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The correlation between CD200 expression and oxidant-antioxidant status in newly diagnosed B-chronic lymphocytic leukemia patients

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***Abstract*---**B cell chronic lymphocytic leukemia (B-CLL) is the highest incidence of lymphoproliferative malignant disease in most adults in the western world. Due to the importance of the pathogenicity and risk factors of this disease, this study was designed to analyze the expression the pattern of CD200 by flow cytometer immunophenotyping (FCI) and the association between this marker and some clinical features and oxidant-antioxidant status in B-chronic lymphocytic leukemia patients. This study was conducted on 60 newly diagnostic patients with B-CLL and 30 apparently healthy individuals as control group. The socio-demographic information including (age and sex) and clinical data were also collected in period 20 February 2021 to 25 December. The result of statistical analysis of the present study showed that out of the 60 patients, 39 were male and 21 females, with 30 controls (12 males and 18 females). The mean age of adults patients was (64.04 ± 5.6) years, and the adults control mean age of (63.33 ± 4.70) years therefore, there was a higher prevalence p value ≤0.05 of newly diagnosed CLL patients in males (65.0 %) in compared with females (35.0 %). Regarding to the results of Binet staging, the present study show the higher percentage were at high-risk (stage C) group, while the lower percentage were at low-risk (stage A) group. The percentage of CD200 expression on leukemic B-cells was high value (97.986% ± 1.334) in B-CLL patients as well as, due to Binet stage, the percentage of CD200 was significantly increase in stage C in compare to other Binet staging. Finally, the result oxidant- antioxidant status show that the B- CLL patients have been

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significantly higher of Malondialdehyde compare to control while the Total antioxidant capacity levels was significantly decrease in B-CLL compare to control group. In conclusion, the clinical profile, Immunophenotypng features of CD200 expression and Oxidant-antioxidant redox status are necessary indicators for predicative diagnosis in all stages and it may contribute in potential therapeutic target for B-CLL.

***Keywords*---**CD200, Clinical profile, Oxidant- antioxidant.

Introduction

B-chronic lymphocytic leukemia (B-CLL), is one of the most common lymphoproliferative malignant diseases that are characterized by uncontrolled proliferation and accumulation of immunologically incompetent B lymphocytes that mature in the bone marrow, peripheral blood and secondary lymphoid organs [1]

A diagnosis of CLL can be relies on a detection of a progressive accumulation of more 5,000 circulating B lymphocytes /µl that have been maintained in peripheral blood for more than three months and can be noted in complete blood count and blood or in lymph nodes by using complete blood count and blood smear examination in addition by specially expressed CLL by of CD5, CD19 and CD23 , dim CD20 and CD79a and weakly express surface IgM and IgD. by using the characteristic morphology and immunophenotyping profile [2,3]

Once a diagnosis is ascertain, clinical staging can be accomplished based on the combination of fundamental diagnostic criteria established from two widely accepted methodologies. Currently, Two staging systems are used in CLL patients to characterize disease burden and therapeutic indication to predict CLL disease outcome are: Rai and and Binet system. These two staging systems have several advantages as simple to define, relying due to a complete blood cell count and physical examination, and they have been demonstrated the effective prognostic predictors for CLL [4,5].

Recently, CD200 was a potential antigen for flow cytometric immunophenotyping of chronic lymphoproliferative disorders in a number of leukemias, especially those of the B lineage. [6]. According to this prognostic role of the CD200 expression , CD200 is a highly conserved membrane glycoprotein that interacts with its structurally related receptor in different hematolymphoid cancers such as B lymphocytes, a subset of T lymphocytes, thymocytes and endothelial cells; this influence and probably because of its immunosuppressive effect on the individual's immune system. However, the diagnostic utility and potential prognostic importance of CD200 expression have not been rigorously examined [7,8]. Several harmful factors that increase the incidence of B-CLL have been reported and these factors led to increase the risk of tumor growth [9]. Common genotoxic factors that induce B hematologic malignancies include oxidative stress (OS) and lipid peroxidation products.[10] and considerably significantly contribute to the pathogenesis of B-CLL [11]. Whenever the unbalance between oxidative

stress and antioxidants may lead to cellular damage [12]. In contrast, the functions of immune cell are associated with ROS production and antioxidant system . Therefore the deficiency of antioxidant can be a cause of immune function suppression, affecting both the innate T cell-mediated immune response and the adaptive immune response [13].

Although the toxic effects of OS and overproduction of lipid peroxidation are well-established, their importance in the pathophysiology of B- CLL remains debatable. There are conflicting views demonstrating varying alterations in the oxidative stress indicators in B- CLL [14].

Meterials and Methods

Five ml peripheral blood samples were taken from 60 patients and 30 control group and then distributed into two parts; one part was collected in tube containing ethylene diamine tetra-acetate, to laboratory investigations for performed immunophenotypic profile analysis of membrane molecule Cluster of differentiation markers expression (CD 19 and CD200) by using flow cytometer using Flow cytometer (BD FACS Canto II) the other part was collected in gel tube. Serum was then obtained after centrifugation of blood in a gel tube, and transferred to a new clean tube and stored in refrigerator at -20^{o}c then used to laboratory investigations for measurement the levels of Malondialdehyde and Total antioxidant capacity. Additionally, the socio-demographic information including (age and sex) and before collection of blood sample, consent of patients and approval of study were also obtained.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD) and considered statistically significant at p ≤0.05. Statistical analysis was done using the statistical analyzing system GraphPad Prism and SPSS Software to make the statistical analysis and for comparisons between B-CLL patients and control group.

Results and Discussion

Demographic and characteristics distribution of our studies

This study was conducted on 60 newly diagnosis B-CLL, there was a slightly higher prevalence p value ≤0.05 in males (65.0 %) over females (35.0 %) .Out of the 60 patients, 39 were male and 21 females, with 30 controls (12 males and 18 females). The mean age of patients was (64.54 ± 4.743) years, while the mean age of control was the (63.33± 4.700) years. (Table 1).

Table 1: The demographic and characteristics of study population

Personal characteristics	Newly diagnosis CLL patients (n=60)	Control (n=30)
Gender, n (%)		
Male	39 (65.0 %)	12 (40.0 %)
Female	21 (35.0 %)	18 (60.0 %)

Age (years), mean ± SD	64.04 ± 5.6	63.33 ± 4.70

The analysis of patients' sex in present study show the distribution of newly diagnosed B-CLL was a significant difference between the incidence of males and females patients. When male have been higher prevalence p value ≤0.05 in compared to female ones, in addition, the mean age of the patients lies in the elderly age. These findings are consistent several studies, Jakšić *et al* showed the incidence of CLL is higher in men than in women, with the male/ female ratio being about 2:1 [15].furthermore study reported that incidence of CLL increase with an aging in elderly phase and ratio of CLL is predominant in males more than female addition to the onset of disease at adult individual [16,17].

The distribution of the patients according to Binet stage and clinical characteristics

In the present study, the results of Binet staging showed that (50.66%) of newly diagnosed B-CLL were present at high risk (stage C) group, (43.3 %) at intermediate risk (stage B) group, and 6.7 (%) at low risk (stage A) group (Table 2).

Table 2: The clinical characteristics data for the newly diagnosed B-CLL

Characteristics	**Newly diagnosed B-CLL (n=60)**
Binet staging , n (%) A B C	 4 (6.7%) 26 (43.3 %) 30 (50.0 %)
Hepatomegaly – and /or splenomegaly, n (%) Yes No	 31(51.7 %) 29 (48.3 %)
Lymphadenopathy, n (%) Yes No	 39(65.0%) 21(35.0%)

For the most of malignancies, staging is the process to determine how far the disease has progressed. Essentially, stages are important because they can guide treatment. Most of the malignancies are classified based on the size of the cancer and the degree to which it has spread [18].The specific staging criteria for B-CLL includes these noted by Rai and Binet system.Generally, patients have a good performance status at the time of diagnosis. Both consider the degree of anaemia, thrombocytopenia and Lymphadenopathy spread. Anemia and thrombocytopenia may be observed in 15-30% of patients while Lymphadenopathy can be detected in approximately 80% of cases, with bilateral and symmetrical enlargement of the cervical and axillary lymph nodes. In around 50 percent of cases, mild to moderate splenomegaly is present, but hepatomegaly is less common and It is evident that there is a wide range of variability in each result of the clinical staging of CLL newly diagnosed patients distribution. This may be attributed to

the fact that some of the patients were with stage B and other patients with higher percentage in stage [4,5].

Immunophenotyping profile of CD200, CD19

In the present study, Immunophenotyping analysis of peripheral blood sample in our study by flow cytometer was showed the level of circulating leukemic B-cells was evaluated in peripheral blood in newly diagnosed B-CLL by the positive percentage of CD19 (as gating) and CD200 expression on these cells .It has been found that circulating leukemic B-cells frequencies of B-CLL were high (98.996% ± 0.3181), and the percentage of CD200 expression on leukemic B-cells was high value (97.986% ± 1.334) in newly diagnosed patients with B-CLL and based on Binet staging , the percentage of CD200 was (96.433%±1.150) in stage A , (97.628% ± 1.031) in stage B and (98.530%±1.270) in stage C (Table 3 and Figure (1).

Table 3 :percentage of CD200 expression on leukemic B-cell based on Binet stage

Immunophenotyping profile	Binet staging			Total
	Stage A	Stage B	Stage C	
CD200	96.433%±1.150	97.628%± 1.031	98.530%±1.270	98.996%±0.3181

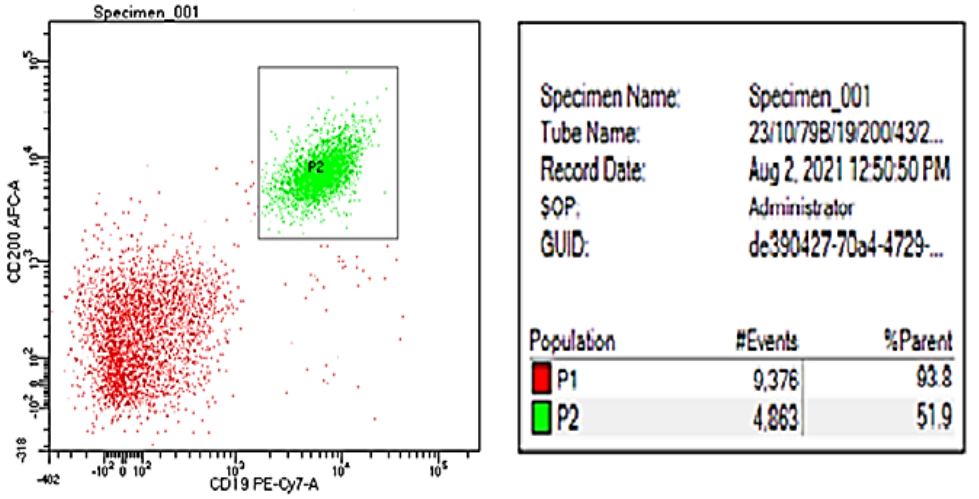

Figure 1: Flow cytometry analysis of B-CLL case, Dot plot to identify CD19 expression as gating and CD200 + expression as a diagnostic marker.

Standard Clinical, morphologic, and immunophenotypic criteria were used to diagnose and stages B-CLL. Although the most of B-CLL are asymptomatic at the time of diagnosis, some may have systemic symptoms (hepatomegaly, splenomegaly, lymphadenopathy, and cytopenias due to leukemic infiltration of bone marrow and other organs [19]. CLL Immunophenotyping profile by Flow

cytometry is an essential element that plays a major role in the CLL diagnostic process. No pathognomonic factor that allows us to identify a diagnosis has been identified yet. Additionally, efforts will continue to develop an international agreement regarding the essential markers for diagnosing CLL [20-22]. Consequently, immunophenotyping profile changes can be effective in predicting clinical course, patient survival, and determining first line therapy. Therefore, investigation of CD200 as diagnostic marker in newly diagnosed B-CLL patients is an essential step in selecting the appropriate management strategy for patients with B-CLL. Typically, immunophenotypic profile of CLL presents a strong expression of CD200. CD200 (known as OX -2) , is a type I glycoprotein that is expressed on a different cell types and plays essential role in the modulation of the immune system , immune checkpoints that suppress immunoregulatory responses and it is upregulated on the surface of numerous cancer cells , such as B-CLL[23,24] .

The results of the present study are in agree with two previous studies that showed the CD200 is an accurate diagnostic marker that is consistently overexpressed on the surface of CLL cells, as well as a prognosis predictor and a novel treatment target in CLL[25,26].and in agree with another two recent studies that proved the surface glycoprotein CD200 have valid role to be a a diagnose predictor of various lymphoproliferative disorders especially CLL. CD200 was especially useful for identifying atypical CLL immunophenotypes from MCL and other CD5+ B-cell tumors . [27,28]. Fouad et al. reported the absent of CD200 expression in mature B- leukemic cells , together with a CD5 presence, as being sufficient to exclude B-CLL from differential diagnosis as well as found the patients with CLL are strong CD200 expression (> 50% of B cells) and was associated with advanced age, lymphocytosis, hepato-splenomegaly, and more advanced Rai and Binet stage [29]. Overexpression of CD200 is generally associated with advanced stage and earlier time to progression, and a study found that CD200 expression was higher in high-risk in compare to intermediate and low-risk patients, suggesting that it can provide diagnostic and prognostic information [30].Therefore, the overall picture reflected an interplay between values attributed to each stage.

Oxidant – Antioxidant status

The results of present study show the concentration that of Malondialdehyde (MDA) was significantly increase in newly diagnosed B-CLL (4.331 ± 0.3095) compared to control group (2.275 ±0.2706) while The results of Total antioxidant capacity (TAC) show a significantly lower in newly diagnosed patients with B-CLL (11.74 ± 0.9114) compared to control group (17.93 ± 0.8864) (Figure 2 and Figure 3).

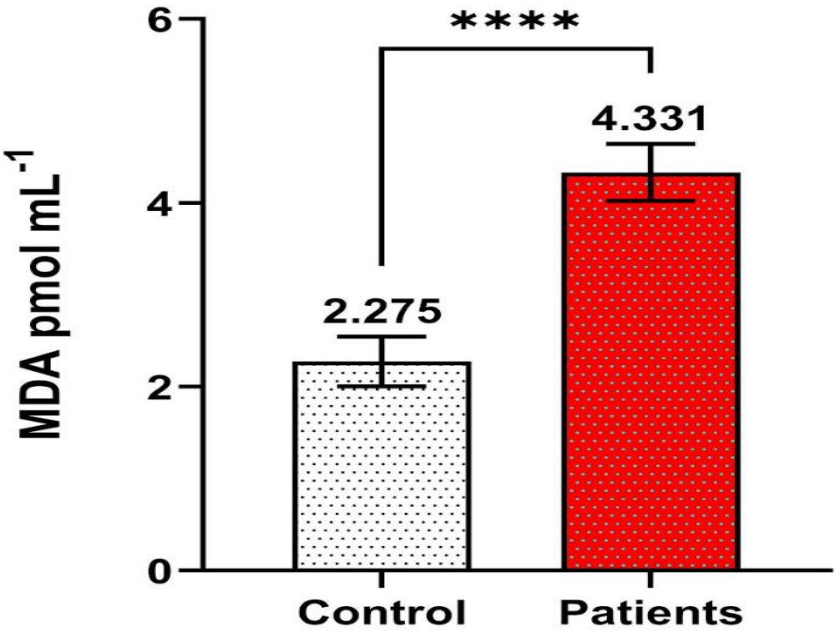

Figure 2: Assessment of Malondialdehyde levels between patients and control group

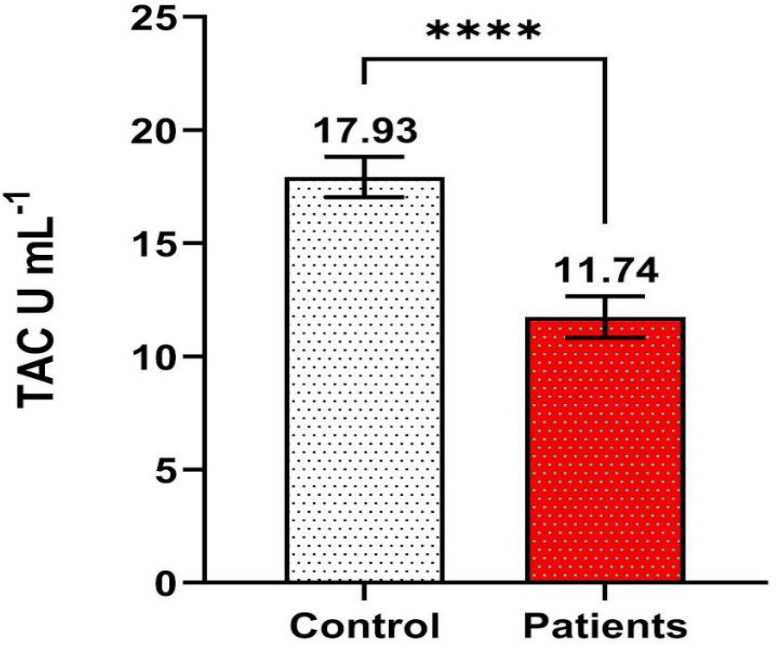

Figure 3: Assessment of Total antioxidant capacity levels between patients and control group

The correlation between CD200 and Oxidant- antioxidant indicators

The results of correlation analysis in present study show that positive CD200 expression had strong direct significant correlation with MDA ($r = 0.612^{**}$, $p = < 0.00001$) while CD200 expression had a strong inverse significant correlation with TAC ($r = - 0.626^{**}$, $p = < 0.00001$) in patients with B-CLL (Table 4).

Table 4: Comparison of the CD200 expression Oxidant- antioxidant indicators in B-CLL patients

Biomarkers	Patients	
	Correlation coefficient (*r*)	*P* value
CD200 vs. MDA	0.612^{**}	< 0.00001
CD200 vs. TAC	$- 0.626^{**}$	< 0.00001

** Correlation is significant at the 0.01 level

The biology of cancer cells, metabolic reprogramming is necessary to survive a harsh environment, including nutrient deprivation, reactive oxygen species (ROS) production, and oxygen withdrawal. Several other metabolic fluxes, most notably the pentose phosphate pathway, folate, and mitochondrial metabolism, are emerging as relevant for cancer in supporting redox balance, in addition to the well-studied glycolytic metabolism. [31].The results of this study are in agree with several recently studies , Zhevak et al they indicated that patients with B-CLL experience increased oxidative stress(OS) by concomitantly increasing reactive oxygen metabolites production and the relative deficiency of the antioxidant defence system [32]. and also agree with Bakan et al. they demonstrated increasing MDA levels in the plasma of CLL patients, which may not only be the result of increased production but also a failure of the antioxidant defence [33]. Furthermore , MDA levels were shown to be higher in B-CLL lymphocytes than in healthy lymphocytes, mainly because of a decrease in the antioxidant enzyme activities of intracellular protection enzymes such as SOD and CAT, indicating that the intracellular level of MDA is a biomarker of disease progression [34].

Results obtained from the current study are in accordance with a previous study, Salimi et al reported that total antioxidant capacity levels were significantly decrease in patients with CLL compare to control group [35]. The combination of endogenous and food-derived antioxidants represents the total antioxidant capacity of extracellular fluids, which integrates the cumulative impact of all antioxidants present in the plasma and body fluids, which may provide more relevant biological evidence than individual factors [36].Several suggested mechanisms contribute to OS in cancer patients. Symptoms such as anorexia/cachexia, nausea and vomiting may be the result of an altered energy metabolism. These prevent a normal diet and, therefore, a normal supply of nutrients such as glucose, proteins, and vitamins, which all contribute to the accumulation of ROS and OS [34,37].The mechanisms by which OS may promote

cancer development have not yet been completely understood, and in CLL, as in other neoplasms, it is still unknown whether oxidative stress is a fundamental cause of the disease or only a downstream effect. OS in CLL is concurrently caused by increased reactive oxygen species generation, primarily attributable to the mitochondrial activity of CLL cells, and deficient antioxidant defences. [38]. The decreased antioxidant capacity found in the sera of our CLL patients may be related to a consumption of antioxidants and/or to a reduced efficacy of antioxidant defences due to the systemic excess of ROS that is common in cancer[39].

Accumulating evidence by two important studies suggested that increased OS and the relative deficiency of the antioxidant defence system are involved in crucial steps of CLL development [40,41]. Resultantly, it may be involved in both initiation and promotion of multiple stage carcinogenesis, cell apoptosis, proliferation, differentiation, and immune function suppression, and it contributes to genomic DNA, RNA instability that resulted in gene mutations through cell division, and a final result is occurrence of B-CLL [42-44].

Identification and determination of antioxidant activities and adequate oxidative stress biomarkers of malignant cell metabolism may be important for the early diagnosis of CLL patients and evaluation of tumour progression. In addition, they serve as prognostic factors for predicting clinical disease outcomes and the course of CLL. Antioxidants play a vital and important role as a protective system against these free radicals and in preventing the risk and complications of blood cancer related to CLL disease. Therefore, it is recommended that antioxidants be evaluated simultaneously in order to improve the identification of B-CLL disease [45].

Conclusion

In conclusion, the clinical profile features, overexpression of CD200 and unbalance between Oxidant- antioxidant status are important indicators for predicative diagnosis in all stages of B-chronic lymphocytic leukemia and it may be have a role in potential target for B-CLL therapeutic.

Conflicts Of Interest

The authors declare no conflict of interest.

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References

1. El-Sharkawy, E. A., Sayed, D. M., Abdel Hamid, W. M., Moness, H., & Yusuf, S. M. (2022). Demographic data in patients with Chronic Lymphocytic Leukemia. *Minia Journal of Medical Research*, 260-261.
2. Hallek, M., Cheson, B. D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., ... & Kipps, T. J. (2008). Guidelines for the diagnosis and

treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute–Working Group 1996 guidelines. *Blood, The Journal of the American Society of Hematology*, *111*(12), 5446-5456

3. Quinquenel, A., Aurran-Schleinitz, T., Clavert, A., Cymbalista, F., Dartigeas, C., Davi, F., ... & Troussard, X. (2020). Diagnosis and treatment of chronic lymphocytic leukemia: recommendations of the French CLL Study Group (FILO). *Hemasphere*, *4*(5).
4. Rai, K. R., Sawitsky, A., Cronkite, E. P., Chanana, A. D., Levy, R. N., & Pasternack, B. S. (1975). Clinical staging of chronic lymphocytic leukemia.
5. Binet, J. L., Auquier, A., Dighiero, G., Chastang, C., Piguet, H., Goasguen, J., ... & Gremy, F. (1981). A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer*, *48*(1), 198-206.
6. D'Arena, G., De Feo, V., Pietrantuono, G., Seneca, E., Mansueto, G., Villani, O., ... & Laurenti, L. (2020). CD200 and Chronic Lymphocytic Leukemia: Biological and Clinical Relevance. *Frontiers in Oncology*, *10*, 2611.
7. Staub, R. B., Marcondes, N. A., & Rotta, L. N. (2021). CD200 expression in hematopoietic neoplasms: Beyond a marker for diagnosis of B-cell neoplasms. *Critical Reviews in Oncology/Hematology*, *167*, 103509.
8. Kotwica-Mojzych, K., Jodłowska-Jędrych, B., & Mojzych, M. (2021). CD200: CD200R interactions and their importance in immunoregulation. *International Journal of Molecular Sciences*, *22*(4), 1602.
9. Ames, B. N., & Gold, L. S. (2000). Paracelsus to parascience: the environmental cancer distraction. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *447*(1), 3-13.
10. Eckl, P. M., & Bresgen, N. (2017). Genotoxicity of lipid oxidation compounds. *Free radical biology and medicine*, *111*, 244-252.
11. Leone, A., Roca, M. S., Ciardiello, C., Costantini, S., & Budillon, A. (2017). Oxidative stress gene expression profile correlates with cancer patient poor prognosis: identification of crucial pathways might select novel therapeutic approaches. *Oxidative Medicine and Cellular Longevity*, *2017*.
12. Sies, H. (2020). Oxidative stress: Concept and some practical aspects. *Antioxidants*, *9*(9), 852.
13. Hileman, E. A., Achanta, G., & Huang, P. (2001). Superoxide dismutase: an emerging target for cancer therapeutics. *Expert Opinion on Therapeutic Targets*, *5*(6), 697-710.
14. Zhevak, T., Shelekhova, T., Chesnokova, N., Tsareva, O., Chanturidze, A., Litvitsky, P., ... & Budnik, I. (2020). The relationship between oxidative stress and cytogenetic abnormalities in B-cell chronic lymphocytic leukemia. *Experimental and Molecular Pathology*, *116*, 104524.
15. Jakšić, B., Vitale, B., Hauptmann, E., Planinc-Peraica, A., Ostojic, S., & Kusec, R. (1991). The roles of age and sex in the prognosis of chronic leukaemias. A study of 373 cases. *British journal of cancer*, *64*(2), 345-348.
16. Yamamoto, J. F., & Goodman, M. T. (2008). Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997–2002. *Cancer Causes & Control*, *19*(4), 379-390

17. Porter, R. S., & Kaplan, J. L. (2011). The Merck Manual of Diagnosis and Therapy Nineteenth Edition. Merck sharp & Dohme corp., A subsidiary of Merck & Co. Inc., Whithouse station, 2868.
18. Zwiebel, J. A., & Cheson, B. D. (1998, February). Chronic lymphocytic leukemia: staging and prognostic factors. In Seminars in oncology (Vol. 25, No. 1, pp. 42-59).
19. Nagieb, R. M., El-Ashmawy, H. E. S. A., Hashim, A. M., & Bakry, R. (2022). Role of immunophenotyping in diagnosis of CLL patient at South Egypt Cancer Institute. *Minia Journal of Medical Research*, 71-76.
20. Rawstron, A. C., Kreuzer, K. A., Soosapilla, A., Spacek, M., Stehlikova, O., Gambell, P., ... & Montserrat, E. (2018). Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: An European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytometry Part B: Clinical Cytometry, 94(1), 121-128.
21. Urbaniak, M., Iskierka-Jażdżewska, E., Majchrzak, A., & Robak, T. (2022). Atypical immunophenotype of chronic lymphocytic leukemia. Acta Haematologica Polonica, 53(1), 48-52.
22. Hamad, H. M., Shabeeb, Z. A., & Awad, M. M. (2022). Expressions of CD274 (PD-L1) and CD47 rReceptors on the sSurface of bBlast cCells in AML pPatients. Iraqi Journal of Science, 2373-2387.
23. Srisurapanont, K., Thepchinda, T., Kasirawat, C., & Rattanathammethee, T. (2020). Prognostic Role of CD200 Expression in Hematologic Malignancies: A Systematic Review and Meta-Analysis. Available at SSRN 3559591.
24. Zahedi, M., Khorasani, S. S. K., Nasirzadeh, A., Abouali, R., Abounoori, M., Mortazavi, P., ... & Mohseni, A. R. (2019). Promising Role of CD200 Expression in Diagnosis and Prognosis of Chronic Lymphocytic Leukemia: A Review. *International Journal of Medical Investigation*, *8*(4), 16-22.
25. Miguet, L., Mayeur-Rousse, C., Eischen, A., Galoisy, A. C., Rolland, D. C., Gervais, C., ... & Mauvieux, L. (2021). The Contribution of CD148, CD180 and CD200 Combination in the Diagnosis of Chronic B-Cell Lymphoproliferative Disorders. Blood, 138, 3520. El Desoukey, N. A., Afify, R. A. A., Amin, D. G., & Mohammed, R. F. (2012). CD200 expression in B-cell chronic lymphoproliferative disorders. Journal of investigative medicine, 60(1), 56-61.
26. Wang, X., Zhang, Z., Liu, Y., Wang, L., Yuan, H., Xie, P., & Qu, J. (2014). Expression of CD200 in the bone marrow of chronic lymphocytic leukemia patients and its correlations with clinical prognosis. Xi bao yu fen zi mian yi xue za zhi= Chinese journal of cellular and molecular immunology, 30(1), 75-78.
27. Rahman, K., Kumar, P., Gupta, R., Singh, M. K., & Nityanand, S. (2017). Role of CD 200 in differential diagnosis of mature B-cell neoplasm. International journal of laboratory hematology, 39(4), 384-391.
28. Divya, P., Vangala, N., Uppin, M. S., Uppin, S. G., Gundeti, S., Bacchu, S., ... & Paul, T. R. (2022). Utility of CD200 expression by flow cytometry in lymphoproliferative disorders and plasma cell dyscrasias.
29. Fouad, N. B. E. D., Ibrahim, N. Y., Aziz, R. S. A., & Ibrahim, S. K. (2018). CD200 expression in diagnostic and prognostic assessment of mature B cell lymphophoproliferative neoplasms. Asian Pacific journal of cancer prevention: APJCP, 19(12), 3383.

30. Baraka, A., & Salem, H. M. (2012). Study of CD200 in chronic lymphocytic leukemia. The Egyptian Journal of Haematology, 37(2), 111.
31. De Santis, M. C., Porporato, P. E., Martini, M., & Morandi, A. (2018). Signaling pathways regulating redox balance in cancer metabolism. Fro [30]D'arena, G., Vitale, C., Perbellini, O., Coscia, M., La Rocca, F., Ruggieri, V., ... & Iorio, E. L. (2017). Prognostic relevance of oxidative stress measurement in chronic lymphocytic leukaemia. European journal of haematology, 99(4), 306-314.
32. Zhevak, T., Shelekhova, T., Chesnokova, N., Tsareva, O., Chanturidze, A., Litvitsky, P., ... & Budnik, I. (2020). The relationship between oxidative stress and cytogenetic abnormalities in B-cell chronic lymphocytic leukemia. Experimental and Molecular Pathology, 116, 104524.
33. Bakan, N., Taysi, S., Yilmaz, Ö., Bakan, E., Kuşkay, S., Uzun, N., & Gündoğdu, M. (2003). Glutathione peroxidase, glutathione reductase, Cu–Zn superoxide dismutase activities, glutathione, nitric oxide, and malondialdehyde concentrations in serum of patients with chronic lymphocytic leukemia. Clinica Chimica Acta, 338(1-2), 143-149.
34. Oltra, A. M., Carbonell, F., Tormos, C., Iradi, A., & Sáez, G. T. (2001). Antioxidant enzyme activities and the production of MDA and 8-oxo-dG in chronic lymphocytic lerkenia. Free Radical Biology and Medicine, 30(11), 1286-1292.
35. Salimi, A., Roudkenar, M. H., Sadeghi, L., Mohseni, A., Seydi, E., Pirahmadi, N., & Pourahmad, J. (2016). Selective anticancer activity of acacetin against chronic lymphocytic leukemia using both in vivo and in vitro methods: key role of oxidative stress and cancerous mitochondria. Nutrition and cancer, 68(8), 1404-1416.
36. Mehde, A. A., & Yousif, A. M. (2014). Estimation of Malondialdehyde, Total Antioxidant Capacity and Some Biochemical Parameters in CSF and Sera of Patients with Acute Lymphoblastic Leukemia. Australian journal of basic and applied sciences, 8(15), 329-333.
37. Mantovani, G., Macciò, A., Madeddu, C., Mura, L., Gramignano, G., Lusso, M. R., ... & Elsener, B. (2002). Quantitative evaluation of oxidative stress, chronic inflammatory indices and leptin in cancer patients: correlation with stage and performance status. International Journal of Cancer, 98(1), 84-91.
38. D'Arena, G., Seneca, E., Migliaccio, I., De Feo, V., Giudice, A., La Rocca, F., ... & Ruggieri, V. (2019). Oxidative stress in chronic lymphocytic leukemia: still a matter of debate. Leukemia & Lymphoma, 60(4), 867-875.
39. D'arena, G., Vitale, C., Perbellini, O., Coscia, M., La Rocca, F., Ruggieri, V., ... & Iorio, E. L. (2017). Prognostic relevance of oxidative stress measurement in chronic lymphocytic leukaemia. European journal of haematology, 99(4), 306-314.
40. Gaibullaeva, N. N. (2021). The role of clinical examination early diagnosis of glaucoma. International Journal of Health & Medical Sciences, 4(3), 333-337. https://doi.org/10.31295/ijhms.v4n3.1745
41. Zhevak, T., Shelekhova, T., Chesnokova, N., Tsareva, O., Chanturidze, A., Litvitsky, P., ... & Budnik, I. (2020). The relationship between oxidative stress and cytogenetic abnormalities in B-cell chronic lymphocytic leukemia. Experimental and Molecular Pathology, 116, 104524.

42. Collado, R., Ivars, D., Oliver, I., Tormos, C., Egea, M., Miguel, A., ... & Carbonell, F. (2014). Increased oxidative damage associated with unfavorable cytogenetic subgroups in chronic lymphocytic leukemia. BioMed Research International, 2014.
43. Zelen, I., Djurdjevic, P., Popovic, S., Stojanovic, M., Jakovljevic, V., Radivojevic, S., ... & Arsenijevic, N. (2010). Antioxidant enzymes activities and plasma levels of oxidative stress markers in B-chronic lymphocytic leukemia patients. J buon, 15(2), 330-336.
44. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. International Journal of Health Sciences, 6(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
45. Hwang, A. B., & Lee, S. J. (2011). Regulation of life span by mitochondrial respiration: the HIF-1 and ROS connection. Aging (Albany NY), 3(3), 304.
46. Kumar, S., Yedjou, C. G., & Tchounwou, P. B. (2014). Arsenic trioxide induces oxidative stress, DNA damage, and mitochondrial pathway of apoptosis in human leukemia (HL-60) cells. Journal of experimental & clinical cancer research, 33(1), 1-12.
47. Gaman, A. M., Buga, A. M., Gaman, M. A., & Popa-Wagner, A. (2014). The role of oxidative stress and the effects of antioxidants on the incidence of infectious complications of chronic lymphocytic leukemia. Oxidative Medicine and Cellular Longevity, 2014.