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The Role of *KRAS* GTPase Activation and Epstein-Barr Virus Antibodies in the Molecular Etiology and Immune Dysregulation of Lung Cancer

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Abstract

Lung cancer is the largest cause of cancer-related mortality worldwide, constituting 18.4% of all cancer deaths, hence causing considerable economic and societal repercussions. The Epstein-Barr virus (EBV) is the first recognized human oncogenic virus capable of establishing asymptomatic, lifelong persistence. The KRAS gene, a member of the Rat sarcoma oncogene family, accounts for 85% of RAS mutations identified in human cancers and is present in 35% of lung adenocarcinomas (LUADs). This work examines the relationship between EBV IgG seropositivity, KRAS mutation-related immunogenicity, and the genetic polymorphism rs121913238, emphasizing their collective influence on oncogenic signaling, immune evasion, and modifications in the tumor microenvironment. Serum and whole blood samples (n=100) were evaluated via ELISA to measure EBV IgG and KRAS antibody levels, while conventional PCR and Sanger sequencing were utilized for SNP identification in 30 samples. EBV IgG levels were markedly higher in patients than in controls (P=0.0003); conversely, KRAS antibody levels did not exhibit a significant difference between the groups (P=0.187), and no link was detected between KRAS and EBV levels. The rs121913238 polymorphism was absent in the examined samples. Nonetheless, another SNP, rs11836509, was detected in eight samples (six patients and two controls) and was initially linked to lung cancer, necessitating more exploration.

Keywords: Lung cancer, EBV, SNP, gene polymorphism, *KRAS*.

دور تنشيط *KRAS GTPase* والأجسام المضادة لفيروس إبشتاين–بار في الإمراضية الجزيئية واضطراب المناعة المرتبط بسرطان الرئة

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الخلاصة

سرطان الرئة هو السبب الرئيسي لوفيات السرطان على مستوى العالم، حيث يشكل 18.4% من إجمالي الوفيات الناجمة عن السرطان، مما يترتب عليه آثار اقتصادية ومجتمعية كبيرة. يُعد فيروس إبشتاين–بار (EBV)أول فيروس بشري مسرطن تم التعرف عليه، وهو قادر على التسبب في عدوى كامنة تستمر مدى الحياة دون أعراض واضحة. أما جينKRAS ، وهو جزء من عائلة الجينات الورميةRAS ، فهو مسؤول عن

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85% من الطفرات في جينات RAS المرتبطة بالسرطان، ويوجد في 35% من حالات سرطان الغدية الرئوية. يهدف هذا البحث إلى دراسة العلاقة بين إيجابية المصل لـEBV IgG ، والاستجابة المناعية المرتبطة بطفراتKRAS ، وتعدد الأشكال الجينيrs121913238 ، مع التركيز على تأثيرهم المشترك على الإشارات المسرطنة، والتهرب المناعي، والتغيرات في البيئة المكروية للورم. تم تحليل عينات من المصل والدم الكامل (n=100)باستخدام تقنية المقايسة الامتصاصية المناعية للإنزيم المرتبط (ELISA) لقياس مستويات EBV IgGوالأجسام المضادة لـKRAS ، بينما تم استخدام تفاعل البوليميراز المتسلسل التقليدي (PCR) وتقنية سانجر لتسلسل الحمض النووي (Sanger sequencing) لتحديد تعددات الأشكال الجينية (SNPs) في 30 عينة.أظهرت النتائج ارتفاعًا ملحوظًا في مستويات EBV IgG لدى المرضى مقارنةً بعينة الضبط (P=0.0003)، في حين لم تكن هناك فروق ذات دلالة إحصائية في مستويات الأجسام المضادة لـ KRAS بين المجموعتين(P=0.187) ، كما لم يتم الكشف عن أي ارتباط بين مستويات KRAS و .EBVعلاوة على ذلك، لم يتم العثور على تعدد الأشكال الجيني rs121913238 في العينات المدروسة، إلا أنه تم اكتشاف تعدد أشكال جيني آخر، وهوrs11836509 ، في ثماني عينات (ستة مرضى واثنان من مجموعة الاصحاء)، حيث ارتبط مبدئيًا بسرطان الرئة، مما يستدعي مزيدًا من الدراسات الاستقصائية.

1.Introduction

Cancer remains a significant global health concern despite advancements in diagnostic and therapeutic techniques[1]. It is a complex array of carcinogenic diseases capable of metastasizing or invading other regions of the body. It progresses through a multi-stage procedure[2]. Our investigation illuminated the most common type of cancer, lung cancer, which is the primary cause of cancer-related mortality globally. Nonetheless, the incidence and mortality rates of lung cancer vary significantly worldwide, indicative of diverse patterns of tobacco use, environmental risk factor exposure, and genetic predispositions[3]. The delayed identification of this cancer significantly contributes to advanced-stage diagnoses and unfavorable outcomes[4].Tobacco smoking is the primary risk factor for lung cancer. Lung cancer is a condition characterized by uncontrolled cellular proliferation in the lungs, which may metastasize to lymph nodes or other organs, including the brain. Malignancies originating from different organs may metastasize to the lungs[5]It is divided into two major groups, Small-Cell Lung Cancer (SCLC) and Non-Small-Cell Lung Cancer (NSCLC), classified into three types: squamous cell carcinoma, large-cell carcinoma, and adenocarcinoma[6]. Small cell lung cancer (SCLC) constitutes 15–20% of all lung cancer instances, and the rest, 80–85%, have non-small cell lung cancer (NSCLC)[7]. Mutations affecting proto-oncogenes and tumor suppressors, along with the emergence of host immunological dysregulation, are the root causes of the genetic and epigenetic abnormalities that propel lung cancer [8]. Lung cancer generally has a five-year survival rate of less than 20%[9]. The postoperative 5-year survival rate for micro-invasive carcinoma and carcinoma in patients with early-stage lung cancer was about 100% [10]. The 5-year relative survival rate for all lung cancers (non-small cell lung cancer [NSCLC] and small cell lung cancer [SCLC] combined) is 19%, and it is higher for NSCLC (23%) than for SCLC (6%).

Annually, over 1.4 million malignancies generated by viruses are diagnosed. The viruses linked to the highest incidence of cancer include human papillomaviruses (HPVs) and hepatitis viruses HBV and HCV. Additional oncoviruses comprise Epstein–Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), human T-cell leukemia virus (HTLV-I), and Merkel cell polyomavirus (MCPyV)[11]. The Epstein-Barr virus (EBV) is a prevalent, carcinogenic virus linked to several human cancers and autoimmune diseases[12].

EBV belongs to the subfamily Gamma herpesvirinae of Lymphocryptovirus, which is an enveloped gamma herpesvirus, it is a double-stranded DNA virus linked to the onset of

several malignancies, including lymphoma, nasopharyngeal carcinoma, and gastric cancer[13].

The Epstein–Barr virus (EBV) is the first recognized human oncogenic virus, responsible for approximately 200,000 cancer cases and about 1.8% of annual cancer-related fatalities. In the last four decades, accumulating data has substantiated a causal relationship between EBV infection and a subset of lung malignancies (LCs)[14]. The infectious virions are primarily distributed through saliva interchange, although body fluids like blood or urine can convey them. Like most human herpesviruses, vaccine-based blockage of EBV infection is still in the research and development stage and is not a possibility, except for varicella-zoster virus (VZV). It is estimated that around 90% of adult people worldwide are currently infected with EBV[15] According to one theory, EBV might first reach the oral cavity's replication-permissive epithelial cells, where it would then initiate active lytic replication. EBV infects B lymphocytes of the immune system and epithelial cells. The initial epithelial cells subsequently release the propagated infectious virions to infect the surrounding infiltrating B cells, which synthesize the complete spectra of viral latency transcripts, also called type III latency. An alternate theory holds that EBV infects B cells after transmigrating across polarized human oral epithelial cells by apical to basolateral transcytosis without producing lytic replication [16].EBV affects lung cancer by altering cellular metabolism via viral proteins such as LMP1 and LMP2A, enhancing glycolysis, glutaminolysis, and lipid metabolism, facilitating carcinogenesis, viral replication, and immune evasion in the tumor microenvironment [17].

The *KRAS* gene belongs to the family of rat sarcoma viral oncogenes (*RAS*), which also includes the human Harvey and neuroblastoma rat sarcoma viral oncogenes (*HRAS*, *NRAS*) isoforms[18]It is the most frequently mutated RAS isoform. KRAS constitutes 85% of RAS mutations detected in human malignancies and is seen in 35% of lung adenocarcinomas (LUADs)[19]. In 1982, the *KRAS* was identified in lung cancer located on the short arm of chromosome 12 (12p11.1–12p12.1)[20] .80% of cancers linked to the RAS gene are caused by KRAS mutations, which are the most prevalent form of *RAS* mutation[21]*KRAS* mutations are dominated by single-base missense mutations, 98% of which are found at codon 12 (G12), codon 13 (G13), or codon 61 (Q61) [22]. KRAS GTPase is integral to lung cancer progression, facilitating cell proliferation and survival through mutations seen in 25% to 30% of non-small cell lung cancer cases, resulting in dysregulated signaling within the RAS pathway [23]. KRAS GTPase proteins are essential for cellular proliferation and differentiation. In lung cancer, especially non-small-cell lung cancer, KRAS mutations are prevalent, affecting tumor characteristics, therapy efficacy, and patient prognoses, frequently co-existing with additional molecular abnormalities [24]It functions as a molecular switch, with mutations resulting in unregulated cell growth and mitosis [25]. The study aims to analyze the complex molecular interactions among Epstein-Barr virus (EBV) IgG seropositivity, *KRAS* mutation-induced immunogenicity, and the genetic polymorphism rs121913238, emphasizing their combined effects on driving oncogenic signaling pathways, evading immune surveillance, and altering the tumor microenvironment to promote lung cancer initiation, progression, and phenotypic diversity.

Materials and Methods

Subjects and Sampling

Between November 2023 and August 2024, fifty patients infected with lung cancer were recruited from Al-Amal National Hospital for Oncology in Baghdad, Iraq, and the Euphrates Center for Cancerous Tumors in Kufa, Najaf, Iraq. Fifty healthy individuals (25 smokers / 25

nonsmokers) representing the control group with no previous history of any cancer diagnosis were collected from the Baghdad governate. Blood samples were taken and obtained from each patient and control via the vein puncture method. 5 ml of each blood sample was divided into two groups to obtain serum and whole blood, which were used later to detect anti-EBV antibody (serum)/ *KRAS* levels in the serum and for human DNA extraction (whole blood) for subsequent *KRAS* single nucleotide polymorphism detection, and the process was as follows:

Two mL of blood were placed in an anti-coagulated tube containing EDTA to be used to extract human DNA. The extraction of human DNA was done one week to three weeks after sample collection. Separation of serum, 3 mL of blood was transferred into a clot activator gel tube and allowed to clot for ~30 minutes; these tubes were centrifuged for 10 min. at 4000 rpm and stored at -20˚C till used to detect anti-EBV antibodies and *KRAS* levels in the serums.

Detection of Anti-EBV IgG and KRAS level by Enzyme-Linked Immunosorbent Assay (ELISA)

In the serum samples from the entire study group (44 lung cancer patients and 32 for controls), anti-EBV antibody (IgG) and *KRAS* levels were tested via ELISA technique; the immunological part for evaluation of Anti-EBV IgG was accomplished by using the ELISA technique by the manufacturing companies (SunLong/China) and (My bioSource / USA).

Molecular Detection by Conventional PCR

DNA was extracted using a Blood Genomic DNA Extraction Kit (Spin column) (China). The molecular analysis was first achieved by conventional PCR using a specific primer (forward: 5’-TCCACTGCTCTAATCCCCCA-3’ / reverse: 5’-CCCACCAGCAATGCACAAAG-3’ for (rs121913238). The PCR methodology for amplifying the KRAS gene (rs121913238) commenced with an initial denaturation step at 95°C for 5 minutes to guarantee the complete separation of the DNA template strands. This was succeeded by 35 cycles of denaturation at 95°C for 15 seconds to promote strand separation. Annealing transpired at 58°C for 30 seconds, facilitating the precise hybridization of primers to the target sequence. The extension step occurred at 72°C for 1 minute, during which Taq polymerase created the complementary DNA strand. A concluding extension at 72°C for 5 minutes guaranteed the resolution of any residual incomplete DNA fragments. The cycle parameters were refined to improve the amplification of the KRAS gene area for subsequent investigation.

After PCR, the amplicons were validated by gel electrophoresis employing a 0.5–1.5% agarose gel. Before sequencing, DNA purification was conducted utilizing the GenepHlow™ Gel Extraction Kit (Taiwan) to eliminate impurities and unincorporated primers. The concentration and purity of DNA samples were assessed using a nanodrop spectrophotometer and Quantus flurometer. The concentration of DNA samples was from 5.26-27.62 ng/µl, and the purity was from 1.8-2.

Sequencing

Sanger sequencing was performed on PCR amplicons using an ABI 3730xl automated sequencer at Macrogen Corporation, South Korea. Geneious software was then used to evaluate the sequencing data that were produced for the detection of *KRAS* single nucleotide polymorphisms (rs121913238).

Statistical Analysis

The Kolmogorov-Smirnov and Shapiro-Wilk tests were conducted to assess the normality of the data distribution. Categorical data were represented as counts and percentages, whereas nonparametric variables were presented as median and interquartile range (IQR). The Kruskal-Wallis H test and the Mann-Whitney U test were utilized to evaluate significant differences among the medians of the research groups. One-way ANOVA and two-way ANOVA, accompanied by Tukey's test as a post hoc analysis, were conducted to evaluate the significant differences among the means of the research groups. Receiver operating characteristic (ROC) analysis was conducted to determine the area under the curve (AUC), 95% confidence interval (CI), cut-off value, sensitivity, and specificity. The likelihood ratio was employed to refine the cut-off value. Differences were deemed significant when the P value was less than or equal to 0.05. The statistical analysis was conducted with GraphPad Prism 9.5 [26]. The Hardy-Weinberg equilibrium analysis was used to predict the distribution of genotypes and calculate allele frequencies.

Results

Distribution of the studied samples according to their age

The statistical analysis revealed a significant difference (p-value <0.0001) between patients and controls. The highest occurrence of lung cancer diagnoses was noted in individuals above 65 years of age (Figure 1). The mean value in the healthy control was (37.36) ng/ml, while that of the patient group was (61.68) ng/ml, the median value in patients was (64), and for the controls, it was (39) ng/ml. The standard deviation for patients was (15.10) ng/ml and (12.9).

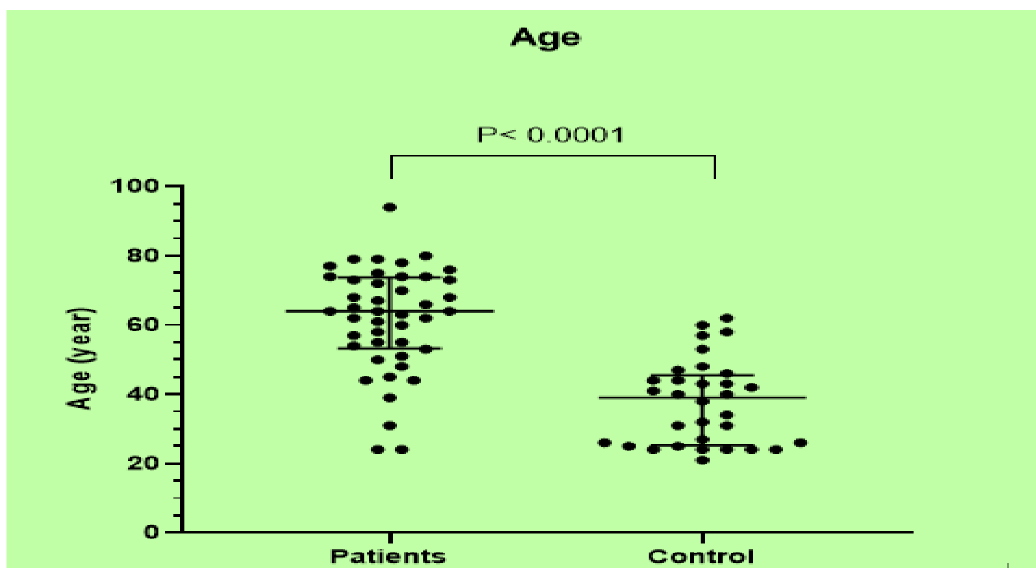


Figure 1: The age distribution between lung carcinoma patients and healthy individuals.

Detection of the immune response (IgG) to EBV by ELISA

The Enzyme-Linked Immunosorbent Assay was employed to detect anti-EBV antibodies (IgG) in blood samples from the study groups (patients and controls) stratified by age, sex, smoking status, and chemotherapy treatment. The statistical analysis indicates a highly significant difference in EBV IgG concentrations among the groups (Figure 2), with a p-value of 0.003. The mean concentration for patients was 14.96 ng/ml, while for the control group, it was 11.54. The median values were 12.52 ng/ml for patients and 8.90 for controls. The interquartile range (IQR) was 4.884 for patients and 3.511 for controls, reflecting greater variability in the control group (Figure 3), revealing a significant difference between female patients and male controls, with a p-value of 0.0042, and between male patients and male

controls, with a p-value of 0.014. The mean values for female patients, male patients, female controls, and male controls were (15.5, 14.55, 11.98, and 11.03) ng/ml, respectively. There was a statistically significant difference in smoking status among smoking patients compared to smoking controls, smoking patients versus non-smoking controls, non-smoking patients against non-smoking controls, and non-smoking patients in comparison to smoking controls (Figure 4), with p-values of 0.02. The mean values for smoking patients and non-smoking patients were 14.32 and 11.41, respectively. The statistical analysis revealed no significant difference in chemotherapy responses in lung carcinoma patients (Figure 5), with a mean value of (14.57) ng/ml for patients who received chemotherapy and (18.03) ng/ml for patients who hadn't received it.

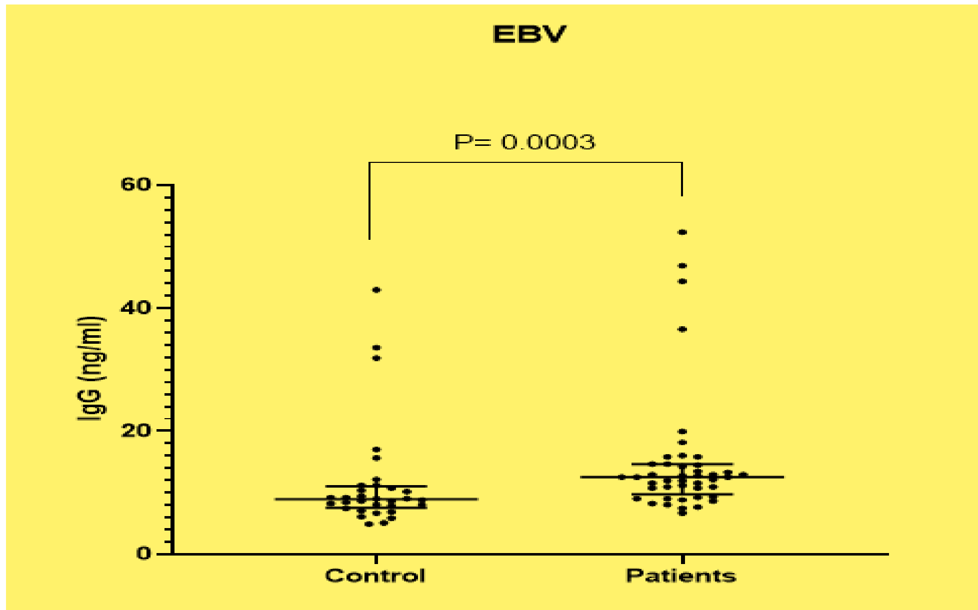


Figure 2: Comparison of EBV IgG levels between patients and controls, p-value = 0.0003 (significant).

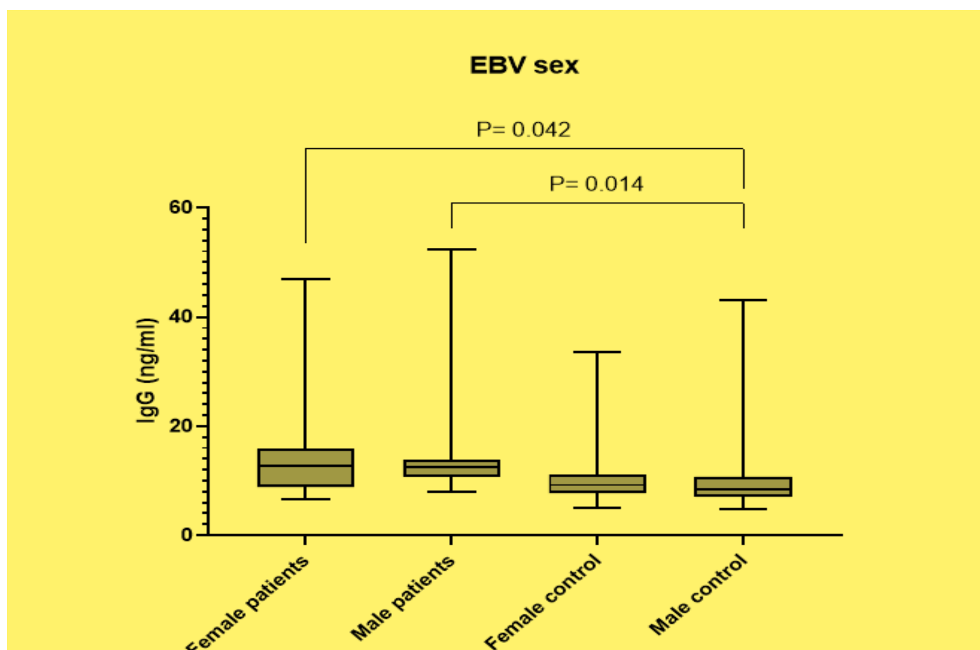
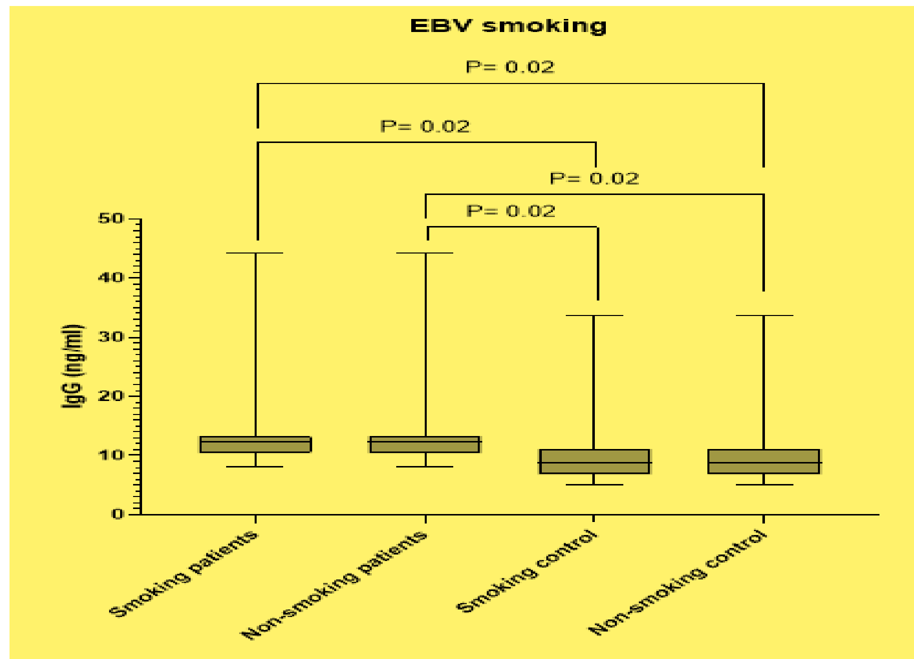


Figure 3: Comparison between patients and controls EBV IgG levels according to sex, p-values = 0.042 and 0.014 (significant)



. Figure 4: Comparison between patients and controls EBV IgG levels according to their smoking status, p-value = 0.02 (significant).

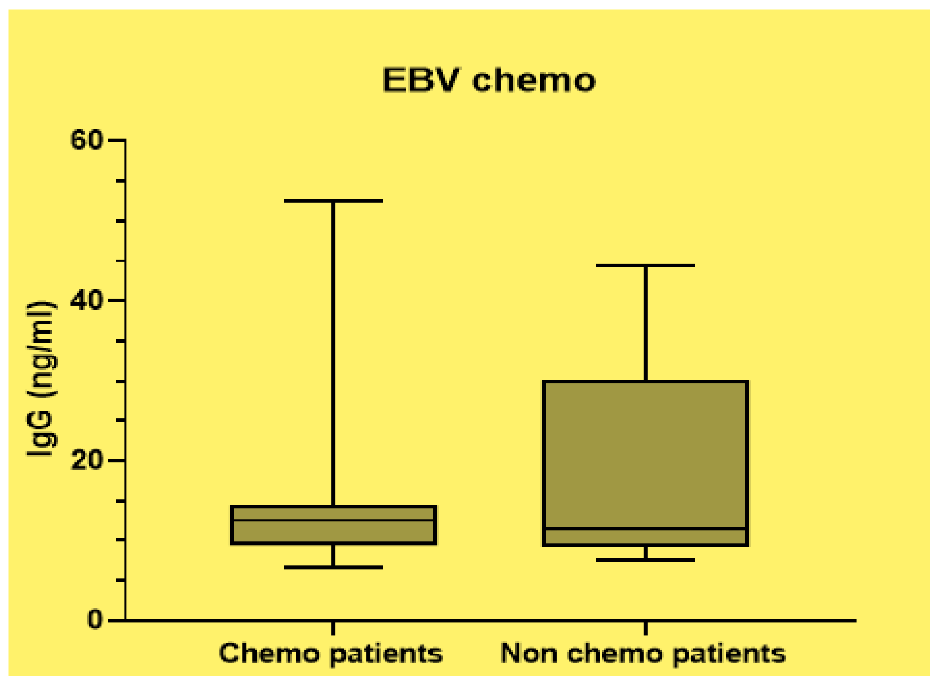


Figure 5: Comparison between patients and controls EBV IgG levels according to chemotherapy treatment p-value = 0.9787 (not significant).

Detection of the immune response (IgG) to KRAS by ELISA

In contrast to EBV, *KRAS* IgG levels did not differ significantly between patients and controls. The median concentration was 8.911 ng/ml for patients and 14.00 ng/ml for controls, with mean values of 12.32 ng/ml and 14.81 ng/ml, respectively (P = 0.187). The interquartile range (IQR) was 11.862 for patients and 15.279 for controls (Figure 6). The statistical analysis showed no significant differences when analyzed according to sex (Figure 7), smoking status (Figure 8), and chemotherapy treatment (Figure 9). When analyzed according

to sex, the mean values for the female patients, male patients, female controls, and male controls were (11.92, 12.63, 13.78, and 15.97) ng/ml, respectively. For the smoking status, the mean values were (12.83 ng/ml) for smoking patients, (11.81ng/ml) for non-smoking patients, (15.77ng/ml) for smoking controls, and (14.06 ng/ml) for non-smoking controls. For the chemotherapy treatment, the mean for patients who received chemotherapy was (12.05 ng/ml), and (14.41 ng/ml) for patients who hadn't received chemotherapy.

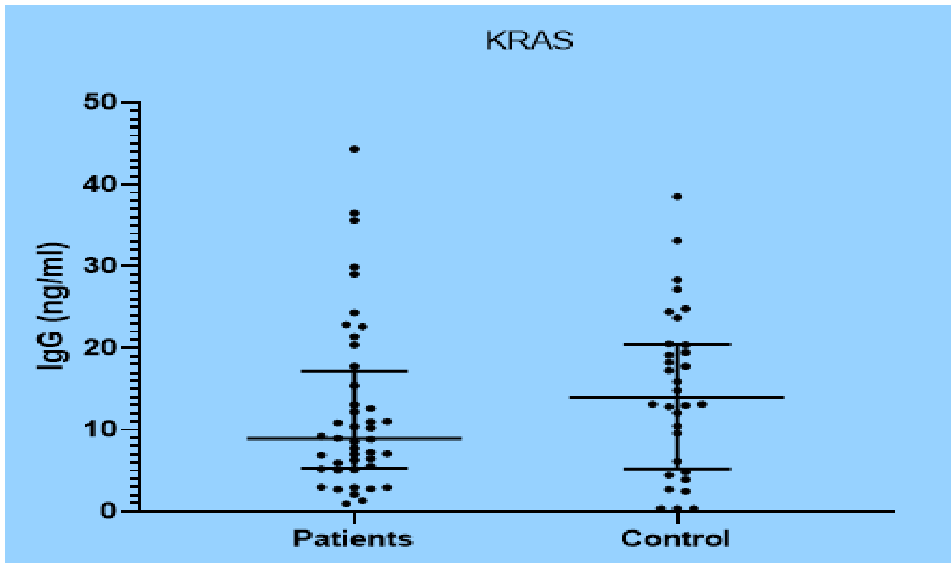


Figure 6 : Comparison of *KRAS* IgG levels between patients and controls p-value = 0.1870 (not significant).

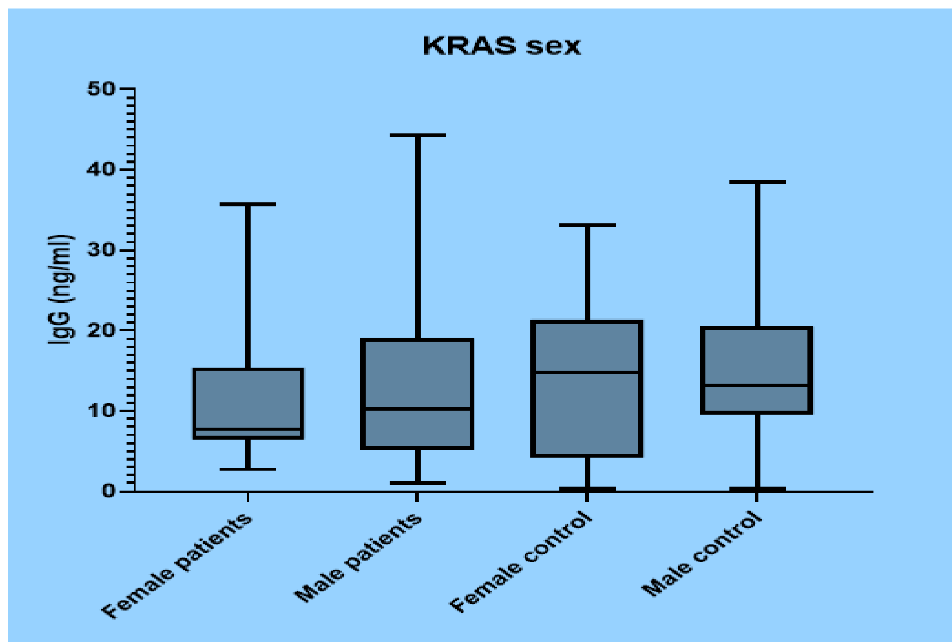


Figure 7: Comparison between patients and controls *KRAS* IgG levels according to sex p-value = 0.532 (not significant).

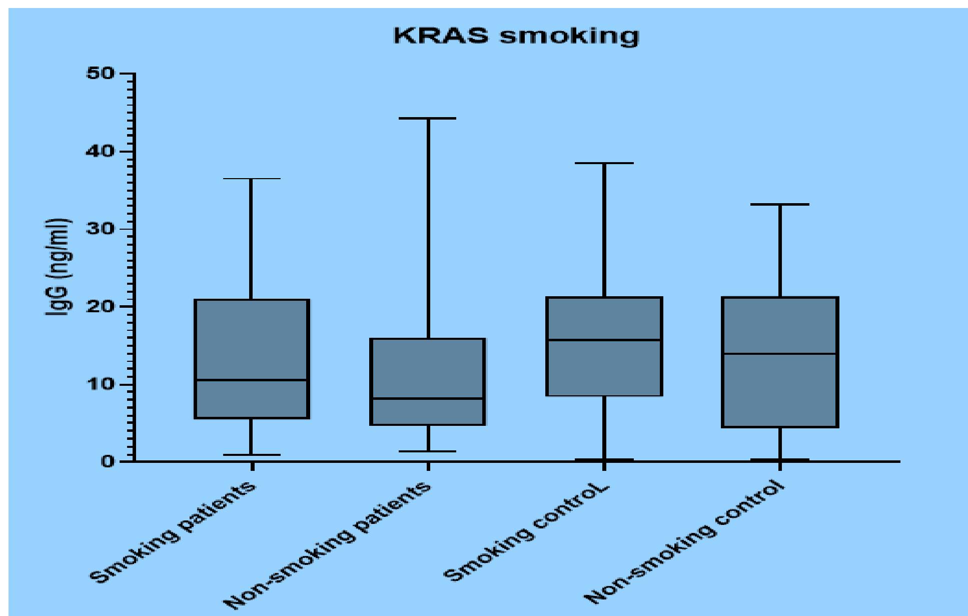


Figure 8: Comparison between patients and controls *KRAS* IgG levels according to their smoking status p-value = 0.5119 (not significant)

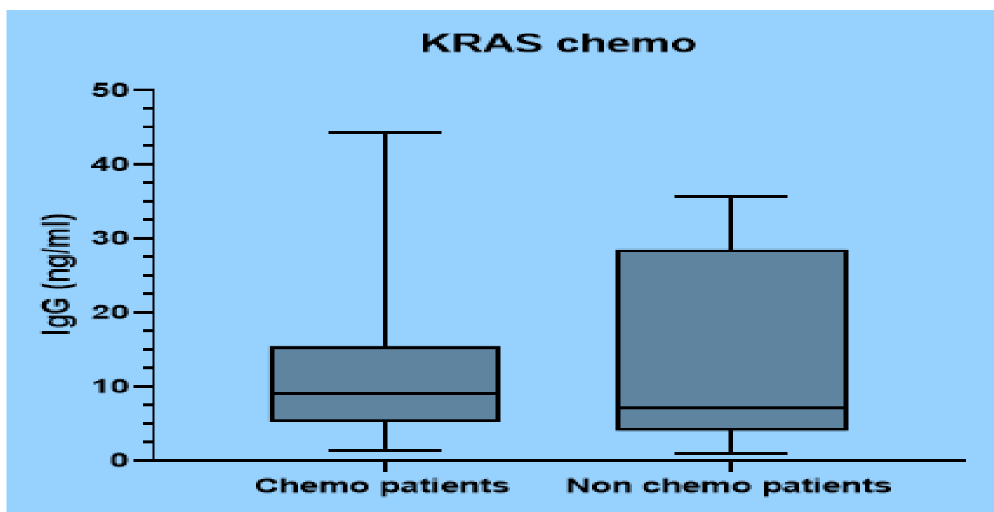


Figure 9: Comparison between patients and controls *KRAS* IgG levels according to chemotherapy treatment p-value = 0.9971 (not significant)

Receiver operating characteristics ROC for EBV and KRAS in lung carcinoma patients and healthy controls

KRAS exhibits limited diagnostic utility, evidenced by an AUC of 0.5895 and non-significant results (P = 0.185) (Figure 10) with a sensitivity of 54.55% and specificity of 71.88%. In contrast, EBV shows superior performance with an AUC of 0.7397 (P = 0.0004) (Figure 11) with a sensitivity of 663.64% and specificity of 81.25%.

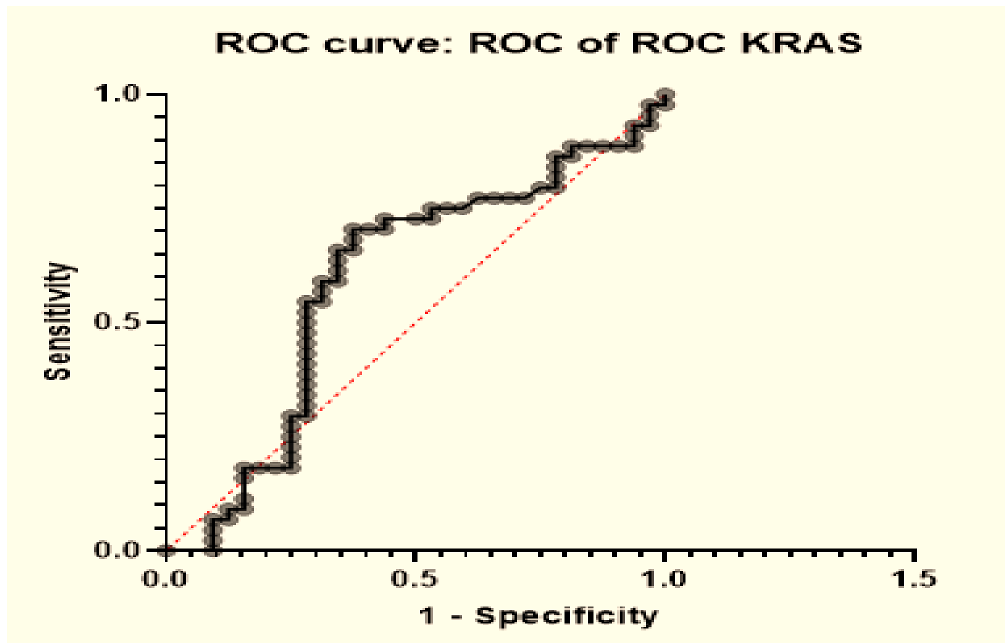


Figure 10 : Receiver operating characteristics curve of *KRAS* IgG antibody in the samples collected from lung carcinoma patients and healthy controls

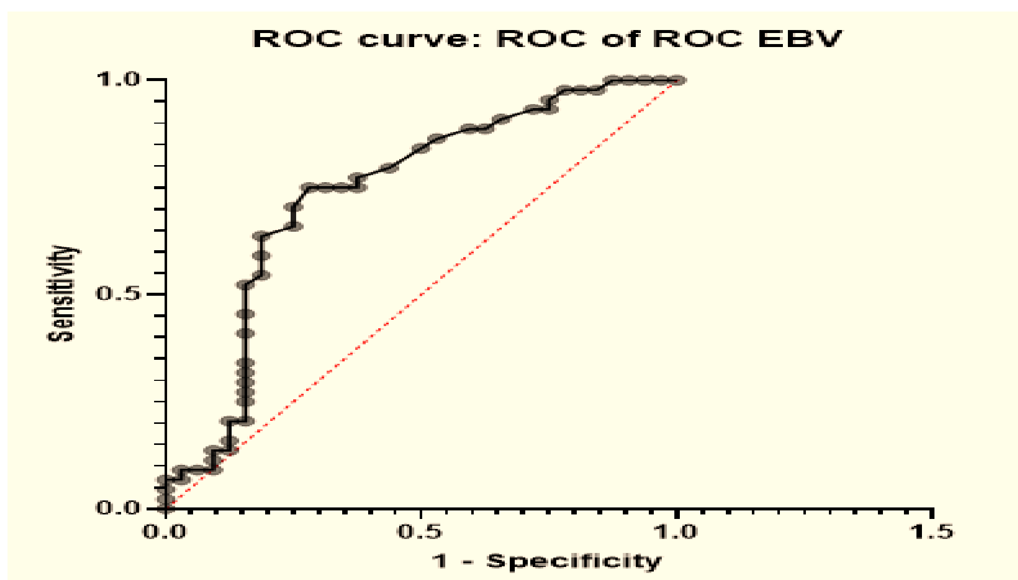


Figure 11: Receiver operating characteristics curve of EBV IgG antibody in the samples collected from lung carcinoma patients and healthy controls

Studying the correlation between EBV and KRAS in the study samples

The study analyzed *KRAS* and EBV levels in control and patient groups, focusing on statistical comparisons and potential relationships. Normality tests revealed distinct distributions for *KRAS* and EBV in both groups. The Mann-Whitney test was used to compare *KRAS* and EBV levels between groups. In the control group (Figure 12), no significant difference was observed between *KRAS* and EBV (p-value =0.0723). In contrast, the patient group demonstrated a statistically significant difference between *KRAS* and EBV levels (Figure 13) (p-value=0.0074). In the control group, *KRAS* had a median value of 14.00 ng/ml and a mean value of 14.81ng/ml, while EBV had a median of 8.906 ng/ml and a mean of

11.54 ng/ml. In the patient group, *KRAS* had a median of 8.911 ng/ml and a mean of 12.32 ng/ml, and EBV had a median of 12.52 ng/ml and a mean value of 14.96 ng/ml. EBV levels were consistently higher in patients than controls, with less variability than *KRAS*.

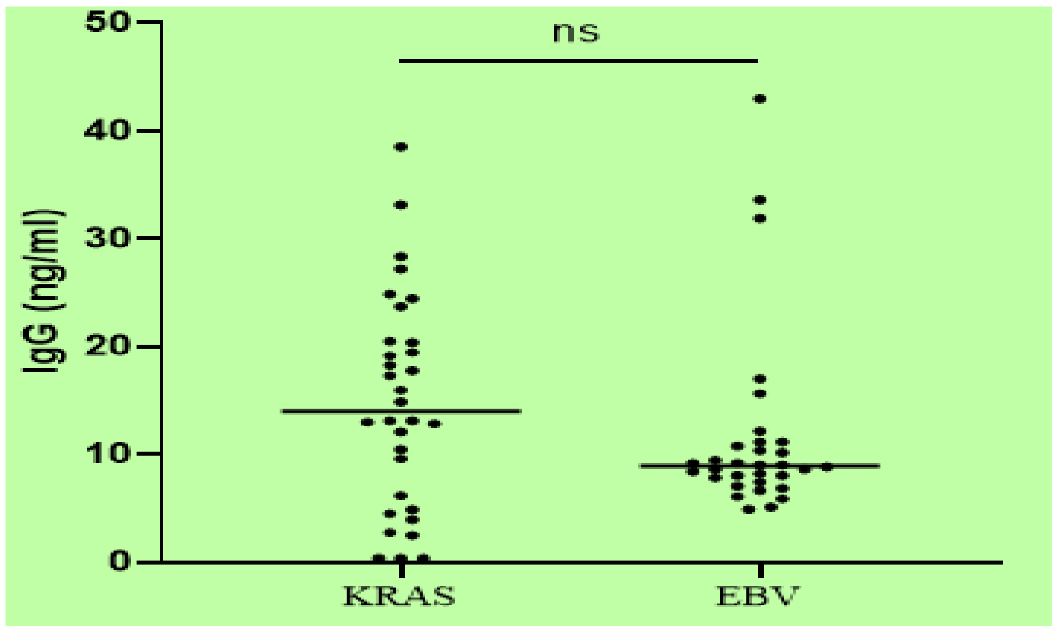


Figure 12: Comparison of *KRAS* and EBV levels in the control group

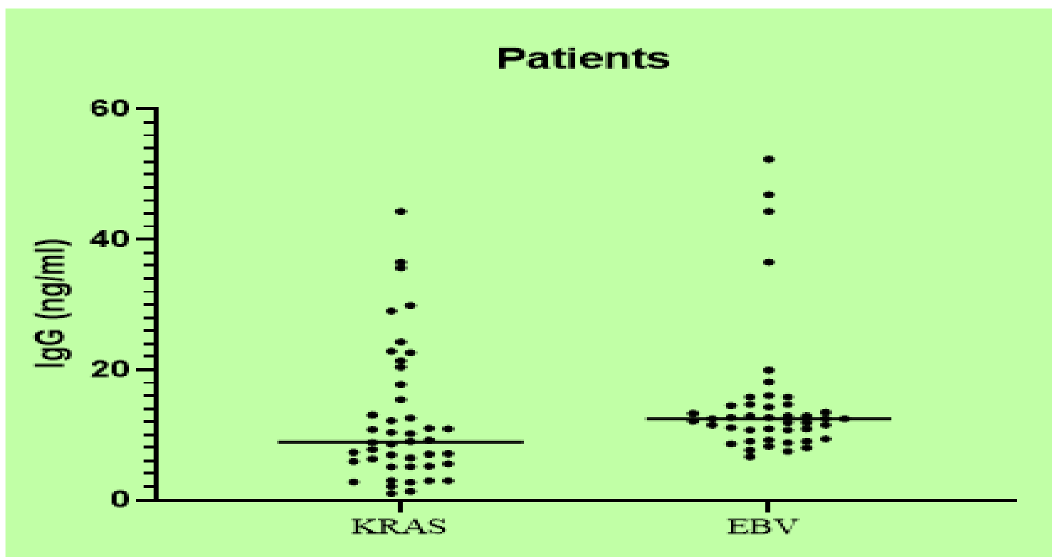


Figure 13 : Comparison of *KRAS* and EBV levels in lung carcinoma patients

Detection of the (rs121913238) of KRAS gene in Lung carcinoma patients and healthy controls by Conventional PCR and gene polymorphism by Sanger sequencing

The detection of the SNP at location 915 in the selected participants from both studied groups (15 patients and 15 controls), who were previously tested for anti-*KRAS* antibodies via ELISA, was accomplished through conventional PCR amplification of the target gene. All samples exhibited homozygosity for GG (Figure 14); However, another SNP at position 709 was identified in 8 samples (6 patients and 2 controls) within a distinct gene region, designated as rs11836509 (Figure 15). The -709 G/G genotype ratio is 0.6 (60%). -709 T\G 0.2 (20%) and -709 T\T 0.2 (20%). The genotype ratio of -709 G\G in the control group is 0.866 (86.6%). -709 T\G 0.066 (6.6%); -709 T\T 0.066 (6.6%). The allele frequency of -709

G in patients was 0.7, whereas in controls, it was 0.9. For the -709 T allele, the frequency in patients was 0.3, while in controls, it was 0.1. Patients have a lower frequency of the *G* allele and a higher frequency of the *T* allele compared to controls. This suggests that the *-709 T* allele may be more common in patients. Similarly, the G/G genotype was much more common in controls (86.6%) than in patients (60%). Conversely, the T/G and T/T genotypes were more frequent in patients. The unexpected SNP that appeared in our analysis was associated with lung carcinoma, according to a study carried out by *Khono et al.,* [27] in Japan and another study done in Russia by Antontseva *et al* [28] confirmed its association.

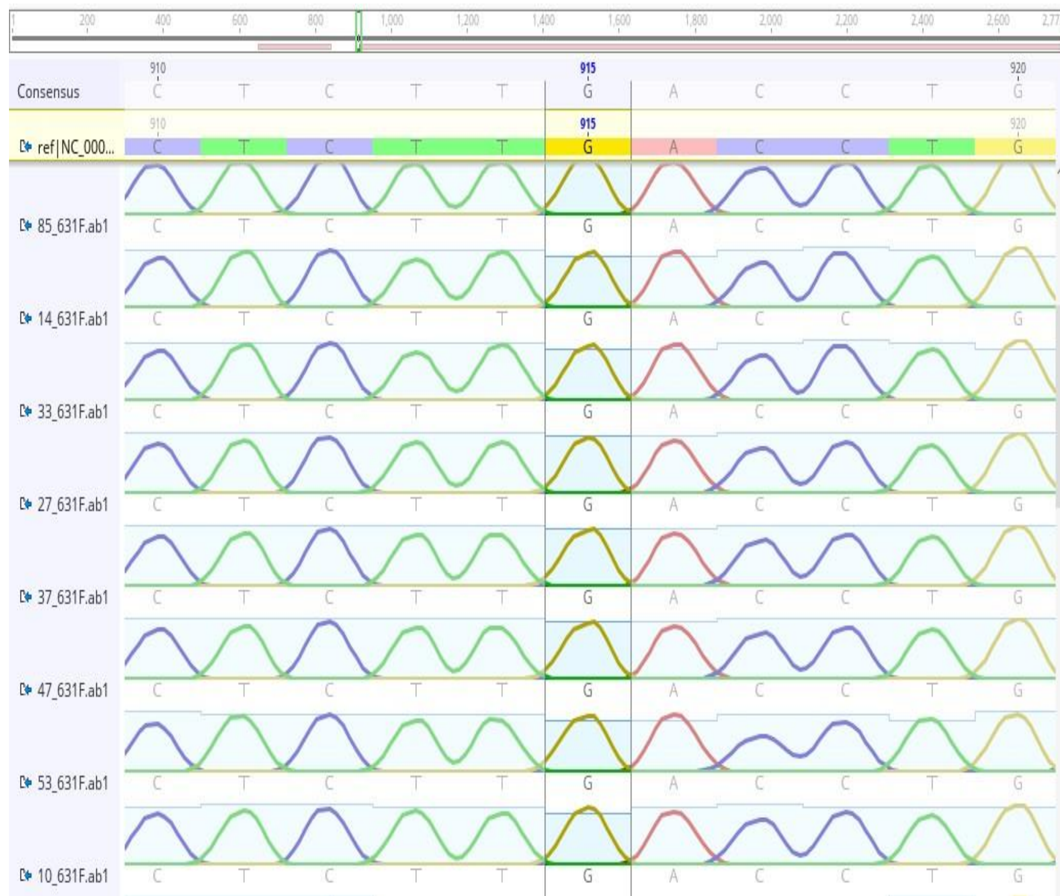


Figure 14 : Analysis of the SNP rs121913238 of the *KRAS* gene using Sanger sequencing. A single “G” peak is indicative of a G homozygous allele. All samples were homozygous GG.

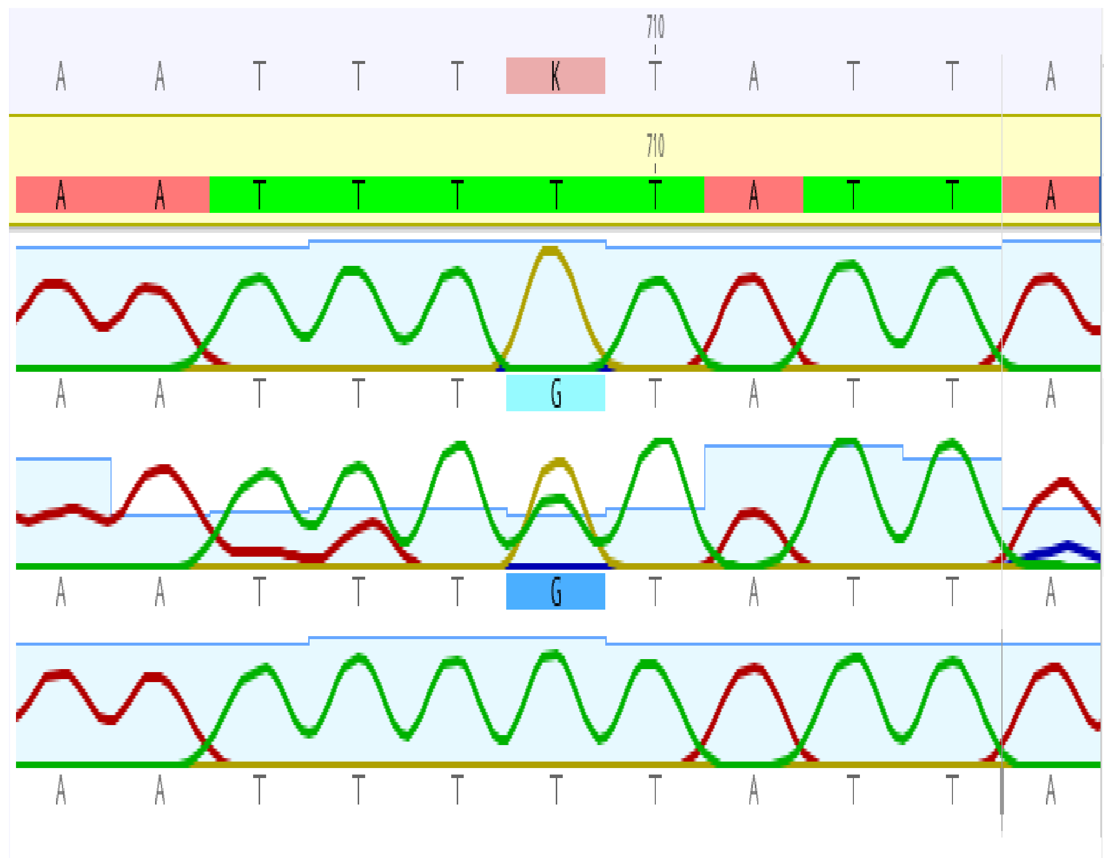


Figure 15: Analysis of the SNP rs11836509 of the *KRAS* gene using Sanger sequencing. A single "G" peak is indicative of a *G* homozygous allele. A Single "T" peak is indicative of a *T* homozygous allele. The presence of the "G" and "T" peaks is indicative of the G/T heterozygous allele, also known as *K*.

Discussion

This work offers new insights into the relationship between Epstein-Barr virus (EBV) IgG and KRAS IgG levels in lung cancer patients, a hitherto unexamined region. The results demonstrated markedly elevated EBV IgG levels in patients relative to controls, with a median of 12.52 ng/ml compared to 8.906 ng/ml and a mean of 14.96 ng/ml versus 11.54 ng/ml, respectively. The statistical significance (P = 0.0003) highlights the possible role of EBV reactivation in the development of lung cancer. The interquartile range (IQR) was 4.884 in patients, signifying reduced variability relative to controls (IQR = 3.511). The findings align with the concept that persistent EBV reactivation plays a role in oncogenesis via mechanisms such as inflammation, immunosuppression, and genomic instability [29]and elevated EBV IgG levels are frequently observed in malignancies, autoimmune disorders, and chronic inflammatory diseases due to an immunological response[30]

The *KRAS* IgG levels exhibited no significant difference between patients and controls, with median values of 8.911 and 14.00 and mean values of 12.32 and 14.81, respectively (P = 0.187). The variability was indicated by the standard deviations (SD), which were 10.34 for patients and 9.88 for controls. The area under the curve (AUC) for *KRAS* in the receiver operating characteristic (ROC) study was 0.5895, signifying restricted diagnostic efficacy. The AUC for EBV was 0.7397 (P = 0.0004), demonstrating its enhanced sensitivity and specificity as a biomarker for lung cancer.
No significant link between EBV and *KRAS* was identified in control samples (P = 0.0723), but a significant difference was noted in patient samples (P = 0.0074). This discovery

indicates that EBV and *KRAS* may interact variably based on the illness state, however, they seem to operate via separate and independent carcinogenic pathways. Our data may suggest that EBV-associated lung cancer and KRAS-driven lung cancer are separate molecular subgroups of the illness rather than part of a unified pathway. The heightened EBV IgG levels indicate a vigorous immunological response to EBV reactivation in lung cancer patients, potentially facilitating tumor formation via mechanisms such as persistent inflammation and immune evasion. KRAS-driven lung tumors predominantly advance via oncogenic signaling, with minimal contribution from the adaptive immune response, which accounts for the lack of raised KRAS IgG levels in patients.

Another possible explanation is that EBV infection may indirectly influence the course of lung cancer without directly impacting KRAS-driven carcinogenesis. Prior research indicates that viral infections may affect the tumor microenvironment, disrupt immune surveillance, and enhance genomic instability, thereby facilitating KRAS-mediated oncogenesis.

The specific SNP (rs121913238) was absent in all samples analyzed. Additionally, another SNP (rs11836509) was detected in 8 samples (6 patients and 2 controls). The genotype distributions indicated that the G/G genotype was more prevalent in controls (86.6%) compared to patients (60%). In contrast, the T/G and T/T genotypes were more prevalent in patients (T/G = 20%, T/T = 20%) than in controls (T/G = 6.6%, T/T = 6.6%). The allele frequency of T was greater in patients (30%) compared to controls (10%), suggesting a potential risk link.

The amalgamation of EBV IgG, *KRAS* IgG, and SNP data yields significant insights into the pathophysiology of lung cancer. Elevated EBV IgG levels indicate a robust immune response, perhaps associated with viral reactivation. The new link of rs11836509 with lung cancer underscores the necessity for further exploration of its functional role. Although there is no association between EBV IgG and *KRAS* IgG levels, the interaction between viral reactivation and genetic predisposition (e.g., SNP rs11836509) may produce a synergistic effect that aids in disease progression.

Statistical studies were conducted using Mann-Whitney U tests, Kolmogorov-Smirnov tests, and Shapiro-Wilk tests.The Kolmogorov-Smirnov test indicated significant differences between groups for EBV IgG (KS distance = 0.3216, $P < 0.0001$). The Shapiro-Wilk test showed that EBV IgG did not conform to a normal distribution ($W = 0.6004$, $P < 0.0001$). The absence of normalcy for *KRAS* IgG was similarly validated ($W = 0.8451$, $P < 0.0001$), while no significant differences were seen across the groups. The statistical validations emphasize the study's robustness.

Study Strengths and Limitations

The merits of this work are in its originality and thorough examination. This work represents the inaugural investigation of the link between EBV IgG and KRAS IgG levels in lung cancer patients, addressing a significant gap in the literature. The utilization of ELISA for serological assessments and PCR/Sanger sequencing for genetic analysis guarantees dependable and accurate data acquisition.

Nonetheless, some limits must be recognized: (I) Sample size: an expanded patient cohort is necessary to corroborate these findings and enhance statistical power. (II) Absence of functional investigations. Although this work establishes a link (or lack thereof) between

EBV and KRAS, the mechanistic interactions between these factors in lung cancer have not been investigated.

No longitudinal follow-up. Evaluating EBV and KRAS IgG levels over time may yield insights into their influence on illness progression and therapy efficacy. Prospective Trajectories

Our findings indicate many critical topics for future research that warrant consideration: Examining the molecular connections between Epstein-Barr virus infection and KRAS mutations through cell culture and animal models. Investigating the impact of EBV infection on tumor immune evasion in KRAS-driven lung malignancies. Evaluating the diagnostic and prognostic significance of EBV IgG levels in extensive, multi-center investigations. Additional characterization of the rs11836509 polymorphism to ascertain its influence on KRAS gene regulation and immunological function.

Conclusion

This research emphasizes the importance of EBV IgG as a prospective biomarker for lung cancer and identifies the rs11836509 SNP as a genetic risk factor. Although *KRAS* IgG demonstrated restricted diagnostic value, its genetic variants provide significant insights into lung cancer susceptibility. The incorporation of numerical data enhances the evidence base. It highlights the complex nature of lung cancer, stressing the necessity for additional study into these interconnections to advance diagnoses and treatment techniques.

Ethical Approval

This study was approved by the local ethical committee in the University of Baghdad, College of Science, Department of Biology, Reference No. CSEC/0222/0007) on (29/ 10/ 2023) and the Iraqi health ministry.

Conflict of interest statement

The authors declare they have no conflict of interest.

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