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# Detection of HTLV-1 and HTLV-2 and their association with soluble Human Leukocyte Antigen-G in adult Iraqi lymphoma patients.

Abdulrahman H. Al-Rawi [1] , Zainab A. Hamid [2] , Alaadin S. Naji [3]
[1,2,3] Collage of Medicine, University of Baghdad, Baghdad-Iraq
[1)]Email: abd.abd2310m@comed.uobaghdad.edu.iq [2)]Email: Zainaba.hamid@comed.uobaghdad.edu.iq
[3)]Email: dr.alaa_1972@yahoo.com

**Abstract:**

**Background:** Hodgkin (HL) and non-Hodgkin lymphoma (NHL) are major lymphoid malignancies. HTLV-1 has been more strongly linked to oncogenesis than HTLV-2. Soluble HLA-G (sHLA-G) is an immunoregulatory molecule implicated in tumor immune evasion. **Objectives**: Detection presence of HTLV-1 and HTLV-2 genomes in Iraqi lymphoma patients, depending on correlation with immunological factor SHLA-G. **Methods:** This Cross-sectional study was carried out between November 2024 to March 2025. A total of 180 patients with lymphoma were divided into 114 non-Hodgkin lymphoma and 66 Hodgkin lymphoma. The patients were recruited from the National Center for Blood Diseases, Blood and Bone Marrow Transplantation Center at the Teaching Hospital, Medical City, Baghdad. We use a sandwich ELISA test to determine SHLA-G quantity in patient serum samples. We compared sHLA-G levels with RT-PCR results to assess associations between the viral (HTLV-1 and -2) and immunological marker. HLA-G ELISA kit, Reed Biotech, RE3072H. The kit procedure, and use Human T-lymphotropic-1 (HTLV-1) Nucleic Acid Detection Kit Shanghai Keshun Science and Technology Co.,Ltd KS52112-48. Human T-lymphotropic virus 2 (HTLV-2) Nucleic Acid Detection Kit.Shanghai Keshun Science and Technology Co.Ltd KS51112T. RNA Viral Genome Extraction Kit, Solarbio, Cat No.: R2000. **Inclusion criteria**: 1-Adult Iraqi patient (male and female) with Hodgkin lymphoma and non-Hodgkin lymphoma. 2-Lymphoma patient with Hepatitis B virus, Hepatitis C virus, or Human immunodeficiency virus (HIV). **Excluding criteria**: 1- Patients have or have had previously any malignancy, except lymphoma. , 2- Haemato-oncology patients with non-lymphoid malignancies were excluded. **Results:** The number of lymphoma patients shows different concentrations of soluble human leukocyte antigen-G. We selected 29 patients with the highest concentration of soluble human leukocyte antigen-G. After viral genome extraction used RT-PCR to test 16 patients for HTLV-1 and none for HTLV-2.Additionally, the majority of lymphoma patients tested negative for HBV and HCV. Regarding sHLA-G profiles, no significant differences were observed between HTLV-1-positive and negative lymphoma participants. **Conclusion:** Depending on the results of my study, we don't have a real correlation between viral infection and the level of soluble human leukocyte antigen-G in non-Hodgkin nor Hodgkin lymphoma, while HTLV-2 didn't show any relationship. Regarding HBV and HCV, the screening tests reduced the total number of

**Keywords**: Lymphoma, HTLV-1, sHAL-G, TaqMan probe RT-PCR.

## الكشف عن عدوى فيروسات الخلايا اللمفاوية التائية البشرية من النوع الاول والثاني فيما يتعلق بعامل المناعة القابل للذوبان – G في مرضى الليمفوما العراقيين البالغين

عبد الرحمن حمدي الراوي[1)] ، زينب عبدالحسين حامد[2)] ، علاء الدين سهام ناجي[3)] // كلية الطب، جامعة بغداد، بغداد - العراق [1,2,3)]

**مستخلص:**

**الخلفية:** يُعدّ كلٌّ من ليمفوما هودجكين (HL) وليمفوما اللاهودجكين (NHL) من الأورام اللمفاوية الخبيثة الرئيسية. وقد ارتبط فيروس HTLV-1 بتكوين الأورام بشكل أقوى من HTLV-2. يُعدّ HLA-G القابل للذوبان (sHLA-G) جزيئًا منظمًا للمناعة، ويُشارك في التهرب المناعي من الأورام. **الأهداف:** تحديد وجود جينات HTLV-1 وHTLV-2 باستخدام اختبار تفاعل البوليميراز المتسلسل العكسي (RT-PCR) باستخدام مسبار TaqMan، وارتباطه بتعبير HLA-G لدى مرضى الليمفوما العراقيين. **المنهجية:** أُجريت هذه الدراسة المقطعية بين نوفمبر 2024 ومارس 2025. وقُسّم ما مجموعه 180 مريضًا مصابًا بالليمفوما إلى 114 مريضًا مصابًا بالليمفوما اللاهودجكينية و66 مريضًا مصابًا بالليمفوما الهودجكينية. وقد جُنّدَ المرضى من المركز الوطني لأمراض الدم، ومركز زراعة الدم ونخاع العظم في المستشفى التعليمي، مدينة الطب، بغداد. نستخدم اختبار الإليزا الشطيري لتحديد كمية SHLA-G في عينات مصل المرضى. قارنا مستويات sHLA-G بنتائج تفاعل البوليميراز المتسلسل العكسي (RT-PCR) لتقييم الارتباط بين الفيروس (HTLV-1 وHTLV-2) والعلامة المناعية.

طقم اختبار الإليزا HLA-G، ريد بيوتك، RE3072H. إجراءات الطقم، واستخدام طقم الكشف عن الحمض النووي البشري T-lymphotropic-1 (HTLV-1)، شركة شنغهاي كيشون للعلوم والتكنولوجيا المحدودة، KS52112-48. طقم الكشف عن الحمض النووي البشري T-lymphotropic-2 (HTLV-2). شركة شنغهاي كيشون للعلوم والتكنولوجيا المحدودة، KS51112T. طقم استخلاص الجينوم الفيروسي RNA، سولاربيو، رقم الكتالوج: R2000. **معايير الإدراج:** 1. مريض عراقي بالغ (ذكر وأنثى) مصاب بلمفوما هودجكين ولمفوما لاهودجكين. ، 2. مريض لمفوما مصاب بفيروس التهاب الكبد الوبائي ب، أو فيروس التهاب الكبد الوبائي ج، أو فيروس نقص المناعة البشرية (HIV). **معايير الاستبعاد:** 1. وجود أورام خبيثة سابقة لدى المرضى، باستثناء اللمفوما. ، 2. استُبعد مرضى أورام الدم المصابين بأورام خبيثة غير لمفاوية. **النتائج:** أظهر عدد مرضى اللمفوما تركيزات مختلفة من مستضد الكريات البيضاء البشرية الذائب-G. اخترنا 29 مريضًا لديهم أعلى تركيز من مستضد الكريات البيضاء البشرية الذائب-G. بعد استخلاص الجينوم الفيروسي، استخدمنا تفاعل البوليميراز المتسلسل العكسي العكسي لاختبار 16 مريضًا للكشف عن فيروس التهاب الكبد الوبائي ب-١، ولم نختبر أي مريض للكشف عن فيروس التهاب الكبد الوبائي ج-2. بالإضافة إلى ذلك، جاءت نتائج اختبارات غالبية مرضى اللمفوما سلبية لفيروس التهاب الكبد الوبائي ب وفيروس التهاب الكبد الوبائي ج. وفيما يتعلق بملفات sHLA-G، لم تُلاحظ أي فروق جوهرية بين مرضى اللمفوما الإيجابية والسلبية لفيروس التهاب الكبد الوبائي ب-1. الاستنتاج: بناءً على نتائج دراستي، لا توجد علاقة حقيقية بين العدوى الفيروسية ومستوى مستضد الكريات البيضاء البشرية الذائب-G في لمفوما هودجكين ولا هودجكين، بينما لم يُظهر فيروس HTLV-2 أي علاقة. فيما يتعلق بفيروس التهاب الكبد B وفيروس التهاب الكبد C، قللت اختبارات الفحص من العدد الإجمالي للمشاركين، ولا يُظهر مستضد HLA-G الذائب أي علاقة مباشرة في التسبب في لمفوما هودجكين لدى المصابين بفيروس HTLV-1.

## Introduction:

Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) are separate types of blood malignancies that originate in lymphocytes. [1]

HTLV-1 is a retrovirus that has significant health concerns; however, it is often overlooked, with little public health initiatives aimed at preventing its transmission and alleviating its consequences upon infection (2).

Human T-cell lymphotropic virus type 2 (HTLV-2) is a delta-retrovirus closely related to HTLV-1, sharing many genomic and structural similarities. However, unlike HTLV-1, which is associated with severe diseases such as adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[3]

Some neurological disorders and atypical hairy-cell leukaemia have been associated with HTLV-2[4].

Amerindian tribes and intravenous drug users are endemic for HTLV-2, which is transmitted similarly to HTLV-1.[5]

HLA is the Human Leukocyte Antigen System, regulated by genes on chromosome six's short arm.[6]

In viral infections, the non-classical HLA class I molecule soluble human leukocyte antigen-G (sHLA-G) facilitates immunological tolerance and may help viruses evade the immune system [7].

Various viral infections have elevated sHLA-G levels, indicating its role in illness etiology and development [8].

This study examined HTLV-1 and -2 infection rates and risk factors. Due to the global expansion of HTLV-1 and HTLV-2, it also assessed the risk of getting other blood-borne viruses.

## Materials and Methods:

Sample size and study design: This cross-sectional study of 180 adult lymphoma patients (98 male and 82 female). Involved 114 Non-Hodgkin lymphoma and 66 Hodgkin lymphoma admitted to blood and bone Marrow implantation centres at the Teaching Hospital at Medical City in Baghdad, National Centre for Blood Diseases / AL-Mustansiriya University, AL-Imamain AL-Kadhimain Medical City

between November 2024 and March 2025 was conducted. Patients were 18–83 years old.

The University of Baghdad College of Medicine Department of Microbiology. The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics Committee authorized this research (Institutional Review Board-Reference Number (0251 – 3/7/2025). All individuals provided informed consent before blood sampling. All patients' information, including lymphoma type and sociodemographic information, was obtained from a 5-10 ml blood sample using standard medical protocols. A gel separation tube was used to collect each blood sample, which was then centrifuged at 3000 rpm for 20 minutes. T

**Soluble Human Leukocytes Antigen-G kit information.**

The micro-ELISA plate in this kit is pre-coated with an antibody specific to Human MHCG/HLA-G. Add samples or standards to micro-ELISA plate wells and mix with the appropriate antibody. Next, a biotinylated detection antibody for Human MHCG/HLA-G and an Avidin-Horseradish Peroxidase (HRP) conjugate are added to each microplate well and incubated. Free parts wash away. The substrate solution is applied to each well. Blue colour is only seen in wells using Human MHCG/HLA-G, biotinylated detection antibody, and Avidin-HRP conjugate.

**TaqMan probe-based Real-Time fluorescence PCR kit information.**

Human T-cell Lymphotropic Virus -1 TaqMan probe real-time fluorescence PCR to create targeted primers for the highly conserved region of HTLV-1. A one-step fluorescent RT-PCR approach was utilised to identify and amplify HTLV-1 nucleic acid in vitro. Including the HTLV-1 nucleic acid template in the reaction system led to a PCR reaction and the release of fluorescence. In real time, the equipment monitored and output the signal strength of the PCR channel, enabling qualitative and quantitative examination of detection findings.

Human T-cell Lymphotropic virus -2 TaqMan probe-based real-time fluorescence PCR is used in the reagent kit to create primers targeting highly con-

served areas of the HTLV-2 genome. In a one-step fluorescent RT-PCR approach, HTLV-2 nucleic acids are amplified and detected in vitro. When the HTLV-2 nucleic acid template is present, the PCR process releases fluorescent signals. In real-time, the equipment monitors and outputs signal strength in PCR channels, enabling qualitative and quantitative examination of test findings.

**Statistical Package:**

for the Social Sciences 27 (SPSS) was used for descriptive statistics, T-tests, normality tests, and group comparisons. Mean and standard deviation were employed for normal distribution data, fisher exact test for categorical variables with limited observation in some cells, and chi-square for variables with ample observation in each cell.

## Results:

Sociodemographic study

The majority of lymphoma participants had Non-Hodgkin Lymphoma, accounting for 63.3%.

Table 3-1 Distribution of participants according to the type of Lymphoma.

| | Frequency | Percent % |
|---|---|---|
| NHL | 114 | 63.3 |
| HL | 66 | 36.7 |
| Total | 180 | 100.0 |

Sex of Patients: As presented in Table 3-2, the majority of overall lymphoma participants were males 54.4 %.

Table 3-2 Sex distribution among Lymphoma participants

| | Frequency | Percent |
|---|---|---|
| Males | 98 | 54.4 |
| Females | 82 | 45.6 |
| Total | 180 | 100.0 |

Spousal status: as shown in table 3-3 a very small proportion of lymphoma participants were singles 11.7 % while the other 88.3 were married.

Table 3-3 Spousal state distribution among Lymphoma participants

| | Frequency | Percent |
|---|---|---|
| Married | 159 | 88.3 |
| Single | 21 | 11.7 |
| Total | 180 | 100.0 |

Smoking: Table 3-4 shows that 92.8% of Lymphoma participants were non-smokers.

Table 3-4 Smoking distribution among Lymphoma participants

| | Frequency | Percent |
|---|---|---|
| Non-Smoker | 167 | 92.8 |
| Smoker | 13 | 7.2 |
| Total | 180 | 100.0 |

Intravenous Transfusion:

The largest proportion of participants in this study didn't receive bloods 86.7 % while the small proportion were blood recipients, 13.3 % as shown in Table 3-5.

Table 3-5 Intravenous Transfusion distribution among Lymphoma participants

| | Frequency | Percent |
|---|---|---|
| Yes | 24 | 13.3 |
| No | 156 | 86.7 |
| Total | 180 | 100.0 |

Age Distribution: Table 3-6 shows that there is non-significant differences between the mean age of NHL 46.91±19.89 years and the age of HL 44.57±14.02 years, $p > 0.05$. but the trends in age groups shows a significantly difference between the group who is older than 40 years, $p < 0.001$.

Table 3-6 Age distribution in Non-Hodgkin and Hodgkin Lymphoma Patients.

| Age / years | NHL | HL | P value |
|---|---|---|---|
| mean±SD | 46.91±19.89 | 44.57±14.02 | > 0.05 |
| Minimum | 18 | 18 | |
| Maximum | 83 | 75 | |
| ≤ 40 | 37 | 39 | < 0.001 |
| > 40 | 77 | 27 | |

NHL= non-Hodgkin lymphoma, HL= Hodgkin lymphoma
> 0.05 = non-significant, < 0.001= highly significant

Hepatitis B virus (HBV) PCR Result: the largest proportion 90% of lymphoma participants were negative to Hepatitis B virus (HBV) infection only 10% were infected with the Hepatitis B virus (HBV), as shown in Table 3-7.

Table 3-7 Hepatitis B virus (HBV) PCR distribution among Lymphoma participants

| | Frequency | Percent |
|---|---|---|
| Positive | 18 | 10.0 |
| Negative | 162 | 90 |
| Total | 180 | 100.0 |

Hepatitis C virus (HCV) PCR Result: Table 3-8 shows that the largest proportion 94.4% of lymphoma participants were negative to Hepatitis C virus (HCV) infection only 6.5% were infected with the Hepatitis C virus (HCV).

Table 3-8 Hepatitis C virus (HCV) PCR distribution among Lymphoma participants

| | Frequency | Percent |
|---|---|---|
| Positive | 10 | 5.6 |
| Negative | 170 | 94.4 |
| Total | 180 | 100.0 |

Human T-lymphotropic virus 1 (HTLV-1) RT-PCR Results:

the smallest proportion 8.9 % of lymphoma participants were infected with the HTLV-1 virus while the largest proportion were negative to HTLV-1, as shown in Table 3-9.

Table 3-9 Human T-lymphotropic virus 1 (HTLV-1) RT-PCR distribution among Lymphoma participants

| | Frequency | Percent |
|---|---|---|
| Positive | 16 | 8.9 |
| Negative | 164 | 91.1 |
| Total | 180 | 100.0 |

3-10 Molecular Genotyping Result of HTLV-1

| No. | Genotype | Cycling A.Green | Cycling A.Yellow | Number of cases |
|---|---|---|---|---|
| 1 | Control Positive | Reaction | Reaction | 1 |
| 2 | Control Negative | Reaction | Reaction | 1 |
| 3 | Negative | No Reaction | Reaction | 13 |
| 4 | Positive | Reaction | Reaction | 16 |

This table illustrate the RT-PCR results. From 180 patient samples, we selected 29 samples based on the sHLA-G result to confirm viral infection in these patients. Samples result divided to High, Medin and Low concentration we select (10) samples from High concentration, (10) samples from Medin concentration and (9) samples from Low concentration. All patient sample under the gene extraction process for the RT-PCR test. Table 3-10 illustrated reading of RT-PCR (13) patient samples have a negative result, so no viral gene is present, and (16) patient samples have a positive viral gene.

Human T-lymphotropic virus-2 (HTLV-2) RT-PCR distribution among Lymphoma participants result. The table below indicates that no one of lymphoma participants is infected with HTLV-2.

Table 3-11, part one: HTLV-2 RT-PCR distribution among Lymphoma participants.
Part two of Table 3-11: Molecular genotyping results of HTLV-2.

| | Frequency | Percent |
|---|---|---|
| Negative | 180 | 100 |

Table 3-11 Molecular Genotyping Result of HTLV-2

| No. | Genotype | Cycling A.Green | Cycling A.Yellow | Number of cases |
|---|---|---|---|---|
| 1 | Control Positive | Reaction | Reaction | 1 |
| 2 | Control Negative | No Reaction | Reaction | 1 |
| 3 | Negative | No Reaction | Reaction | 29 |

HLA-G according to the HTLV-1 presence result:

The table below represents the non-statistically significant $p > 0.05$ differences in the mean±SD of lymphoma participants who are HTLV-1 positive, 0.634±0.2, vs lymphoma participants who are HTLV-1 negative, 0.701±0.2.

Table 3-12 HLA-G according to HTLV-1 presence

| Group Statistics | | | | |
|---|---|---|---|---|
| group | | N | Mean±Standard Deviation | P value |
| HTLV-1, HLA-G | Positive | 16 | 0.634 ± 0.2 | > 0.05 |
| | Negative | 164 | 0.701±0.2 | |

HLA-G according to HTLV-1 antigens based on gender results.

Table 3-13 shows that males with lymphoma who are HTLV-1 negative show significantly higher levels of HLA-G than females, 0.74 ± 0.26 vs 0.66 ± 0.22, $p < 0.04$.

Table 3-13HLA-G according to HTLV-1 antigens based on gender

| HLA-G | | P value (<0.05) | | | |
|---|---|---|---|---|---|
| Group | M±SD (ng/ml) | HTLV-1 Positive Female | HTLV-1 Negative Male | HTLV-1 Negative Female |
| HTLV1 Positive Male | 0.59 ± 0.2 | > 0.05 | 0.05 | > 0.05 |
| HTLV1 Positive Female | 0.73 ± 0.1 | | > 0.05 | > 0.05 |
| HTLV1 Negative Male | 0.74 ± 0.2 | | | > 0.04 |
| HTLV1 Negative Female | 0.66 ± 0.2 | | | |

**Discussion:**

Table 3-1 illustrates that an Iraqi study aligns with my findings, indicating significant regional variations in the distribution of lymphoma across Iraqi provinces, especially between northern and southern Iraq. Studies indicate that non-Hodgkin lymphoma (NHL) is more prevalent than Hodgkin lymphoma (HL) across various regions.[9] The combination of regional epidemiological patterns and environmental factors that illustration why Non-Hodgkin lymphoma (NHL) is more distribution than Hodgkin lymphoma (HL) in Iraq. [10]As well-known NHL risk increased by factors such as obesity and tobacco use are associated with increased. [11] Immunological factors include immunosuppression from conditions like HIV/AIDS or organ transplantation, and autoimmune diseases, which impair immune system function [12].

Regarding the sex distribution in table 3-2 the males were higher han females this finding agrees with Radkiewicz et al. Lamb et al. and Traoré et al [13], a cohort study made by Crehan et al, found that males are predominant

in NHL, and this is in agreement with the result of this study.[14] while for HL the males are predominant as indicated by Shah et al. Yıldırım et al, Aslani et al.[15]

Table 3-3 indicates that the majority of participants were married, corroborating the findings of a study conducted by Omoti et al. [16] . Earlier diagnosis among married patients, facilitated by care and support from their spouses, may result in improved survival rates. Marriage is a cultural universal, which may explain why the married population exceeds that of the unmarried. [17]

Table 3-4 Taborelli et al discovered that the majority of lymphoma participants were non-smokers, a finding that corresponds with the results presented in this investigation in[18] . Battaglioli et al. reveal a clear correlation between the timing of smoking cessation and mortality risk among lymphoma participants. [19]

Concerning the blood transfusion data in Table 3-5, the majority had no prior history of blood transfusion, and several studies verify my findings, indicating no association between blood transfusion and lymphoma. [20]

There was no statistically significant difference in the mean ages of NHL and HL individuals. In patients with NHL, the youngest was 18 years old, and the oldest was 83 years old. In the HL group, the youngest participant was 18 years old, while the oldest was 75 years old. The patterns among NHL and HL participants were markedly different, with the majority of NHL players above 40 years of age. Conversely, most HL participants were aged 40 or younger, as seen in Table 3-6.

Adolescent and young adult patients with lymphoma may exhibit greater intensity and severity of disease than younger patients, which may result in a worse prognosis attributed to factors such as severe illness at diagnosis and other health inequities. [21]

Studies demonstrate that lymphoma diagnoses are more prevalent in older persons, especially non-Hodgkin lymphoma (NHL), which has seen a surge in prevalence among those aged over 55 years. The mean age of diagnosis for lymphomas varies, with notable studies reporting a median age of 55 years for non-Hodgkin lymphoma patients. [22]

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are linked to a heightened risk of lymphoma in affected individuals. As seen in Tables 3-7 and 3-8. The majority of lymphoma participants tested negative for HBV by PCR, a finding consistent with previous studies. [23] The proportion of HL individuals who tested PCR-positive for HBV and HCV was lower than that of NHL participants who tested PCR-positive for both viruses, consistent with previous findings.

The majority of lymphoma participants were negative for HTLV-1, as seen in tables 3-9; this may be explained by These patients often display distinctive phenotypic and karyotypic characteristics, suggesting that other variables may contribute to the emergence of T-cell malignancies independent of HTLV-1 infection. [24]

The limited incidence of HTLV-1 in certain communities requires careful screening, particularly for migrants from endemic areas, to identify individuals at risk for ATLL. [25] In addition to Insufficient information exists on Iraq. [26]

The presence of HTLV-1-negative cases underscores the complexity of lymphoma etiology, suggesting that other viral, genetic, or environmental factors may significantly contribute to the emergence of T-cell malignancies. [27]

Table 3-10, pertaining to the genetic distribution of HTLV-1, corroborates my findings, indicating that the optimal diagnostic tool for detecting HTLV-1 is RT-PCR, which is essential for identifying and controlling illnesses caused by these viruses. Real-time RT-PCR was used to quantify HTLV-1 mRNA variations, clarifying viral gene expression and pathogenicity. This approach demonstrated considerable amplification efficiency and a wide dynamic range, making it suitable for thorough viral studies. [28]

Real-time PCR methods have been proposed as confirmatory tests for HTLV in blood banks, offering improved sensitivity and lower costs than Western blot testing. These tests effectively identified HTLV-2 infections and explained unclear circumstances. [29]

Table 3-11 indicates that all subjects tested negative for HTLV-2, which may be supported by an unclear association

with haematologic cancers, with only occasional occurrences documented. [30] HTLV-2 also targets CD8+ T cells, which often do not result in the same carcinogenic consequences as CD4+ T cells. [31] The Tax oncoprotein of HTLV-1 significantly contributes to T-cell transformation and proliferation, increasing its oncogenic potential. (32) Conversely, the Tax-2 protein of HTLV-2 lacks certain domains essential for comparable carcinogenic functions, which explains the greater prevalence of HTLV-1 in our study relative to HTLV-2(33).

Table 3-12 indicates that the mean values of HLA-G in lymphoma patients negative for HTLV-1 exceed those in HTLV-1 positive lymphoma patients. The tumors may evade the immune system owing to the condition of immunological tolerance in a malignant environment, which can be modulated by the expression of HLA-G. [34] Some studies indicate that HLA-G expression correlates with diminished NK cell function, maybe worsened in HTLV-1 positive individuals owing to systemic NK cell deficits. [35] In addition to Polymorphisms in the HLA-G gene, other factors may affect its expression levels. Research suggests that certain polymorphisms are more common in lymphoma patients, likely leading to lower HLA-G levels in individuals who are HTLV-1-positive.[36]

Reduced HLA-G levels in HTLV-1-positive individuals may be linked to unfavourable clinical outcomes, since HLA-G expression has been connected with overall survival rates in lymphoma and corresponds with worse clinical outcomes in tumour patients. [37] .The presence of HLA-G levels may indicate an impaired immune response in HTLV-1-positive individuals; however, this decrease may facilitate a more effective anti-tumor response by reducing immunological inhibition. This paradox underscores the complexity of immunological interactions in relation to viral infections and cancers (38).

Table 3-13 is divided into two sections. The first observation indicates that HLA-G levels are markedly reduced in HTLV-1-positive men compared to HTLV-1-negative females, attributable to Males often having a distinct immunological response relative

to females, frequently leading to diminished levels of immune regulatory molecules such as HLA-G. [39]

HLA-G polymorphisms may influence expression levels, potentially leading to reduced HLA-G in men with HTLV-1, as these polymorphisms may interact variably with the male immune system.[40] HLA-G is recognized for its immunosuppressive characteristics, enabling tumor cells to avoid immune detection (41). The expression of HLA-G might downregulate T-cell responses, hence facilitating HTLV-1 persistence, which may be more prominent in males owing to their distinct immunological profiles (42). The interplay between HTLV-1 and HLA-G may affect clinical outcomes in lymphoma, with men possibly experiencing more immunological dysregulation. [43] The elevated levels of HLA-G in HTLV-1 positive females suggest a vigorous immunological response, which may imply a more favorable result in the absence of HTLV-1, highlighting the intricate interplay between sex and the immune system in disease development. [44]

The other section of Table 3-13 indicates that HTLV-1 negative men exhibit considerably greater expression of HLA-G compared to HTLV-1 negative females. The explanation may lie in Hormonal Influence, since Testosterone potentially increases the expression of HLA-G, resulting in elevated levels in men (45). Estrogen in females may differentially modify immunological responses, resulting in significantly reduced levels of HLA-G, which may also be influenced by polymorphisms in the HLA-G gene (46). HLA-G's role in immunological tolerance may be more significant in men, facilitating tumour evasion of immune detection more effectively. [47] This may result in elevated HLA-G levels as a compensatory response in male lymphoma patients, but in females, they may exhibit alternative immunological pathways, demonstrating a robust immune response to viral stimuli and malignancies despite reduced HLA-G expression compared to men, resulting in different clinical outcomes (48).

**limitations in this study:**

This work offers significant insights into the identification of HTLV-1 and HTLV-2 viruses in lymphoma patients

and their link with sHLA-G levels; nonetheless, it has some limitations that should be acknowledged when interpreting the data.

The sample size is restricted to a certain number of patients, potentially impacting the generalisability of the results to the broader lymphoma population. Secondly, the research was confined to a single centre or geographic region, thereby diminishing demographic variety and limiting comparative analysis with other communities. Third, viral detection depended on particular laboratory methods, and the absence of supplementary highly sensitive techniques like quantitative PCR may compromise the precision of viral load assessment, particularly as the virus frequently exists as a cell-associated entity rather than as a free virus in plasma.The assessment of sHLA-G levels may be affected by other immunological or inflammatory elements that were not well controlled, thereby impacting the interpretation of the correlation between the viruses and this immune marker.

**Implications in this study:**

The results of this study highlight the potential clinical and immunological significance of detecting HTLV-1 and HTLV-2 viruses in lymphoma patients, particularly when correlated with levels of the immunoregulatory molecule sHLA-G. The findings suggest that viral infection may contribute to alterations in the host immune response, which could help explain certain patterns of tumor behavior or disease progression. Furthermore, the study underscores the potential value of sHLA-G as an immunological biomarker that cannot be reliably used to assess disease status or monitor lymphoma progression, especially in cases where viral infection may exacerbate immune dysregulation. These implications support the need to improve diagnostic and prophylactic strategies and encourage future research to further elucidate the interaction between HTLV infection and the lymphoma microenvironment.

**Conflict of interest:**

There is no conflict of interest

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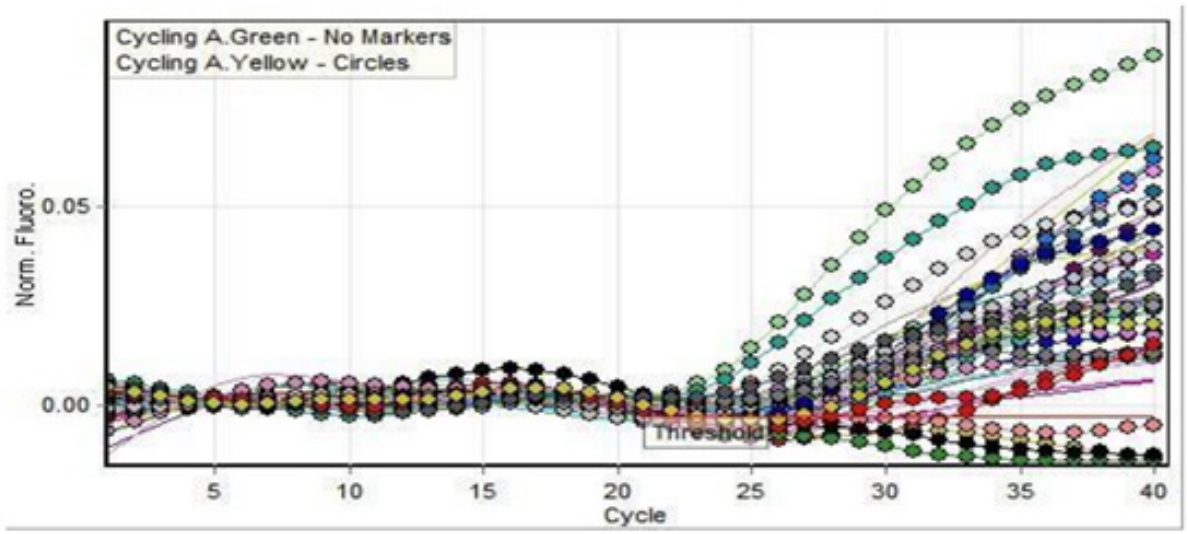


Figure 3-1 Amplification Curve Graph of HTLV-1

HTLV-1 RT-PCR for Allelic Discrimination Information

| | |
|---|---|
| Digital Filter | Light |
| Imported Analysis Settings | |
| Left Threshold | 1 |
| No Template Control Threshold | % 0 |
| Noise Slope Correction | Yes |
| Normalization Method | Dynamic Tube Normalization |
| Reaction Efficiency Threshold | Disabled |
| Start normalizing from the cycle | 1 |
| Threshold | .01887 |

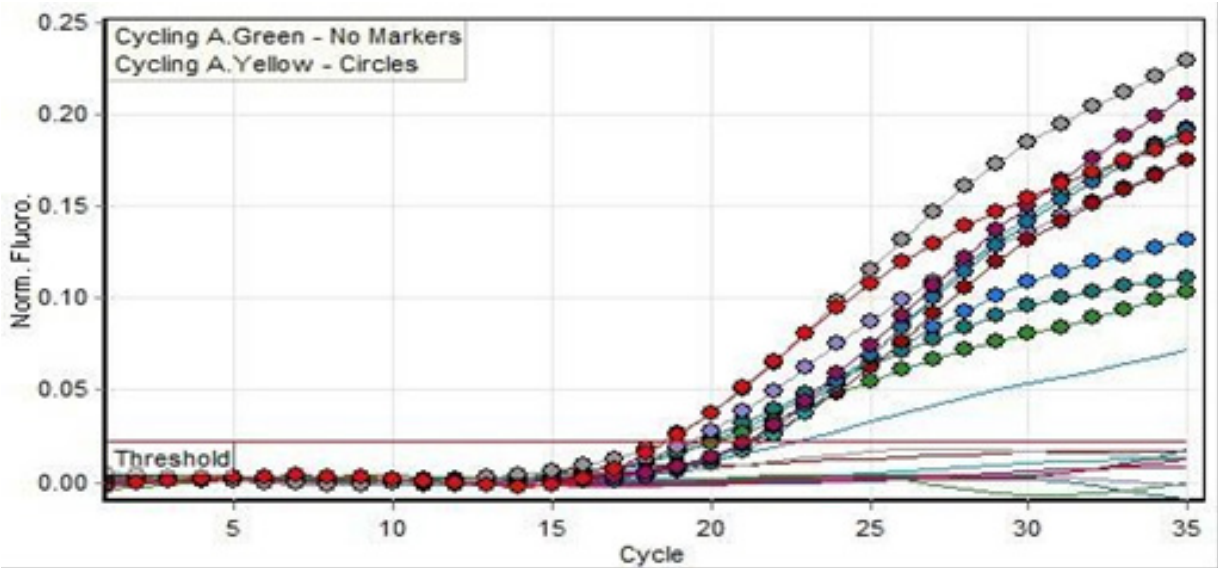


Figure 3-2 Amplification Curve Graph of HTLV-2

HTLV-2 RT-PCR for Allelic Discrimination Information

| | |
|---|---|
| Digital Filter | Light |
| Imported Analysis Settings | |
| Left Threshold | 1 |
| No Template Control Threshold | % 0 |
| Noise Slope Correction | Yes |
| Normalization Method | Dynamic Tube Normalization |
| Reaction Efficiency Threshold | Disabled |
| Start normalizing from the cycle | 1 |
| Threshold | .01887 |