 in exon 1 results in an amino acid substitution from threonine to alanine in the CTLA-4 protein. This specific polymorphism may influence CTLA-4's function and has been studied for its role in immune regulation and cancer susceptibility. Various genetic variant was observed in the CTLA-4 gene, one of them the missense variant rs231775 c.49 A>G, found in exon 1, is a potent polymorphism, showing a potential role in malignancies and their progression [7]. In various studies, it was found A allele (17 Thr) played an increasing breast cancer risk, although the G allele (17Ala) performed a protective role in breast cancer.

Various epidemiological studies were conducted for the investigation of the correlation of CTLA-4 rs231775 +49 A>G polymorphism and its role in cancer progression. Even though CTLA-4 is located in the exonic region for cancer risks, their presence, and functional effects of rs231775 +49 A>G polymorphism in the Saudi Arabian population in any type of cancer have yet not be elucidated.

The current study aims to determine the frequency distribution of CTLA-4 rs231775 +49 A>G polymorphisms among the healthy normal population of Saudi Arabia and compare it with various other epidemiological investigations carried out worldwide.

## 2. Materials and Methods

### 2.1. Gene variants search criteria

The literature search was conducted using databases including PubMed, CGEMS, Web of Science, Embase, and EBSCO, employing keywords such as “CTLA-4,” “rs231775,” “+49 A>G,” and “polymorphisms.” The search included studies on human subjects reported in any language. Eligible studies reported genotype frequencies for the control population. However, studies providing only allele frequencies without genotype data were excluded from this analysis to ensure consistency and accuracy in assessing genotype distributions across populations.

The first author's name, publication years, nationality of subjects, type of research, number of controls, criteria of inclusions and exclusions, alleles, and genotype frequencies of the subjects were abstracted for each study that qualified those criteria. If various reports were found from the same race, the most recent published data were included. Data on the population of Saudi Arabia were included in the most recent reports.

In this study, the prevalence rate of CTLA-4 rs231775 +49 A>G polymorphisms was extracted from nineteen studies [5, 8-24] (Table 1) that the gene variants of CTLA-4 rs231775 (+49 A>G) were analyzed in different populations and it was matched with the population of Saudi Arabia [8] (Table 2), the Observed and expected genotype frequency of CTLA-4 rs231775 (+49 A>G) polymorphisms in the control groups of the study.

### 2.2. Statistical analysis

For the comparison of the allelic and genotypic frequencies, Pearson's $\chi 2$ test was applied to different populations by applying the statistical software program SPSS (version 21). The Hardy-Weinberg Equilibrium (HWE) was applied in this study by using the Court Lab (a software program based on the web). In the analysis, the p-value was found to be $p \leq 0.05$ is considered statistically significant.

## 3. Results

In the study conducted by Al-Harbi et al., [8] was used to extract data about the prevalence of the CTLA-4 exon 1 Thr17Ala (+49 A>G) polymorphism in the Saudi Arabian population. Eighteen (Shabbir et al., [9], Ali et al.,

**Table 1.** Studies included in the CTLA-4 rs231775 (+49 A>G) gene variant analysis in different populations.

| Study | Country/ Ethnicity | Race | Disease/ Cancer types | Total no. of subjects (n) |
|---|---|---|---|---|
| Al-Harbi et al., 2023 [8] | Saudi Arabia | Asian | CRC | 97 |
| Shabbir et al., 2022 [9] | Pakistan | Asian | HCC | 216 |
| Ali et al., 2022 [10] | Egypt | African | HCV | 162 |
| Babteen et al., 2020 [11] | Egypt | African | BC | 179 |
| Zou et al., 2018 [5] | China | Asian | CRC | 1303 |
| Enciso-Vargas et al., 2018 [12] | Mexico | North America | HCV | 215 |
| Chang et al., 2017 [13] | China | Asian | DTC | 350 |
| Diler., 2017 [14] | Turkey | Caucasian | PC | 119 |
| Arikan et al., 2017 [15] | Turkey | Caucasian | GC | 105 |
| Isitmangil et al., 2016 [16] | Turkey | Caucasian | BC | 76 |
| Tang et al., 2016 [17] | China | Asian | GCA | 608 |
| Wang et al., 2015 [18] | China | Asian | CRC | 389 |
| Farbod et al., 2015 [19] | Iran | Asian | BC | 100 |
| Liu et al., 2015 [20] | China | Asian | HCC | 78 |
| Minhas et al., 2014 [7] | India | Asian | BC | 250 |
| Antczak et al., 2013 [21] | Poland | European | NSCLC | 104 |
| Bharti et al., 2013 [22] | India | Asian | OC | 180 |
| Welsh et al., 2009 [23] | USA | Caucasian | NMSC | 819 |
| Dilmec et al., 2008 [24] | Turkey | Caucasian | CRC | 162 |

CRC, Colorectal Cancer; HCC, Hepatocellular carcinoma; NSCLC, Non-Small-Cell Lung Cancer; BC, Breast Cancer; CC, Cervical cancer; PC, Prostate Cancer; OC, Oral Cancer; GC, Gastric Cancer; HCV, Hepatitis C Virus infection; GCA, Gastric cardia adenocarcinoma; NMSC, Non-Melanoma Skin Cancer; DTC, Differentiated Thyroid Carcinoma

**Table 2.** Observed and expected genotypic frequencies of CTLA-4 rs231775 (+49A>G) polymorphism in the control group.

| Study | Observed Genotype (n) | | | Expected Genotype (n) | | | MAF | p-value (HWE) |
|---|---|---|---|---|---|---|---|---|
| | AA | AG | GG | AA | AG | GG | | |
| Al-Harbi et al., 2023 [8] | 38 | 54 | 05 | 44 | 43 | 11 | 0.330 | 0.01 |

[10], Babteen et al., [11], Zou et al., [5], Enciso-Vargas et al., [12], Chang et al., [13], Diler., [14], Arikan et al., [15], Isitmangil et al., [16], Tang et al., [17], Wang et al., [18], Farbod et al., [19], Liu et al., [20], Minhas et al., [7], Antczak et al., [21], Bharti et al., [22], Welsh et al., [23], Dilmec et al., [24]) studies were included for the comparative analysis of the Saudi population with Asian, African, Caucasian, North American and European populations, respectively.

According to genotype distribution, the minor allele frequency (MAF) of CTLA-4 rs231775 +49 A>G polymorphisms was found to be 33.0% in the population of Saudi Arabia, which was found consistent with the HWE accordingly as shown in Table 2. The different MAF was found in the genotypes (AA, AG, and GG), and the distribution of allelic frequencies of polymorphisms studied in several populations is shown in Table 3. The different MAFs were observed significantly for the ethnicity of Pakistan ($p = 0.02$), China ($p = 0.05$), China ($p = 0.00$), Iran ($p = 0.00$), Poland ($p = 0.00$), the USA ($p = 0.00$) and Turkey ($p = 0.02$) to that of CTLA-4 rs231775 +49 A>G observed frequencies in the populations of Saudi Arabia were matched with other population groups ($p = 0.01$).

This CTLA-4 genetic variant in the exon 1 is a missense variation leading to a threonine to alanine substitution at codon 17 (Thr17Ala). However, CTLA-4 prevents immune response and its tumor-killing activity. More than 100 SNPs are found in the CTLA-4 gene, out of which AG dysmorphism, situated on +49 of exon 1, is due to an amino acid change (threonine to alanine) in the CTLA-4 protein. Various genetic variants were observed in the CTLA-4 gene; one of them, the missense variant rs231775 c.49 A>G, located in exon 1, is a potent polymorphism that may play a role in malignancies and their progression [7].

## 4. Discussion

Cancer is a multifactorial genetic disease that results from the interplay of genetics and various environmental factors, which influence disease progression throughout a person's lifetime. The etiology of cancer is a multistep process and is not fully understood, although several factors, including genetic and immunological factors, play an important role. The immune system's network is a complex pathway, and its activation by tumor antigens can destroy tumor cells, as reported in some cases. Commonly known cells that play a role in the rejection of tumors, such as natural killer cells and T-cells. CTLA-4 is an immunoregulatory molecule involved in the process of homeostasis of the immune system. The genomic polymorphism of this molecule affects the level of transcription, translation, and its functions. The apoptotic process and cellular proliferation are the fundamental processes that play a vital function, leading to tissue homeostasis [25]. Alteration in the apoptotic response may cause intimal cell accumulation by the process of therogenesis [26]. In humans, es-

**Table 3.** CTLA-4 rs231775 (+49A>G) gene variant genotype and allele frequency distribution in different populations and p-values in contrast to the Saudi Arabian population.

| Study | Disease /tumor type | Total no. of subjects (n) | Genotype | | | Allele | | Total Alleles | A Allele frequency | G Allele frequency | p-value | MAF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | AA | AG | GG | A | G | | | | | |
| Al-Harbi et al., 2023 [8] | CRC | 97 | 38 | 54 | 05 | 130 | 64 | 194 | 0.670 | 0.330 | **Ref. 0.01** | 33.0 |
| Shabbir et al., 2022 [9] | HCC | 216 | 00 | 55 | 161 | 55 | 377 | 432 | 0.128 | 0.872 | **0.02*** | 87.2 |
| Ali et al., 2022 [10] | HCV | 162 | 112 | 48 | 02 | 272 | 52 | 324 | 0.840 | 0.160 | 0.24 | 16.0 |
| Babteen et al., 2020 [11] | BC | 179 | 67 | 92 | 20 | 226 | 132 | 358 | 0.631 | 0.369 | 0.17 | 36.9 |
| Zou et al., 2018 [5] | CRC | 1303 | 116 | 564 | 623 | 796 | 1810 | 2606 | 0.305 | 0.695 | 0.45 | 69.5 |
| Enciso-Vargaset al., 2017 [12] | HCV | 215 | 93 | 89 | 33 | 275 | 155 | 430 | 0.640 | 0.360 | 0.14 | 36.0 |
| Chang et al., 2017 [13] | DTC | 350 | 49 | 142 | 159 | 240 | 460 | 700 | 0.342 | 0.658 | **0.05*** | 65.8 |
| Diler., 2017 [14] | PC | 119 | 67 | 43 | 09 | 177 | 61 | 238 | 0.743 | 0.257 | 0.63 | 25.7 |
| Arikan et al., 2017 [15] | GC | 105 | 50 | 46 | 09 | 146 | 64 | 210 | 0.696 | 0.304 | 0.65 | 30.4 |
| Isitmangil et al., 2016 [16] | BC | 76 | 34 | 36 | 06 | 104 | 48 | 152 | 0.685 | 0.315 | 0.35 | 31.5 |
| Tang et al., 2016 [17] | GCA | 608 | 50 | 272 | 286 | 372 | 844 | 1216 | 0.306 | 0.694 | 0.18 | 69.4 |
| Wang et al., 2015 [18] | CRC | 389 | 101 | 147 | 141 | 349 | 429 | 778 | 0.449 | 0.551 | **0.001*** | 55.1 |
| Farbod et al., 2015 [19] | BC | 100 | 28 | 69 | 03 | 125 | 75 | 200 | 0.625 | 0.375 | **0.002*** | 37.5 |
| Liu et al., 2015 [20] | HCC | 78 | 07 | 33 | 38 | 47 | 109 | 156 | 0.301 | 0.699 | 1.00 | 69.9 |
| Minhas et al., 2014 [7] | BC | 250 | 105 | 121 | 24 | 331 | 169 | 500 | 0.662 | 0.338 | 0.18 | 33.8 |
| Antczak et al., 2013 [21] | NSCLC | 104 | 49 | 33 | 22 | 131 | 77 | 208 | 0.630 | 0.370 | **0.003*** | 37.0 |
| Bharti et al., 2013 [22] | OC | 180 | 75 | 80 | 25 | 230 | 130 | 360 | 0.638 | 0.362 | 0.56 | 36.2 |
| Welsh et al., 2009 [23] | NMSC | 819 | 318 | 353 | 148 | 989 | 649 | 1638 | 0.603 | 0.397 | **0.001*** | 39.7 |
| Dilmec et al., 2008 [24] | CRC | 162 | 108 | 43 | 11 | 259 | 65 | 324 | 0.800 | 0.200 | **0.02*** | 20.0 |

CRC, Colorectal Cancer; HCC, Hepatitis-related hepatocellular carcinoma; HCV, hepatitis C virus infection; BC, Breast Cancer; CC, Cervical cancer; HCC, Hepatocellular carcinoma; PC, Prostate cancer; OC, Oral Cancer; GC, Gastric Cancer; GCA, Gastric cardia adenocarcinoma; DTC, Differentiated Thyroid Carcinoma

trogen and progesterone are regulatory hormones for the developmental process of the mammary which results in proliferation and differentiation of cell growth. Although these hormones promote the tumorigenic process [27]. In the case of blood cancer, leukemia progression has been reported for many years; however, the actual mechanisms of hematological cancer remain unknown [28].

The CTLA-4 gene rs231775 (+49 A>G) is a widely studied polymorphism that has been identified as a susceptibility locus for autoimmune diseases. The fundamental function of the immune system is to control the progression of malignancies [24]. Immune responses are attenuated by CTLA-4 after activation of T-cells, and mutation of these regulatory molecules leads to autoimmune disease in humans [29]. Alternatively, the association of CTLA-4 gene variants and their splicing has been observed, and the role of CTLA-4 in the immune checkpoint leads to the downregulation of the immune response. The survival rate can be improved in cases of carcinoma of renal cell, head, and neck squamous cell, non-small cell lung cancer, and melanoma by blocking the immune checkpoint of the CTLA-4, and acts as a target gene for the treatment of cancer in the future. Polyphenols are one of the most effective phytochemicals that have been applied for the treatment of cancer, hepatitis, diabetes, kidney disease, brain disorders, etc., via gene targets that are involved in the pathophysiology of complex diseases [30].

A study reported that high-level of CTLA-4 expression found in breast tumor tissues is a prediction of the worst prognosis via antitumor immunity suppression. It was observed from other studies that CTLA-4 overexpression is more than 50% of breast tumor tissues as compared to that of benign breast tissues. These observations supporting the CTLA-4 involvement could be in the developmental processes of cancer progression. Numerous studies have been conducted on the functions of genes that encode proteins involved in immune regulation, such as CTLA-4 polymorphisms, which are associated with tumors, autoimmune diseases, and hepatitis B [31].

The effect of CTLA-4 polymorphic alleles on the functions and protein expressions. However, various studies have reported a correlation between the risk of breast tumors and CTLA-4 (+49 A>G) polymorphisms. Expression of this gene has different effects in the process of different phases of T-cell response, reaching the level of threshold for T-cell activation, by suppressing T-cell proliferation, which induces the processes of apoptosis. It was observed that the polymorphisms rs231775 (+49 A>G) were found as a potential risk factor in HCV-induced HCC in the population of Pakistan [9].

The SNP rs231775 (+ 49 A>G) is linked to a high risk associated with different types of cancers. Several other studies have linked the rs231775 gene to various cancers, including colorectal, hepatocellular carcinoma, lung, cervical, prostate, oral, gastric, hepatitis C virus infection, gastric cardia adenocarcinoma, non-melanoma skin cancer, and differentiated thyroid carcinoma [5, 9-24]. The SNP rs231775 was observed to be significantly associated with the Saudi Arabian population [8], confirming early findings [9, 13, 18, 19, 21, 23, 24].

According to Shabbir et al. [9] observed that the effect of polymorphism (rs231775) found in the exonic region is susceptible to HCC. It was found that the genotype AG of rs231775 in HCV-induced HCC patients was found at high frequencies; however, in the case of control samples, the dominant genotype was GG. The GG genotype plays a protective role in the case of HCV-induced HCC patients [9].

According to Wang et al. [18], it was found that the correlation of CTLA-4 polymorphism rs231775 (+49 A>G) and risk of colorectal cancer. The associations were observed significantly among the population of Asians, but not in the populations of Caucasians Wang et al., [18].

It was observed by Dilmec et al. [24] that a decreased CTLA-4 rs231775 +49AA genotype frequency in CRC patients, showing a correlation of this polymorphism with a high risk of CRC. However, no significant association was observed with CTLA4 +49 G polymorphisms in colorectal cancer risks in the patients of Italian Caucasians [32] and populations of Iran [33].

A study was carried out by Farbod et al. [19] for the determination of correlation between polymorphisms AA, AG, and GG of the CTLA-4 gene associated with breast cancer risks. It was observed that the AA and GG genotype frequencies in patients with breast cancer are higher as compared to controls, while the genotype AG is commonly found in healthy controls. Such observation confirms the other two studies and their findings conducted in Iran [34] and China [6], which observed that genotype GG is commonly found in patients with breast cancer. Reported in one study by Erfani et al. [35] regarding genotype AG, they did not observe any differences in their study groups. In China, other studies suggest the genotype AG is more prevalent in patients with breast cancer [36].

According to Antczak et al. [21], CTLA-4 +49 A>G Polymorphism analysis showed that there is a limitation in terms of their functions. SNPs located at +49 A>G are associated with the transition of adenine to guanine (A>G) found in exon 1 of the CTLA-4 gene that codes the leading sequences of CTLA-4 protein, corresponding to a substitution of amino acid Thr to Ala. In this way, it can be correlated with antitumor immune responses more effectively and decrease the risk of cancer.

In a study conducted by Welsh et al. [23], the polymorphism rs231775 (+49 A>G) was found to have an impact on the susceptibility of the US population to developing non-melanoma skin cancer.

The discrepancy among various studies is attributed to factors such as the assessed populations being of different ethnic groups; various methods of genotyping may affect the results; several studies have deviated from the HWE; and study design methods for each study were different, resulting in a reduction in consistency. However, cancers and other diseases in humans have different inheritance patterns.

The progression of diseases and their consequences on genetic and epigenetic alterations, copy number variation, and the influences of various environmental factors. The different prevalence of this rs231775 (+49 A>G) SNP among the populations suggests that the factors of susceptibility have various effects on the different population groups.

In the present analysis, the frequencies of alleles and genotypes studied do not show the overall ranges of variants at the same location. However, such a study provides insight into the developmental process of clinical and epidemiological databases for prospects. Genome-wide association studies (GWAS) and genetic correlation analyses

have revealed that common alleles and their frequencies significantly contribute to the heritable components of many prevalent complex diseases. This highlights the importance of understanding allele distribution in relation to disease susceptibility and genetic risk factors.

The association of several genetic model tests is needed for the identification of essential genes and their corresponding SNPs participating in the progression and early development of therapeutic interventions of the disease progression, leading to possible treatments.

However, there are several shortcomings, including statistical applications and computational analysis, as well as reproducibility factors, that must be considered for essential genetic markers used in gene-disease research associations that could be identified [37].

The allelic variants of the rs231775 (+49 A>G) polymorphism in Saudi Arabia's population substantially vary from different populations in the world. The extracted findings can be applied for the screening of cancer populations, as well as in the assessment and prediction of disease progression significantly, and could be used as a potential biomarker in the progression of cancer.

**Acknowledgments**
The research was conducted at the Department of Basic Medical Sciences, Faculty of Applied Medical Sciences, Al-Baha University, Al-Baha, Saudi Arabia.

**Funding**
No fund was received for this study.

**Availability of data and materials**
The data generated in this study may be requested from the corresponding author.

**Authors contributions**
MSA and RAA conceptualized the study. AT, AAAS and DKM retrieved the data from NCBI and arranged the same. FK, MAS, and AAZ help in writing the manuscript and making modifications. AMK and AMA cross-checked the manuscript and modified the English language. MSA and RAA- have seen and can confirm the authenticity of the raw data. All authors read and approved the final version of the manuscript. MSA, Mohammad Salman Akhtar; RAA, Raed A. Alharbi; AT, Arshi Talat. AAAS, Abdulmajeed AA Sindi; DKM, Dina K. Marghani; FK, Faisal Klufah; MAS, Mohammad A. Shanawaz; AAZ, Ali A Zaeri; AMK, Abdulbaset M Kabli; and AMA, Abdulmohsen M Alruwetei.

**Ethics approval and consent to participate**
Not applicable

**Patient consent for publication**
Not applicable.

**Competing interest**
The authors declare that they have no conflict of interest.

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