DISCUSSION

Human Leukocyte Antigen (HLA)-G is a non- classical HLA class I molecule, originally described essential for promoting fetus-maternal tolerance. It is now clear that HLA-G is involved in promoting beneficial tolerance in several settings, such as autoimmunity and organ transplantation, and in contributing to detrimental tolerance in viral infections and cancer. (92)

HLA-G could inhibit function(s) of immune effector cells through binding to diverse types of receptors, such as ILT2, ILT4 and KIR2DL4. Although its initial significance had been established in foetal–maternal immunotolerance, HLA-G has been suggested to provide tumor cells with an effective pathway to escape from anti-tumor immune responses.(93)

In the past few years, making use of HLA-G protein expression in tissues and circulating levels in body fluids as a tumor marker have been the focus of extensive research in the diagnosis and prognosis of several human malignancies. In addition, this molecule might be a promising target for future immune therapeutic approaches based on its immune tolerant functions and its highly specific expression for malignant transformation. Few studies had been performed on HLA-G expression in different types of leukemia patients; however, data are limited and conclusions remain controversial. (94)

The present study included 30 patients with AML subdivided into 2 groups. Group 1 consisted of 15 de novo AML patients while group 2 consisted of 15 relapsed AML patients. In addition to 15 normal persons of matched age and sex and were considered control group. The mean value of s-HLA-G among the three groups was 329.8 ± 57.54 ng/L among controls, 451.15 ± 163.99 ng/L among newly diagnosed and 551.63 ± 109.08 ng/l among relapsed patients. We detected statistically significant difference between the three studied groups (controls, newly diagnosed AML and relapsed AML as regarding the mean value of sHLA-G ($P < 0.05$). In this study this cutoff value was considered the level at which HLA-G was considered within normal. Using this cutoff point it was noted that HLA-G expression was 24/30

(80%) of patients had levels higher than the cutoff point among all AML patients. The newly diagnosed AML were 10/24 whereas the relapsed AML were 14/24.

In a previous study on 28 AML patients, HLA-G was undetectable in all 28 AML patients; however, a prior incubation of AML cell samples with IFN-γ induced HLA-G expression in 21% of AML patients. [95] Poláková K et al [96] analyzed HLA-G expression in 25 AML patients but did not find HLA-G expression;, in a later study, they showed that HLA-G expression in AML samples could be detected by Western blot. [97] In a larger cohort, HLA-G expression was analyzed in 54 patients with AML where 18.5% (10/54) of the leukemic blast cells in AML patients was positive for HLA-G expression. The proportion of HLA-G expression on leukemic cells varied from 3.47% to 99.69% for the AML samples.[93] In a study done by Guo et al, the percentage of HLA-G expression in leukemic blasts varies from 0% to 93.96% (median: 0.42%; 95% CI: 0–89.0%). When using the cut-off value of 0.5%, HLA-G expression in AML blast cells at 0.5% or less was considered as negative, the median for the HLA-G positive patients with 2.0% (95% CI: 0.52–93.15%).[97]

As regard the influence of age and sex on the level of sHLA-G, there was no significant correlation detected in our study between sHLA-G levels and age of patients. This is similar to that reported by Locafaro et al [92] and Guo et al [97] who reported that HLA-G expression status on leukemic blasts was not associated with patient age at diagnosis. Also, there was no significant difference detected in the present study as regards gender in the same group but sHLA.G expression in males and females in newly diagnosed and relapsed groups was higher than that in control group that explained by tumour burden (p=0.004) for males: and (p=0.022) for females:.

The analysis of the association between the expression of HLA-G on blasts and clinical parameters, including patient age, gender and subtype of AML revealed that HLA-G expression is independent of all of the above mentioned variables .it was different from that reported by Locafaro et al **[92]** who reported that all males showed HLA-G+ blasts.

It cannot be excluded that this discrepancy can be due to the different ethnic population analyzed. This discrepancy can be also due to the fact that the levels of sHLA-G (shed HLAG1 and HLA-G5) are significantly higher in plasma treated with EDTA as compared to those in plasma treated with heparin or in serum. [92]

In acute leukemias, several factors are relevant to prognosis, including cytogenetic abnormalities and myelodysplasia, which are now included in the World Health Organization classification. In our study there is no evidence of dysplastic features among our cases. Gros et al [98] study failed to reveal any correlation between sHLA-G level at diagnosis with prognosis. However, two biologic features could be correlated with a high sHLA-G level: absence of myelodysplasia and high-level leukocytosis. The inverse correlation between sHLA-G level and myelodyplasia may appear surprising, considering the poor prognosis impact of this background. However, this finding can be explained by a potential link between sHLA-G secretion and a de novo acute leukemic process unrelated to chronic pathology, such as dysplasia.

In our study, no correlation was detected between white blood cell count and sHLA-G level ($p=0.126$). A correlation between hyperleukocytosis and sHLA-G merely reflects the frequent hypercellular pattern of pathologies expressing higher levels of sHLA-G.[98]

As regard the correlation between sHLA-G and bone marrow blast percentage, HLA-G was positively correlated with percent blasts in the bone marrow, being higher with higher percent blasts in the bone marrow. This was similar to that reported by Yan et al **[93]** who reported HLA-G-positive patients had a significant higher bone marrow leukaemic blast cell percentage when compared with that of HLA-G-negative patients ($P < 0.01$). It differed from that reported by Locafaro et al [92] who noted no difference. Yet, differed from that reported by Guo et al [97] who reported that HLA-G expression status on leukemic blasts was not associated to percentage of blasts.

Locafaro et al [92] demonstrated the presence of HLA-G-expressing DC-10 and CD4+ T cells in the peripheral blood of leukemic patients. This finding indicates that the frequency of regulatory cells, DC-10 and HLA-G+ CD4+ T cells, is increased in patients with HLA-G-expressing blasts, supporting the hypothesis that the expression of HLA-G on blasts may be a strategy by which leukemia promotes a tolerogenic microenvironment limiting anti-tumor responses. This mechanism of immune escape has been previously proposed for solid tumor where both infiltrating cells and tumor cells can express HLA-G. [99]

As regard to s HLA-G and cytogenetics findings it was noted that sHLA-G was statistically insignificantly related to cytogenetic. However, the number of the studied case is small to reach a solid conclusion. A study done by Yan et al **[93]** showed that all HLA-G-positive AML patients (n = 5) were cytogenetically abnormal, which was markedly different from that of HLA-G-negative patients ($P < 0.01$).

Yan et al **[93]** studied twenty-one AML samples (5 for HLA-G-positive and 16 for HLA-G-negative) for both the HLA-G expression measurement and the cytogenetic karyotyping. All five patients with HLA-G-positive showed cytogenetic karyotype abnormality, including two cases with t(15;17); two cases t(8;21) and 1 case with t(9;11), respectively, while 6 out of 16 HLA-G negative patients are with cytogenetic karyotype abnormality, including two cases with t(7;11); two cases t(15;17), one case with t(6;9) and one case with +8, +8, +15, respectively. When compared, a significant difference was obtained ($P < 0.01$).

In our study s HLA-G was not related to FAB classification. This is similar to that reported by Guo et al [97] who reported that HLA-G expression status on leukemic blasts was not associated with the clinical parameters such as patient age at diagnosis, sex, sub-type of AML, percentage of blasts.

The lowest level of s HLA-G was detected in M2 subtype where its mean value was 365.2 ± 98.9 ng/L while the highest mean value was present in M6 subtype where its level was 605.3 ± 116.5ng/L.

A study done by Gros et al [98] showed that soluble HLA-G antigen were increased during acute leukemia, especially in subtypes of AML-M4 and AML-M5,

as well as in both B- and T-ALL patients. GM-CSF and IL-10 exert an influence on HLA-G secretion for FABM4 cells, in contrast to FABM5. This latter difference in susceptibility could be related to the presence of a mature monocytic component in FABM4, contrary to FABM5, which is defined by immature monocytic proliferation. [98] In Yan study [93], 3 out of 11 cases of AML-M5 patients were HLA-G-positive, but not detected in both AML-M4 and B-ALL patients.

In this study sHLA-G was statistically not related to response to therapy. The lowest mean value of sHLA-G was observed in patients in complete remission (359.67±58.29) when compared with relapsed patients (404.565±160.48) or those refractory to chemotherapy (402.3±95.62). This is unlike that reported by Guo et al [97] whose results of their study indicated that HLA-G expression is of no significance for the prognosis of patients with AML.

CONCLUSION

1- There was statistically significant difference in sHLA-G level between new and relapsed AML cases when compared with controls. sHLA-G level was statistically significant increase in relapsed cases compared to new AML cases.

2- The highest mean value of serum HLA-G was present in M6 subtype while the lowest mean value was observed in M2 in comparison with other subtype.

3- HLA-G had a sensitivity of 100% and a specificity of 62%. in acute myeloid leukemia

4- The HLA-G levels was not affected by age, gender and WBCs, but affected by bone marrow blast percentages.

5- The HLA-G levels was insignificantly related to response to therapy.