ORIGINAL ARTICLE

Circulating Cytotoxic T lymphocytes associated antigen-4 (CTLA-4) levels and CTLA-4 gene polymorphism role in head and neck squamous cell carcinoma

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ABSTRACT

Aim*:* To determine the function of soluble CTLA-4 and the CTLA-4+49A/G gene polymorphism in patients with head and neck squamous cell carcinoma.

Materials and Methods: The current study recruited 180 subjects (90 patients with HNSCCs and comparable number apparently healthful persons as reference) from February 2024 to February 2025.These subjects selected from Medical City, Baghdad, Iraq. All individuals' genomic DNA was extracted from blood samples. To evaluate soluble (sCTLA-4), an enzyme-linked immunoassay kit was used. The Tetra-Primer Amplification Refractory System-Polymerase Chain Reaction (T-ARMS-PCR) was used to genotype and amplification of the CTLA-4 gene using specific primers.

Results*:* As opposed to controls (median = 389.5 ng/ml, range = 160-1966 ng/ml), the median serum level of sCTLA-4 in patients was 1593.5 ng/ml (range = 1118-2000 ng/ml), which was noticeably higher. There was a significant difference (p=0.024) in the frequency of patients with the AA genotype compared to those did not (67.78% *vs.* 50%). Conversely, controls had a significantly higher frequency of the heterozygous genotype (AG) than patients (37.78% vs. 28.89%) (OR= 0.20, 95%CI=0.05-0.76, p=0.018). At allelic level, G allele was far more frequent in controls than patients (31.11% versus 17.78%).

Conclusions: The G allele and the heterozygous genotype (AG) may be regarded as protective factors from HNSCC, and AA genotype is risk factor for HNSCC. Soluble CTLA-4 rise remarkably in patients with HNSCC suggesting a function for this protein in pathogenesis of this disease.

KEY WORDS: Head and neck squamous cell carcinoma, CTLA-4+49A/G, sCTLA-4, polymorphism

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INTRODUCTION

Squamous cell carcinoma is the most common histological type of head and neck malignancies, which are a diverse group of tumors that arise in the head and neck area. The majority of these malignancies originate from the squamous cells lining the mucosal surfaces of the upper aerodigestive tract, collectively referred to as head and neck squamous cell carcinoma [1]. It accounted for about 890,000 new cases and 450,000 fatalities, making it the seventh most prevalent cancer globally. Tobacco and alcohol use, and HPV infections are the primary risk factors for HNSCC [2]. This cancer type's oncogenesis and evolution are significantly influenced by the immune system. Interestingly, the immunosuppressive tumor microenvironment causes immunological escape via a number of methods [3]. In HNSCC immunosuppression, PD-1/PD-L1 and Cytotoxic T lymphocytes antigen-4 (CTLA-4) are the main immunological checkpoints. It is well known that Cytotoxic T lymphocyte-Associated Antigen-4 (CTLA-4; CD152), a homologue of CD28, contributes to the reduction in T cell activation and clonal proliferation. Antigen-presenting cells' ligands, CD80 and CD86 can normally bind to either CD28 or CTLA-4, causing a costimulatory or co-inhibitory response, respectively [4]. Cytotoxic T lymphocytes antigen-4(CTLA-4) is only expressed on the surface of activated T cells, down-regulates the stimulatory signals from CD28 and competes for binding to B7 family members i.e. B7.1 and B7.2. This co-inhibitory molecule also interferes with signals delivered by T cell receptor (TCR). There are two types of CTLA-4 inhibitory tech-

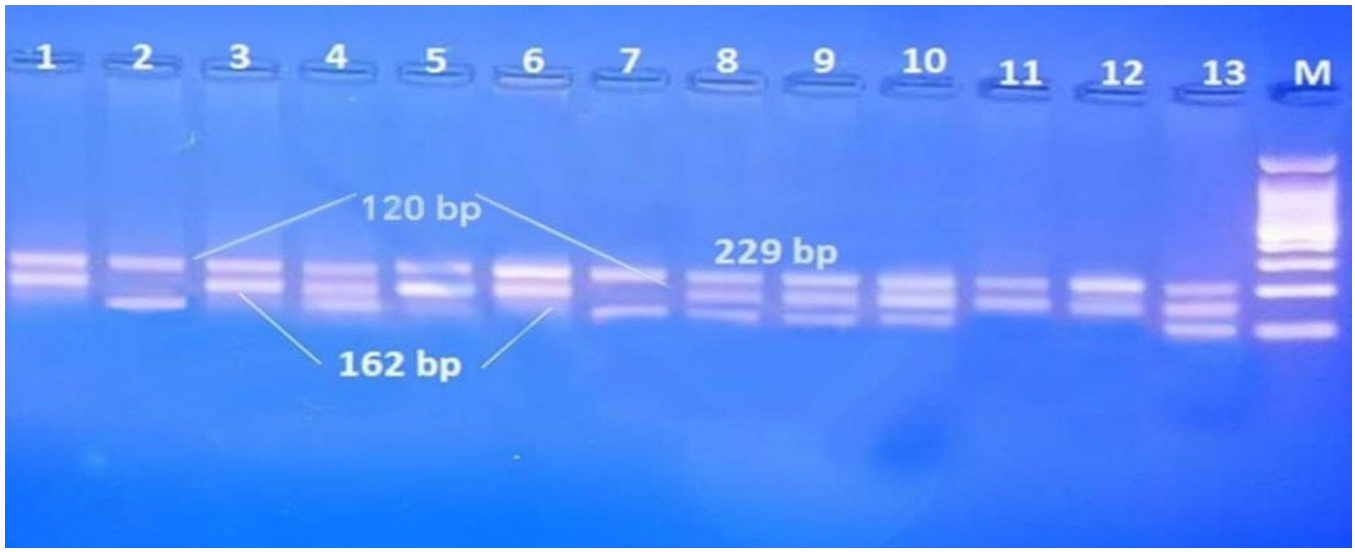

Table 1. The sequences of CTLA-4 +49A/G primers and the length of the resultant fragments employed for T- ARMS-PCR amplification of genes [15]

Primers	Sequence (5'-3')	Fragment length
Outer primers	Forward:49 Fo 5'-GTGGGTTCAAACACATTTCAAAGCTTCAGG-3' Reverse:49 Ro 5'-TCCATCTTCATGCTCCAAAAGTCTCACTC-3'	229bp
Inner primers	49Fi(G) 5'-GCACAAGGCTCAGCTGAACCTGGATG-3' 49Ri(A) 5'-ACAGGAGAGTGCAGGGCCAGGTCCTAGT-3	A162bp G 120bp

Source: Own material

niques: cell-intrinsic (which target the T-cell that expresses CTLA-4) and cell-extrinsic (which target secondary cells) [5]. Chromosome 2q33 is dedicated to the CTLA-4 gene, which has four exons [6]. The CTLA-4+49A/G genotype has been connected to a number of cancer disorders risk e.g., malignant bone tumors [7], nasopharyngeal carcinoma [8], oropharyngeal carcinoma [9-10]. As an alternative, cytotoxic T-lymphocyte protein 4 (s CTLA-4) is produced in a soluble form by spliced mRNA. Serum levels of sCTLA-4 are modest in normal human serum, but they have been discovered to be raised in a number of malignant illnesses [11-12]. Reduced surface expression of CTLA-4 protein and lower amounts of soluble CTLA-4 mRNA are linked to the G allele, a functional polymorphism of CTLA-4 [13–14].

MATERIALS AND METHODS

STUDY POPULATION

180 subjects registered in this case- control study, encompass 90 patients with HSCCs and 90 healthy participants. All subjects enlisted at National Al-Amal Hospital for Oncology and Baghdad Center for Radiation – Medical City (Baghdad, Iraq) during period from February 2024 to February 2025. Diagnosis was executed by examined clinically and assert by histopathological diagnosis.

ETHICAL APPROVAL

Ethical approval to perform the research acquired from scientific and ethical committees/College of Medicine / University of Diyala (No: 2025HHR901 Date: 19/3/2025).

DATA AND SAMPLE COLLECTION

Every individual who has previously participated in the study gave their written consent. Each subject's age, gender, and smoking status were obtained through direct interview using a pre-made formula. Two halves of five milliliters of venous blood were taken out. Serum was separated in a gel tube comprising the first portion (3 ml) that was employed for detection of s CTLA-4 by Enzyme-Linked Immunosorbent Assay. Two milliliters of the second component were placed in an EDTA tube and kept at -80 °C, till needed for DNA extraction for amplification of CTLA-4+49A/G.

SERUM PREPARATION AND STORAGE

After being stored at room temperature (15–25°C) for 20 minutes to guarantee complete clotting, the tubes were centrifuged for 10 minutes at 1900 x g at 4°C. Lastly, to detect sCTLA-4, the supernatant was cautiously moved to a new tube.

Table 2. Demographic features of the study population

Variables	Patients (n=90)	Controls (n=90)	p-value
Age, years Mean ± SD Range	 55.26±15.85 9.0-85	 51.12±10.59 16.0-82	0.041
Sex Male Female	 74(82.22%) 16(17.78%)	 53(58.89%) 37(41.11%)	0.001
BMI (kg/m^2) Mean ± SD Range	 25.03±3.81 18.78-34.37	 25.13±3.76 20.34-31.46	0.819
Smoking Yes No	 41(45.56%) 49(54.44%)	 82(91.11%) 8(8.89%)	<0.001

BMI: Body Mass Index; SD: Standard Deviation
Source: Own material

Table 3. Clinical characteristics of patients with HNSCC

Variables	Frequency	Percentage
Type of cancer		
Laryngeal	30	33.33%
Nasopharyngeal	41	45.56%
Tongue	9	10%
Oropharyngeal	6	6.67%
Others	4	4.44%

Source: Own material

DETERMINATION OF CYTOTOXIC T LYMPHOCYTES ASSOCIATED ANTIGEN-4 (CTLA-4) BY ELISA

Enzyme Linked Immunosorbent Assay (ELISA) (Cat. No: ELK1815, ELK biotechnology, USA) kit for quantitative assessment of human serum CTLA-4. A microtiter plate that has already been coated with an antibody specific to human CTLA4 is included in this kit. After standards or samples have been added to the proper microtiter plate wells, a biotin-conjugated antibody that is specific to human CTLA4 is added. Then, horseradish peroxidase (HRP) conjugated to Avidin is added to each microplate well, and the plates are incubated. When TMB substrate solution is added, only the wells that contain human CTLA4, Color changes will occur in enzyme-conjugated Avidin and biotin-conjugated antibody. A sulfuric acid solution is added to interrupt the enzyme-substrate combination, and the color shift is measured using spectrophotometry at a wavelength of 450 nm ± 10 nm. To determine the amount of human CTLA4 present in the samples, the optical density (OD) of the samples is compared to the standard curve.

ISOLATION DNA AND TETRA-PRIMER AMPLIFICATION REFRACTORY SYSTEM - POLYMERASE CHAIN REACTION (ARMS)

Gsync™ DNA extraction kit fast organizer (Cat. No. GS100, Geneaid, Taiwan) is a commercial kit was used, as instructed by the manufacturer, to extract human genomic DNA. A260/A280, the extracted DNA concentration, was determined by the Biospec Nano spectrophotometer. Using certain primers, the CTLA-4 gene was generated and genotyped (Table 1). The Tetra-Primer Amplification Refractory System-Polymerase Chain Reaction (T-ARMS-PCR) is being used. The PCR settings included a 10-minute initial denaturation at 95°C, 35 cycles of denaturation 94° for 30 sec C, 30 seconds of annealing at 62°C, and a 45-second extension at 72°C. An elongation for seven minutes at 72°C was the last phase [15]. PCR products were appreciated by electrophoresis on a 2% agarose gel and Ethidium bromide was used to stain this results. Following gel electrophoresis, a digital camera and a UV transluminator were used to read the data, sequences of outer and inner primers and length of product illustrated in figure 1.

STATISTICAL ANALYSIS

The statistical software SPSS 25.0 (SPSS, Chicago) was employed to carry out the analyses. Constant statistics were presented as mean and standard deviation unless specified. Student t-test, Mann Whitney U test was used to compare means as required. Categorical variables were expressed as number and percentage and analyzed with Chi-square test. Binary logistic regression was applied to gauge the correlation between HNSCC and the CTLA-4+49A/G gene polymorphism. Based on this test, the odds ratio (OR) and related 95% CI were estimated. Any variation with a p-value below 0.05 was regarded as statistically significant.

AIM

This study's goal was to assess the role of the CTLA4 +49A/G gene variants and soluble CTLA-4 in HNSCC Iraqi population.

Table 4. Median level of sCTLA-4 in patients with HNSCC and controls

Variables	Patients (n=90)	Controls (n=90)	p-value
sCTLA-4, ng/ml			
Mean ± SD	1593.4±228.76	470.5±310.0	
Median	1593.5	389.5	<0.001
Range	1118-2000	160-1966	

Source: Own material

Table 5. The incidence of various genotypes and alleles in patients and controls with CTLA-4 polymorphism

CTLA-4 Polymorphism	Patients (90)	Controls (90)	p-value	OR(95%CI)
Genotypes				
AA	61(67.78%)	45(50%)	0.024	1.0
AG	26(28.89%)	34(37.78%)	0.018	0.20(0.05-0.76)
GG	3(3.33%)	11(12.22%)	0.142	0.36(0.09-1.41)
HWE	0.909	0.260		
Dominant model				
AA+AG	87(96.67%)	79(87.78%)	0.037	1.0
GG	3(3.33%)	11(12.22%)		0.25(0.07-0.920)
Recessive model				
AA	61(67.78%)	45(50%)	0.016	1.0
AG+GG	29(32.22%)	45(50%)		0.48(0.26-0.87)
Alleles				
A	148(82.22%)	124(68.89%)	0.004	1.0
G	32(17.78%)	56(31.11%)		0.47(0.29-0.77)

Source: Own material

RESULTS

THE STUDY POPULATION'S CLINICAL AND DEMOGRAPHIC FEATURES

Table 2 displays the association of demographic factors with HNSCC. Patients were 55.26±15.85 years old on average, which was significantly older than the controls (51.12±10.59 years old). The male-to-female ratio is more equal in controls (58.89% male, 41.11% female), while the majority of HNSSC patients are male 82.22%. The significance of this difference is strong p=0.001. The mean BMI of HNSCC patients 25.03±3.81 kg/m^2 and controls 25.13±3.76 kg/m^2 does not differ significantly p=0.819. There was a highly significant difference p<0.001 between the percentage of smokers among HNSCC patients 45.56% and controls 8.89%.

CLINICAL CHARACTERISTICS OF THE PATIENTS WITH HNSCC

Nasopharyngeal cancer 45.56% is the most common sort among the patients, which is followed by laryngeal cancer 33.33%. The less frequently occurring cancers include tongue 10%, oropharyngeal 6.67%, and other types 4.44% (Table 3).

SERUM LEVEL OF SCTLA-4

It was discovered that the data pertaining to the blood levels of sCTLA-4 was not regularly distributed. As a result, the median and range of these data were used, and the non-parametric Mann Whitney U test was used for analysis. In patients with HNSCC, the median serum level of sCTLA-4 was 1593.5 ng/ml (range = 1118-2000 ng/ml), considerably greater than in controls (median = 389.5 ng/ml, range = 160-1966 ng/ml) (Table 4).

MOLECULAR ASSAYS

This research looked at The SNP CTLA-4+49A/G and HNSCCs: A relationship was examined using the T- ARMS technique. AA, AG, and GG are the three SNP genotypes that were found in both patients and controls (Fig. 1.

CTLA-4 +49A/G GENE POLYMORPHISM WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA

The AA genotype of this polymorphism was more frequent patients than controls 67.78% vs. 50% with a significant difference p=0.024. In contrast, the heterozygous genotype (AG) was more frequent in controls than patients (37.78% *vs.* 28.89%) with a significant difference (OR= 0.20, 95%CI=0.05-0.76, p=0.018). Similarly, the mutant homozygous genotype (GG) was more frequent in controls than patients (12.22 *vs.* 3.33%) although the difference was not significant. The G allele was considerably greater in prevalence

in controls than in patients at the allelic level 31.11% versus 17.78%, with an extremely significant variance (OR=0.47, 95%CI=0.29-0.77, p=0.004) (Table 5).

DISCUSSION

This case-control study was showed the HNSCC patients were significantly older than the controls with mean of age 55 years. In Iraq previous study was demonstrated that head and neck cancer patients increased in the ages from 41 to 60 years old [16]. Globally past report was observed the HNSCCs mean age was in sixth decade of life. Research on carcinogenesis in elderly persons led to the hypothesis that tumor cell transformation is caused by a sequence of spontaneous mutations rather than by increasing exposure to carcinogenetic substances [17]. Males make up a significant part of HNSCC sufferers than females, which agree with another studies substances [18-19]. This may be related to tobacco and alcohol usage. The disparity in working patterns observed in our state may also be a contributing cause to the higher incidence of HNSCC in men than in women. The vast majority of men are engaged in outdoor activities because they work as laborers or farmers, which entails long hours in blazing sun [20]. In comparison to controls, a highly significant difference $p<0.001$ was found in the percentage of smokers among HNSCC patients. This report agrees with other previous studies [19-21]. Numerous carcinogens, including nitrosamines, aromatic amines, aldehydes, and polycyclic aromatic hydrocarbons, are found in tobacco. These compounds, which are created by burning at high temperatures, are known to damage the DNA of oropharyngeal cells, potentially leading to cancer [22]. In current study, the most prevalent cancer type among patients is nasopharyngeal cancer, which is followed by laryngeal cancer, cancers of the tongue, oropharynx, and other forms are less common, this finding comparable with other study in Iraq It revealed that the floor of the mouth had the lowest prevalence and the tongue had the highest [18]. Another study announced the oral cavity accounted for 58.4% of all cancer cases observed in HNSCC patients, with the oropharynx, larynx, hypopharynx, and nasopharynx following closely behind [19]. One of the main elements that stops the autoimmune damage connected to T-cell hyperactivation is the CTLA-4 protein, which acts as an immunological checkpoint. The current study investigated the connection between HNSCC manifestation and one of the circulating T-cell brake and polymorphism rs231775 (c.49A>G) linked to HNSCC susceptibility. This finding demonstrated that the median serum levels of sCTLA-4 in patients were significantly higher than those in controls. Thus, it may be said that sCTLA-4 levels are essential for controlling the immune system. Although sCTLA-4 levels in normal human serum are modest, increased or high levels of the protein are linked to number of cancers [23]. On the other several studies worldwide have showen elevated levels of s CTLA-4 in the patients with certain cancers, like breast cancer acute lymphoblastic leukaemia [24-25] because it accumulates in a wide range of other malignancies, the examination may not be an accurate diagnostic marker even with its relatively high sensitivity and specificity. This result is in contrast to an Iraqi study that involved 45 women with breast cancer diagnoses and 45 healthy women who were paired with patients as a control group. The study indicated a non-significant increase in the serum level of CTLA-4 under $p>0.05$ in patients compared to control in mean ± SD (3.17 ± 0.82 ng/ml) [26]. In the present study, the frequency of various genotypes and allele of CTLA-4+A/G polymorphism in patients and controls revealed significantly more frequent heterozygous AG genotypes in control than patients. Patients were more likely to have the AA genotype than controls, while controls were more likely to have the mutant homozygous genotype (GG), however not statistically considerable distinction was seen. The G allele was much more prevalent in controls than in patients at the allelic level. This study partially agree with previous case-control study discovered single nucleotide polymorphisms (SNPs) in the CTLA-4 gene were genotyped on 301 patients with prostate cancer (PCa) and 301 controls, Carrier of the CTLA4c.49A>G[A] allele and AA genotype were overrepresented in PCa compared to controls ($p=0.082$ and $p=0.13$, respectively) [27]. Globally, another study conducted in Turkey compatible with this finding that observed the homozygous AA genotype for CTLA-4+49A>G were more frequent in patient than controls [23]. Nonetheless, these investigations presented contentious findings regarding the correlation between different cancer types and CTLA-4 polymorphisms [28]. For example, Qi et al. found that a lower risk of colorectal cancer was linked to the CTLA4 +49 AA genotype [29]. Conversely, Cozar et al., [30] found a link between the CTLA4 gene and renal cancer susceptibility and the CTLA4 AA genotype, but no correlation between the CTLA4 gene and colon cancer. According to Sun et al., [31] found Chinese women who carry the A allele are more likely to develop breast cancer and other cancers. Another study by Ghaderi et al. on Iranian women with breast cancer showed that, in comparison to controls, the frequency of the GG genotype was much lower [32]. The CTLA-4+49A/G polymorphism occurs when guanine is substituted for adenine at position 49 in the CTLA-4 gene. Consequently, GCC which encodes alanine, replaces codon 17 (ACC), which encodes threonine. By regulating the total intensity of T-cell activation, the CTLA-4 receptor fulfills a crucial regulatory role during the im-

munological response [33]. Actually, this regulating effect has been explained by two different processes. The first mechanism involves CTLA-4 engaging with its ligands B7.1 and B7.2 to deprive the homologous receptor CD28 of its ligands. The subsequent system involves the signal transduction pathway inhibiting T-cell activation by down regulating T-cell receptor dependent signaling [34]. Numerous phenotypic alterations that impact one or both of these two processes occur when alanine is substituted for threonine. The CTLA-4 protein, which contains alanine, was thought to have a changed spatial structure that results in poor handling of the protein in the endoplasmic reticulum and less effective N-glycosylation [35]. The dimerization and subsequent activation of CTLA4's inhibitory activity depend heavily on this glycosylation [36]. Additionally, some data indicated that patients with autoimmune illnesses had a markedly lower mRNA for the CTLA-4 protein linked to the GG genotype [37]. In addition to downregulation CTLA-4 synthesis, Sun et al., [31] proposed that the G allele produces a protein with a reduced affinity for B7. There is nearly universal consensus that G allele-bearing CTLA-4 has a lower capacity to regulate T-cell activation than A allele-bearing CTLA-4, regardless of the exact mechanism by which this polymorphism influences the immune response. However, this has two sides. The carriers of the +49G variant have the advantage of a strong immunological response and sustained T-cell activation, which may shield them from various infectious agents and even some types of cancer. On the other hand, this variation puts its carriers at risk for a variety of autoimmune conditions. Because it alters the CTLA-4's inhibitory function and results in a Thr/Ala substitution in the leader peptide, the A>G transition at position 49 in exon 1 (CTLA-4c.49A>G) may contribute to antitumor immunity. The presence of [AA] genotype was shown to be associated with significantly lower activation and proliferation of T lymphocytes than [GG] genotype. The protein product coded by CTLA-4c.49A>G[AA] genotype CTLA-4 Thr has more capacity to bind B7.1 and a stronger inhibitory effect on T-cell activation compared with CTLA-4 Ala. The CTLA-4c.49A>G polymorphism in the leader sequence has also been hypothesized to affect intracellular/surface partitioning, CTLA-4 glycosylation, and endocytosis or surface trafficking rates, all of which could change the molecule's inhibitory activity [14, 31, 38, 39].

CONCLUSIONS

CTLA-4 gene polymorphism was significantly associated with HNSCC: CTLA-4 AA genotype consider risk factor for HNSCCs susceptibility. Elevated of serum levels CTLA-4 participate in pathogenesis of HNSCCs. Use sCTLA-4 as Biomarkers: This immune marker shows strong diagnostic value and could assist in early detection or monitoring of HNSCC. Expanding screening methods should be the main focus in future work since it is crucial to better therapy.

RECOMMENDATIONS

Recommended for using another primer in another region of gene for obtain the additional risk factors for HNSCCs and Recommended for diagnose another gene**.** Our findings should be addressed by requiring additional research on a bigger patient population.

REFERENCES

1. Chow LQ. Head and neck cancer. New Eng J Med. 2020; 382(1):60-72. doi: 10.1056/NEJMra1715715. DOI
2. Barsouk A, Aluru JS, Rawla P, Saginala K, Barsouk A. Epidemiology, risk factors, and prevention of head and neck squamous cell carcinoma. Med Sci. 2023;11:42. doi: 10.3390/MEDSCI11020042. DOI
3. Veigas F, Mahmoud YD, Merlo J, Rinflerch A, Rabinovich GA, Girotti MR. Immune checkpoints pathways in head and neck squamous cell carcinoma. Cancers. 2021;13(5):1018. doi: 10.1111/j.1744-313X.2009.00888. DOI
4. Rotte A, Jin J, Lemaire V. Mechanistic overview of immune checkpoints to support the rational design of their combinations in cancer immunotherapy. Ann Oncol. 2018;29:71-83. doi: 10.1093/annonc/mdx686. DOI
5. Van Coillie S, Wiernicki B, Xu J. Molecular and cellular functions of CTLA-4. Adv Exp Med Biol. 2020;1248:7-32. doi: 10.1007/978-981-15-3266-5_2. DOI
6. Brakta C, Tabet AC, Puel M, Pacault M, et al. 2q33 Deletions Underlying Syndromic and Non-Syndromic CTLA4 Deficiency. J Clin Immunol.. 2025; 45(1):1-10. doi: 10.1007/s10875-024-01831-5 DOI
7. Zhang C, Hou WH, Ding XX, Wang X, Zhao H, Han XW Wang WJ. Association of Cytotoxic T-lymphocyte Antigen-4 Polymorphisms with Malignant Bone Tumors Risk a Meta-analysis. Asian Pac J Cancer Prev. 2016;17(8):3785-91.
8. Xiao M, Qi F, Chen X, Luo Z, Zhang L, Zheng C, et al. Functional polymorphism of cytotoxic T-lymphocyte antigen 4 and nasopharyngeal carcinoma susceptibility in a Chinese population. Int J Immunogenet. 2010;37:27-32. doi: 10.1111/j.1744-313X.2009.00888. DOI
9. Kammerer PW, Toyoshima T, Schoder F, Kammerer P, et al. Association of T-cell regulatory gene polymorphisms with oral squamous cell carcinoma. Oral Oncol. 2010;46:543-548 doi: 10.1016/j.oraloncology.2010.03.025. DOI

10. Bharti V, Mohanti BK, Das SN. Functional genetic variants of CTLA-4 and risk of tobacco-related oral carcinoma in high risk North Indian population. Hum Immunol. 2013;74:348-352. doi: 10.1016/j.humimm.2012.12.008. DOI
11. Liu J, Tian X, Wang Y, Kang X, Song W. Soluble cytotoxic T-lymphocyte–associated antigen 4 (sCTLA-4) as a potential biomarker for diagnosis and evaluation of the prognosis in Glioma. BMC Immunol. 2021;22(1):33. doi: 10.1186/s12865-021-00422-y. DOI
12. Liu Q, Hu P, Deng G, Zhang J, et al. Soluble cytotoxic T-lymphocyte antigen 4: a favorable predictor in malignant tumors after therapy. Onco Targets Ther. 2017;2147-2154. doi: 10.2147/OTT.S128451. DOI
13. Ueda H, Howson JM, Esposito L, Heward J, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003;423:506-511. doi: 10.1038/nature01621. DOI
14. Kouki T, Sawai Y, Gardine CA, Fisfalen ME, Alegre ML, DeGroot LJ. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. J Immunol. 2000;165:6606-11. doi: 10.4049/jimmunol.165.11.6606. DOI
15. NarooieNejad M, Taji O, Kordi Tamandani DM, Kaykhaei MA. Association of CTLA-4 gene polymorphisms-318C/T and + 49A/G and Hashimoto's thyroidits in Zahedan, Iran. Biomed Rep. 2017;6(1):108-112. doi: 10.3892/br.2016.813. DOI
16. Idan HM, Motib AS. Incidence of head and neck cancer among Baquba Teaching Hospital Patients. Diyala Journal of Medicine. 2024;27(1):86-96.
17. Sesterhenn AM, Teymoortash A, Folz BJ, Werner JA. Head and neck cancer in the elderly: a cohort study in 40 patients. Acta Oncol. 2005;44(1):59-64. doi: 10.1080/02841860510007431. DOI
18. Al-Kawaz AB. Oral squamous cell carcinoma in Iraq: clinical analysis. MD. 2010;7(1):100-105.
19. Chauhan R, Trivedi V, Rani R, Singh UA. Study of head and neck cancer patients with reference to tobacco use, gender, and subsite distribution. South Asian J Cancer. 2022;11(01):046-051. doi: 10.1055/s-0041-1740601.
20. Pandey A, Singh A, Singh S, Kumar A, et al. Oral smokeless tobacco consumption pattern among rural Indian cancer patients: a prospective survey. South Asian J Cancer. 2020;9(1):17-19. doi: doi: 10.4103/sajc.sajc_40_19. DOI
21. Gislon LC, Curado M P, López RVM, de Oliveira JC, et al. Risk factors associated with head and neck cancer in former smokers: A Brazilian metacentric study. Cancer Epidemiol. 2022; 78: 102143. doi: 10.1016/j.canep.2022.102143. DOI
22. Shunyu NB, Syiemlieh J. Prevalence of Head and Neck Cancer in the State of Meghalaya: Hospital based Study. Int J Head Neck Surg. 2013; 4(1):1-5. doi: 10.5005/jp-journals-10001-1127. DOI
23. Isitmangil G, Gurleyik G, Aker FV, Coskun C, et al. Association of CTLA4 and CD28 gene variants and circulating levels of their proteins in patients with breast cancer. In Vivo. 2016;30(4):485-493.
24. Erfani N, Razmkhah M, Ghaderi A. Circulating soluble CTLA4 (sCTLA4) is elevated in patients with breast cancer. Cancer Invest. 2010; 28(8):828-832. doi: 10.3109/07357901003630934. DOI
25. Simone R, Tenca C, Fais F, Luciani M, et al. A soluble form of CTLA-4 is present in pediatric patients with acute lymphoblastic leukemia and correlates with CD1d+ expression. PLoS One. 2012;7:e44654. doi: 10.1371/journal.pone.0044654. DOI
26. Al-Saadi HHA, Al-Abassi HM, Jawad MM. Role of inhibitory check point (CTLA-4) in Iraqi women with breast cancer. Indian J Foren Med Toxicol. 2021;15(3):2337-2347. doi: 10.37506/ijfmt.v15i3.15664. DOI
27. Karabon L, Tupikowski K, Tomkiewicz A, Partyka A, et al. Is the genetic background of Co-stimulatory CD28/CTLA-4 pathway the risk factor for prostate cancer? Pathol Oncol Res. 2017; 23: 837-843. doi: 10.1007/s12253-016-0180-4.
28. Monne M, Piras G, Palmas A, Arru L, Murineddu M, Latte G, Noli A, Gabbas A: Cytotoxic T lymphocyte antigen-4 (CTLA4) gene polymorphism and susceptibility to non-hodgkin's lymphoma. Am J Hematol. 2004;76:14-18. doi: 10.1002/ajh.20045. DOI
29. Qi P, Ruan CP, Wang H, Zhou FG, Xu XY, Gu X, et al. CTLA-4+ 49A> G polymorphism is associated with the risk but not with the progression of colorectal cancer in Chinese. International journal of colorectal disease. 2010; 25(1): 39-45. https://doi.org/10.1007/s00384-009-0806-z
30. Cozar JM, Romero JM, Aptsiauri N, Vazquez F, et al : High incidence of CTLA4 AA (CT60) polymorphism in renal cell cancer. Hum Immunol. 2007;68(8):698-704. doi: 10.1016/j.humimm.2007.05.002. DOI
31. Sun T, Zhou Y, Yang M, Hu Z, et al. Functional genetic variations in cytotoxic T-lymphocyte antigen 4 and susceptibility to multiple types of cancer. Cancer Res. 2008;68(17): 7025-7034. doi: 10.1158/0008-5472.CAN-08-0806. DOI
32. Ghaderi A, Yeganeh F, Kalantari T, Talei AR, Pezeshki AM, Doroudchi M, Dehaghani AS. Cytotoxic T lymphocyte antigen-4 gene in breast cancer. Breast Cancer Res Treat. 2004;86:1- 7. doi: 10.1023/B:BREA.0000032918.89120.8e. DOI
33. Bour-Jordan H, Grogan JL, Tang Q, Auger JA, Locksley RM, Bluestone JA. CTLA-4 regulates the requirement for cytokine induced signals in Th2 lineage commitment. Nat Immunol. 2003;4(2):182-188. doi: 10.1038/ni884. DOI
34. Van Coillie S, Wiernicki B & Xu J. Molecular and cellular functions of CTLA-4. Regulation of Cancer Immune Checkpoints: Molecular and Cellular Mechanisms and Therapy. 2020; 7:32. doi:10.1007/978-981-15-3266-5_2 DOI
35. Pavkovic M, Georgievski B, Cevreska L, Spiroski M, Efremov DG. CTLA-4 exon 1 polymorphism in patients with autoimmune blood disorders. Am J Hematol. 2003;72(2):147-149. doi: 10.1002/ajh.10278 DOI

36. Darlington PJ, Kirchhof MG, Criado G, Sondhi J, Madrenas J. Hierarchical regulation of CTLA-4 dimer-based lattice formation and its biological relevance for T cell inactivation. J Immunol. 2005;175(2):996-1004. doi: 10.4049/jimmunol.175.2.996. DOI
37. Daroszewski J, Pawlak E, Karabon L, Frydecka I, et al. Soluble CTLA-4 receptor an immunological marker of Graves' disease and severity of ophthalmopathy is associated with CTLA4 Jo31 and CT60 gene polymorphisms. Eur J Endocrinol. 2009; 161(5):787-793. doi: 10.1530/EJE-09-0600. DOI
38. Anjos S, Nguyen A, Ounissi-Benkalha H, Tessier MC, Polychronakos C. A common autoimmunity predisposing signal peptide variant of the cytotoxic T-lymphocyte antigen 4 results in inefficient glycosylation of the susceptibility allele. J Biol Chem. 2002;277:46478-46486 doi: 10.1074/jbc.M206894200. DOI
39. Rashid HH, Salman RE, Ali AY, Khaleq MAA. The significant impact of T-cell immunoglobulin and mucin domain 3 (Tim-3) gene polymorphism on HCV infection and viral load. Wiad Lek. 2025;78(4):845-852. doi: 10.36740/WLek/202971. DOI

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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