ejpmr, 2018,5(4), 21-29

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

STUDY OF CD163 AND B2M AS MARKERS FOR HODGKIN LYMPHOMA IN HUMAN AND ITS RELATION WITH CLINICO–PATHOLOGICAL BEHAVIOR

Hossam Darwish*[1], Lamiaa Barakat[2] and Rania Bondok

[1]Medical Oncology Consultant, Head of Medical Oncology Department of Ismailia Teaching Oncology Hospital, Egypt.

[2]Assistant Professor of Biochemistry, Faculty of Science, Portsaid University, Egypt.

[3]B.Sc Faculty of Science, Mansoura University, Diploma in Biochemistry, Faculty of Science, Port Said University Egypt.

***Corresponding Author: Hossam Darwish**

Medical Oncology Consultant, Head of Medical Oncology Department of Ismailia Teaching Oncology Hospital, Egypt.

Article Received on 22/01/2018 **Article Revised on 12/02/2018** **Article Accepted on 05/03/2018**

ABSTRACT

Background: Hodgkin lymphoma is the most non malignancies at young adult ages, and is a curable disease in most patients. In the present study, our suggestion is to use CD163 and B2M as supplementary markers for the prognosis concerning Hodgkin lymphoma. So, identification of sensitive biomarkers to improve the prognosis related Hodgkin lymphoma is needed. **Aim of the work:** The aim of present study was to discuss the prognosis utility of serum CD163 and serum B2M in patients for Hodgkin lymphoma during and post therapy the clinical advantage of these markers will be decreased or normal during or post therapy. CD163 will be compared with B2M which is a routinely marker used for diagnosis and in follow up the patients for Hodgkin lymphoma. **Results:** Serum CD163 was significantly in most patients for Hodgkin lymphoma. The receiver operating characteristic curve (ROC) showed the best cut off values for CD163 and B2M were 5.0 ng/ml and 2.50 mg/l respectively. Area under the curve of CD163 and B2M were significantly 0.79 and 0.78. The sensitivity of CD163 and B2M were 76.7% and 66.7% respectively. **Conclusion:** Results revealed that serum CD163 may be used as potential prognostic markers for Hodgkin lymphoma patients.

KEYWORDS: Hodgkin lymphoma patients, Cluster of Differentiation 163 (CD163), Beta 2 microglobulin (B2M).

INTRODUCTION

Lymphoma is type of cancer of the lymph system, which is a part of the immunity. (Shankland et al., 2012). Lymphomas can impact any organ in the body with broad range of symptoms, they are divided into Hodgkin lymphoma and non-Hodgkin's lymphoma (NHL), they arise from lymphocytes at different stages of evolution, the features of subtype lymphoma cancer to explain the cell from which they produced (Armitage et al., 2017). Hodgkin's lymphoma ranged for approximately 30% of all malignant lymphoma (Diehl et al., 2003). In Egypt, the recurrence of malignant Lymphoma varies between 7.8% to 12% of cancer cases as the registries of various cancer centers indicated (ElBolkainy et al., 1984). In the NCI-Egypt cancer pathology registry, the ratio of NHL to HL was 2.3: 1 and HL constituted 30.3% of all types of lymphoma cases (Mokhtar and Khaled, 2002).

Hodgkin lymphoma is characterized by the Presence of a rather low number of neoplastic cells, either multinucleated (Reed Sternberg cells, RSCs) or mononucleated (Hodgkin cells and variants, HCs) (viviani et al., 2017). It is chronic, progressive, neo plastic disorder of lymphatic tissue with the painless and enlargement of lymph nodes with progression to extra lymphatic zones such as spleen and liver. It may metastasize to bone marrow and other organs. Zones which more initially influenced are usually the lymph glands of the neck or groin, commonly on one side (Goodman and Snyder, 2007). Around age (25-30) years and after 55 years, men are typically diagnosed for Hodgkin lymphoma more than women. Hodgkin lymphoma can happen in children and adults, but less than 5 years little develop to this disease, but 10% of Hodgkin lymphoma cases happen in children 16 years old and younger (Goodman and Fuller, 2009). There are two subtypes of Hodgkin's lymphoma, classic and nodular lymphocyte-predominant. Classic Hodgkin's lymphoma which classified to four categories based what the cell resemble under a microscope, these are lymphocyte-rich classical Hodgkin lymphoma, Nodular-sclerosis Hodgkin lymphoma, mixed-cellularity Hodgkin lymphoma and Lymphocyte-depleted Hodgkin lymphoma (Goodman and Snyder, 2007). A history of infectious mononucleosis as a result of this infection by Epstein–Barr virus (EBV) may increase the risk of

Hodgkin lymphoma, but the accurate contribution of Epstein–Barr virus remains broadly unknown (Gandhi et al., 2004). Ideal markers must be specific, sensitive, and plain to assay and interpret with rapid turnaround high reproducibility and comparability between laboratories. Serum protein estimation by Enzyme-linked immuno sorbent assay (ELISA) exploits an established technology used in the diagnostic laboratory. Furthermore, while tissue markers could be applied routinely to the diagnostic biopsy to provide prognostic information, they have no applicability in monitoring disease response. In contrast, biomarkers based on blood have possibility to supply information on disease response and exhibit of early relapse (Gandhi et al., 2006). CD163 is an endocytic receptor for haptoglobin-hemoglobin complexes. On account of presence of ectodomain shedding, the extracellular part of CD163 diffuse in blood as a soluble protein (sCD163), the mission of sCD163 is unknown, but during the inflammation and macrophage activation, sCD163 levels increase by reason of metalloproteinase-mediated cleavage near the cell membrane (Moller, 2012). CD163 appears to be related to anti-inflammatory functions associated with macrophages that stimulate tumor cell increase and metastasis (M2 macrophages) in contrast to M1 macrophages that destroy tumor cells (Jensen et al., 2009). An increased plasma concentration of sCD163 is apparent in diseases relating to macrophage activity, containing acute and chronic inflammations (Moller, 2012). Beta 2 microglobulin is existing on the surface of nearly all nucleated cells in the body. In healthy people, lymphocytes are the major production site of B2M, and inflammatory cytokines induce the production of B2M, and increased levels of B2M can are due to increased liberation from immune system activation or proliferation. It is a prognostic marker in many lymphomas, including Hodgkin lymphoma. Elevated levels of B2M can be originate in 5–30% of patients at diagnosis, depend on the stage, and have been appear to be associated with the relapse (Vassilakopoulos et al., 2002).

Subjects and Methods

In this research, patients were grouped samples, in Clinics of Damietta, Mansoura during the interval between November 2015 and April 2016, this study was proceed on eighty four patients they were diagnosed Hodgkin lymphoma during and post-therapy. Included criteria, All Egyptian patients were diagnosed by Hodgkin lymphoma and with age ranging from 18 to 70 years old regardless to sex, social status or occupation. Excluded criteria, Patients who diagnosed as EBV, HIV and auto-immune diseases were positive.

Samples collection

Five ml venous blood was withdrawn from each individual, was collected and coagulated in room temperature 10-20 minutes, centrifugated 20 minutes at the speed of 2000- 3000 rpm, for separation of serum which was transferred into another tube and kept frozen at -20°C to detect CD163 and B2M. Tumor markers, CD163 and B2M were done using ELISA (enzyme-linked immune sorbent assay) kit by (ELISA plate analyzer, ELIZA read well touch, Rebonik, India).

Reagent preparation

All reagents were allowed to reach room temperature (the reagents should not be dissolving at 37^{o}C directly and mixed thoroughly by swirling before pipetting, and avoid foaming.

Statistical analysis

All statistical analyses of the data were done by statistical package for the social sciences (SPSS), version 20.0 on Microsoft Windows XP (SPSS Inc., Chicago, IL, USA). Optimal biomarker cut-off levels for patients were determined by using the Receiver Operating Characteristic (ROC) method. Differences in biomarker levels were calculated with the unpaired t-test. Baseline biomarker levels were correlated to Ann Arbor stage and response for treatment. Kaplan–Meier analysis was used to calculate OS. Boxplot which appears the interquartile range, the whiskers specify the highest and lowest values, and the line across the box indicates the median value.

RESULTS

The study was proceed on eighty four patients for Hodgkin Lymphoma divided according to pathology, stage, symptoms and response to treatment, In the study there were 48 (75.2%) males and 36(24.8%) females all were patients for Hodgkin Lymphoma with average age <35 years 34 patients (40.5%) and >35 years 50 patients (59.5%). Patients were classified to nodular sclerosis 45 (53.5%) and mixed cellularity 23 (27.3%) which were appear as the most frequent subtype followed by lymphocyte depletion 10 (9.5%) then nodular lymphocyte predominant 6 (7.1%), according to the Histological WHO classification. Patients classified to four stages, in the study stage (I) were the most frequent stage 38 (45.5%) patients followed by stage (III) 28 (33.3%) patients then stage (II) 14 (16.7%) patients and 4 (4.8%) patients were distributing for stage (IV). Patients were classified as indicated in Ann Arbor stage to early stage without bulky lesions (I- II) 52 (61.9%) patients and in advanced stage with bulky lesions (III- IV) 32 (38.1%) patients. B symptoms varied between patients, 22 (26.2%) patients were with B symptoms, and 62 (73.8%) patients were with no B symptoms. Most of patients presented with complete response 48 (55.1%) followed by which presented with partial response 34 (24.4%). The poly chemotherapy treatment frequently used was ABVD (adriamycin/bleomycin/vinblastine/ dacarbazine) followed by many types of chemotherapy, patients were treated with ABVD only were 32 (38.1%), patients were treated initially with ABVD and/or other chemo-therapy (following therapy strategies for HL patients BEACOPP (bleomycin / etoposide / adriamycincyclophosphamide / vincristine / procarbazine / prednisone), DHAP (dexamethasone / highdosearaC /

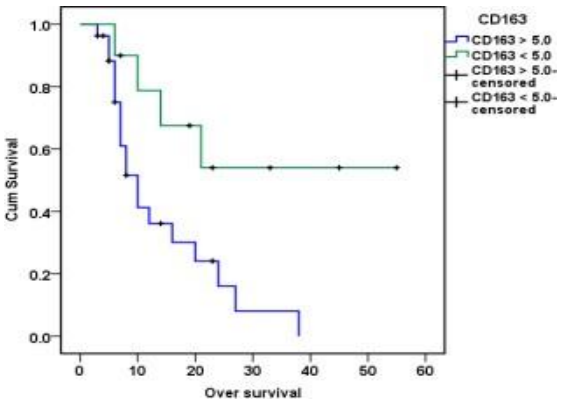
cisplatin), ICE (ifosfamide / carboplatin / etoposide), IGEV (ifosfamide / gemcitabine / vinorelbine / dexamethasone), MINE (mesna, ifosfamide, mitoxantrone, and etoposide) 42 (50.0%) most patients in this group were not treated by radiotherapy ABVD therapy and involved-field radiation Therapy (IFRT) 10 (11.9%), patients were treated by ABVD only were classified according to count of cycles, patients were treated by two cycles 4(12.5%), patients were treated by four cycles 8 (25.0%), patients were treated by six cycles 12 (37.5%) and patients were treated by eight cycles 8 (25.0%) as indicated in (Table1). most frequent subtype Nodular Sclerosis was with a mean value 3.55±1.02 showed in CD163 and 2.37±0.74 showed in B2M, Mixed Cellularity sub type was with a mean value 3.82±1.20 showed in CD163, and 2.68±0.39 showed in B2M, Lymphocyte-Depletion subtype was with a mean value 3.79 ±1.18 showed in CD163 and 2.03± 0.27 showed in B2M and Nodular Lymphocyte Predominant subtype was with a mean value 3.33±1.04 showed in CD163 and 1.98± 0.61 showed in B2M. There is no difference in pathology and parameters in CD163 and B2M were showed significant (p value < 0.05). Early stages (stage I –II) were with a mean value 3.25±1.00 showed in CD163 and 2.00±0.52 showed in B2M and in advanced stages (stage III–IV) were with a mean value 4.77±1.30 showed in CD163 and 3.19±0.92 showed in B2M. The relation between parameters and stages showed that CD163 was very high significant (p value < 0.001**) and B2M (p value < 0.001**). With respect to the values of CD163 and B2M and it is relation to symptoms which are 3.88 ±1.10 and 2.79 ±0.77 in cases of no-B symptoms and 4.87±1.09 and 3.83±1.16 in cases of B-symptom. The relation between parameters and symptoms showed that CD163 was high significant (p value <0.01*) then B2M (p value < 0.05). 48 patients achieved complete response with a mean value 3.51± 1.11 in CD163 and 2.13± 0.62 in B2M and 34 patients with partial response with a mean value 4.83±1.21 in CD163 and 3.47 ±1.02 in B2M. B2M was showed high significant (p value<0.01) and CD163 showed a significant (p value< 0.05) as indicated in (Table2). Kaplan Meier method was to estimate the distribution of over survival was done using individual patient information, the best chosen cut off of CD163 was (5ng/ml), patients with CD163 <5.0 were more response than patients with CD163 >5.0, there was high significant difference (x^2=6.285, p<0.001) among detection of quality of over survival distributions for different levels of CD163_5 in Hodgkin lymphoma as indicated in (Figure1A). The cut off of B2M was (2.5mg/l), Patients with B2M <2.5 were more response than patients with B2M >2.5, there was high significant difference (x^2=5.474: p<0.001) among detection of quality of over survival distributions for different levels of B2M _2.5 in Hodgkin lymphoma as indicated in (Figure 1B). There was significant difference (x^2=4.117: p<0.042) among detection of quality of over survival Comparisons for different levels of symptoms in Hodgkin lymphoma as indicated in (Figure 1C), the cut off of serum CD163 in early stages vs. late stages was 5.0 ng/ml yield AUC=0.79, with sensitivity 62.5% specificity 70.0% as indicated in (Figure 3A), the cut off of serum B2M in early stages vs. late stages was 2.50 mg/l yield AUC=0.78, with sensitivity 75.0% specificity 76.9% as indicated in (Figure 3B). the cut off of serum CD163 in presence of B symptoms vs. no B symptoms was 5.0ng/ml yield AUC=0.774, with sensitivity 76.7% specificity 70.0% as indicated in (Figure 5A), the cut off of serum B2M in presence of B symptoms vs. no B symptoms was 2.50 mg/l yield AUC=0.684, with sensitivity 66.7% specificity 72.4% as indicated in (Figure 5B).

Table (1): Clinicopathological characteristic of patients.

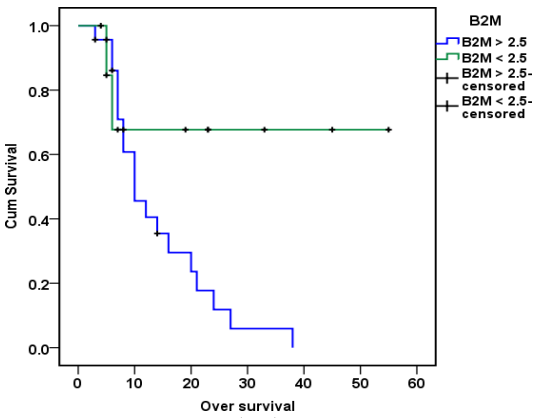
Clinical features		No	Percent
sex	male	48	57.2 %
	female	36	42.8 %
Age	<35	34	40.4%
	>35	50	59.5%
stages	I	88	45.2%
	II	41	16.7%
	III	88	33.3%
	IV	1	4.8%
pathology	Nodular sclerosis	14	(48.5%)
	Mixed Cellularity	88	(27.3%)
	Lymphocyte Depletion	41	(9.5%)
	Nodular Lymphocyte Predominant	6	(7.1%)
B-symptoms	No B-Symptoms	62	73.8 %
	B-Symptoms	22	26.2 %
response	Partial response	48	57.4 %
	Complete response	34	11.4 %
Therapy type	ABVD and/or other chemotherapy	42	50.0%
	ABVD+RT	10	11.9%
ABVD only	Digit of cycles of ABVD		
	2 cycle	4	12.5%
	4 cycle	8	25.0%
	6 cycle	12	37.5%
	8 cycle	8	25.0%
	Total	32	38.1%

Table (2): Demographic characteristic of patients.

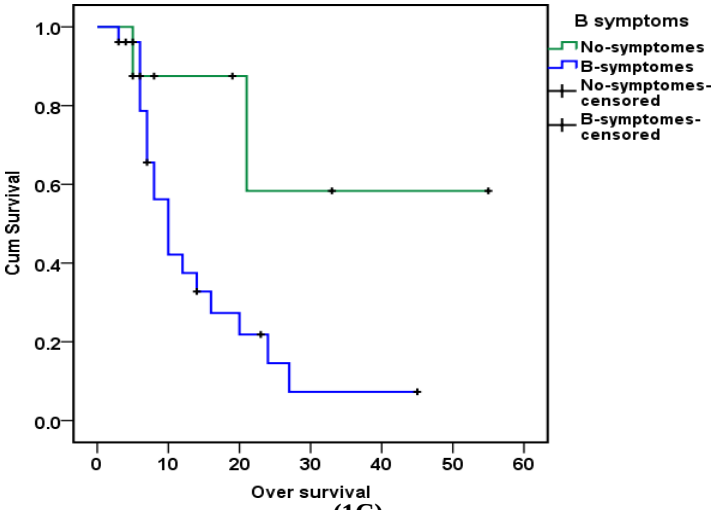
First variable	Second variable	CD163			B2M		
		mean	SD	P value	mean	SD	P value
pathology	Nodular Sclerosis	3.55	1.02	< 0.05	2.37	0.74	< 0.05
	Mixed Cellulariy	3.82	1.20		2.68	0.39	
	Lymphocyte Depletion	3.79	1.18		2.03	0.27	
	Nodular Lymphocyte Predominant	3.33	1.04		1.98	0.61	
Ann prop stage	Early (I – II)	3.25	1.00	<0.001	2.00	0.52	<0.001**
	Advanced (III-VI)	4.77	1.30		3.19	0.92	
symptoms	B-Symptoms	4.87	1.09	<0.01	3.83	1.16	<0.05
	No-symptoms	3.88	1.10		2.79	0.77	
response	Partial response	4.83	1.21	<0.05	3.47	1.02	<0.01*
	Complete response	3.51	1.11	0.05<	2.13	0.62	<0.01*



(1A)

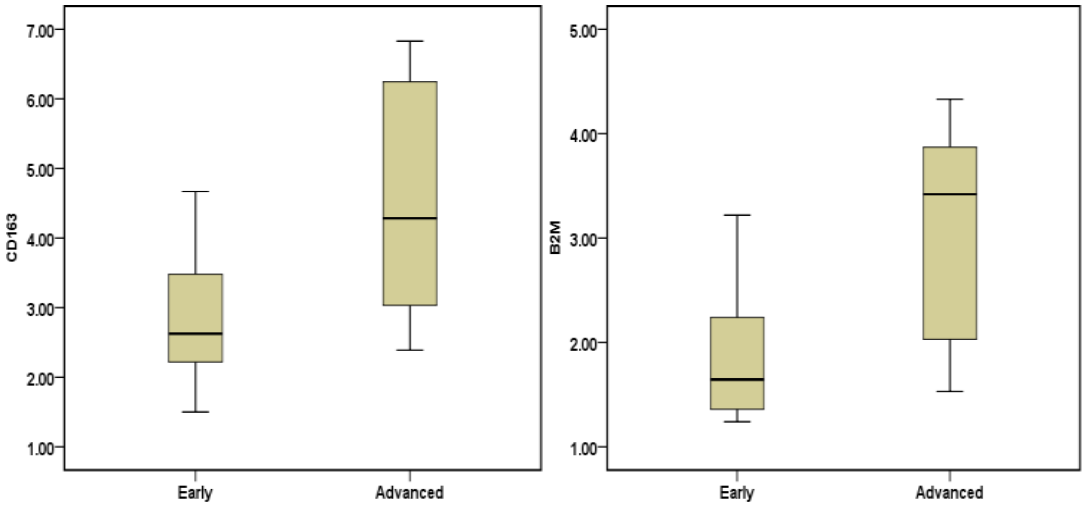


(1B)



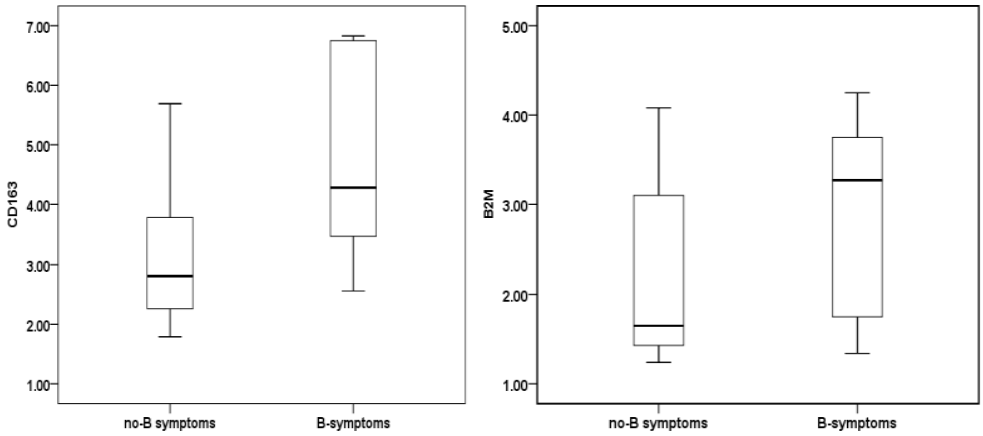
(1C)

Figure (1): Kaplan–Meier curves for estimation of over survival in patients of Hodgkin lymphoma (A) with serum CD163 concentrations<5.0 ng/ml and >5.0 ng/ml at prognosis (p<0.001) (B) with serum B2M concentrations<2.5 mg/l and >2.5 mg/l at prognosis (p<0.001) (C) with symptoms at prognosis (p<0.042).



(2A) (2B)

Figure (2): Boxplot of (A) serum CD163 in patients (B) serum B2M in patients. The box appears the interquartile range. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value.



(3A) (3B)

Figure (3): Boxplot of (3A) serum CD163 in patients (3B) serum B2M in patients. The box appears the interquartile range. The whiskers indicate the highest and lowest values and the line across the box indicates the median value.

DISCUSSION

Hodgkin's lymphoma (HL) is one type of lymphoma which produces from a specific type of white blood cells called B-lymphocytes. The infectious mononucleosis outcome infection by Epstein–Barr virus (EBV) may increase the risk of HL (Gupta et al., 2016). Treatment of the classical Hodgkin's lymphoma (HL) has been a good story with treatment of disease by radiotherapy and treatment in advanced stages with combination of chemotherapy with/without radiotherapy leading to improvements since then (Glimelius and Diepstra, 2017). More than 95% of Hodgkin lymphoma (HL) patients in early stage and 80–90% in intermediate and progressive stages can be treated by standard chemo and radiotherapy. However, this outcome is associated with treatment of a significant proportion of patients; this can cause unnecessary treatment- related toxicity including organ damage and secondary malignancies or early progression and relapse (Sauer et al., 2013). There are an improvements in survival in the treatment of HL, concerns start to around the long-term toxicities of chemotherapy and radiation therapy (Hodgson et al., 2007), serum levels were lower compared to plasma, this makes it unlikely that serum would be more sensitive than plasma (Ouyang et al., 2013). Prognostic biomarkers for cHL can be divided into risk pre- therapy which appears disease response throughout therapy, although tissue biomarkers may be useful before to therapy, it is an impracticable source for repeated measures to observe disease response. Nevertheless, markers elevated in cHL tumor tissues may also be elevated in blood, in contrast to tissue (Shim et al., 2009). Blood- based biomarkers hold to be much more practical, patient friendly and cost-effective and might be used as serial markers during and after treatment to determine early response to treatment and disease recurrence after treatment, these biomarkers can be divided into tumor cell specific markers, secreted by Hodgkin Reed-Sternberg (HRS) cells or markers related to micro-environment (Kuppers et al., 2012 and Liu et al., 2014). The individuals' serum samples were screened for different parameters to prognosis Hodgkin lymphoma including determination of Cluster of Differentiation 163 (CD163) and Beta-2 microglobulin (B2M) that was determined using ELISA technique. This study was conducted on eighty four patients diagnosed with Hodgkin lymphoma during, and post-therapy which indicated that CD163 and B2M during and after treatment in patients with Hodgkin lymphoma related to symptoms (B symptoms n=62 patients and no B symptoms n=22 patients), according to the WHO classification, patients were classified to nodular sclerosis 45 (53.5%) and mixed cellularity 23 (27.3%) which were the most subtypes followed by lymphocyte depletion 10 (9.5%) then nodular lymphocyte predominant 6 (7.1%), stages (early stages (I – II) n=56 patients and in advanced stages (III-VI) n=32 patients, response to treatment partial response n=34 patients and complete response n=48 patients, therapy type ABVD only n=32 patients, ABVD and/or other chemotherapy n=42 patients and ABVD+RT (radiotherapy) n=10 patients and number of cycles of ABVD (2 cycles n=4 patients, 4 cycles n=8 patients, 6 cycles n=12 patients and 8 cycles n=8 patients). some routine parameters were determined as hemoglobin (Hb), white blood cells (WBCs) count, red blood cells (RBCs) count, Platelets count, serum lactate dehydrogenase (LDH), serum Albumin, Serum Creatinine, serum uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and erythrocyte sedimentation rate (ESR) but the more parameters were related to Hodgkin lymphoma were serum lactate dehydrogenase (LDH) and erythrocyte sedimentation rate (ESR) which are also increased in inflammation, pregnancy, anemia, autoimmune disorders (such as rheumatoid arthritis and lupus), infections, some kidney diseases and some cancers (such as lymphoma and multiple myeloma). The ESR is decreased in polycythemia, hyper viscosity, sickle cell anemia, leukemia, low plasma protein (due to liver or kidney disease) and congestive heart failure, the basal ESR is slightly higher in females. LDH is an enzyme which stimulate the transformation of lactate to pyruvate which is an important step in energy production inside the cells, many various types of cells in the body contain this enzyme, some of the organs relatively rich in LDH are the heart, kidney, liver, and muscle tissue, as cells die, LDH is liberated into the blood, some studies suggest LDH Levels as prognostic marker in Hodgkin's disease (Gocheva et al., 2010). The measurement of hematological factors such as Erythrocyte Sedimentation Rate (ESR) and Lactate Dehydrogenase (LDH) help in the prognosis of Hodgkin lymphoma (Kuruvilla, 2009). In our study correlation analysis was determined among LDH and ESR parameters and markers which showed that CD163 which was positively related to LDH (p value: 0. 038, r = 0.342*), ESR1 (p value: 0.0001, r = 0.571**) and ESR2 (p value: 0. 005, r = 0.469**) respectively, while β_2m was positively related to LDH (p value: 0.0001, r = 0.584**), positively related with ESR1 (p value: 0.031, r = 0.443*) and positively related to ESR2 (p value: 0.004, r = 0.488**), sCD163 related to the amount of tumor associated macrophages (TAM) infiltration, The amount of TAM is highly associated with shortened survival in the classical Hodgkin Lymphoma, correlated with probability of relapse for disease-specific survival (Steidl et al., 2010). The M2 macrophage marker soluble CD163 (sCD163) are elevated in patients cHL serum (Jones et al, 2013 and Ouyang et al, 2013). CD163 help in anti-inflammatory functions associated with M2 macrophages, which promote tumor cell growth and metastasis, in contrast M1 macrophages that kill tumor cells (Jensen et al., 2009), the functional characterization of TAM is still to be performed and the differenence in survival among patients could be indicated by the macrophages M1/M2 paired on which these cells differentiate the antigen CD163 is physiologically expressed on the macrophage surface, and it is exact as an additional marker of macrophage infiltration in HL microenvironment (Yoon et al., 2012). Recently, the common fraction of CD163 in

serum (s-CD163) has been estimated in patients at diagnosis and relapse. CD163 are often used to identify macrophages in tissue sections, numerous studies indicated that elevated CD163 expression related to advanced cancer stages, unfavorable prognosis, early distant recurrence and reduced patient survival in various types of cancer, which include meningioma, breast cancer, colorectal cancer, melanoma, oral squamous cell carcinoma ovarian carcinoma, HCC, angiosarcoma, glioma, gastrointestinal stromal tumors and hematopoietic malignancies, such as T cell leukemia/lymphoma (Komohara et al., 2013). Acute myeloid leukemia and classical Hodgkin lymphoma, a recent study showed that relapse of head and neck cancer after chemo- radiotherapy also correlated with CD163+ macrophages, many studies reported that the tumor cell itself in many cancers are associated with metastatic grade, early recurrence and reduced patient survival (Kanno et al., 2013). CD163 not only existing as a membrane-bound form, but also existing as a soluble form (sCD163) in plasma and other tissue fluids CD163 not only existing as a membrane-bound form, but also existing as a soluble form (sCD163) in plasma and other tissue fluids. sCD163 has been reported to be elevated in several inflammatory conditions, such as sepsis, diabetes, liver cirrhosis, rheumatoid arthritis, human immunodeficiency virus and macrophage activation syndrome. However, this not explain the different patterns observed in plasma versus serum samples, the M2 macrophage marker soluble CD163 are elevated in serum of patients with cHL (Jones et al., 2013). Also found a correlation between serum sCD163 and interim response, it must be stressed that there was a large overlap between patients in complete remission compared to partial remission. There are many studies indicated the role of CD163 in tissue but this study indicated the role of CD163 in serum, in the present study CD163 levels significantly correlated with presence of symptoms ($p<0.01$), stages ($p<0.001$) and the response to treatment ($p<0.05$) which is in accordance with the results of (Wouter et al., 2016) who reported CD163 levels only significantly correlated with presence of B symptoms ($p<0.001$). The initial level of B2M has been also confirmed to be an important diagnostic marker in most lympho proliferative diseases of adults, including HL, serum B2M levels correlate with Ann Arbor stage and B symptoms in patients with Hodgkin disease and that elevated levels of this polypeptide predict less favorable prognosis serum B2M levels have been associated with the malignancy of lymphoma (Dimopoulos et al., 1993). Similar results were obtained by (Gupta et al., 2016) who found that levels were significantly raised in HL patients who presented with stages ($p<0.01$) and ($p <0.05$) in HL patients who presented with B symptoms as demonstrated in our study which showed significantly levels to B2M correlated with B symptoms ($p<0.05$) and in stages were very significant ($p<0.001$) which is in accordance with the results of Hodgkin's lymphoma (HL) has changed its prognosis from being relatively incurable to one in which patients have a high likelihood of long- term survival, there are an improvements in survival in the treatment of HL, concerns began to center around the long-term toxicities of chemotherapy and radiation therapy (Hodgson et al., 2007). In patients with advanced-stage of HL, the early response after 2 courses of ABVD chemotherapy, when evaluated for 18FDG-PET scan, shows important prognostic significance (Terasawa et al., 2009). Favorable outcomes with ABVD, reporting complete response rates of 73-89%, 5-year freedom from progression (FFP) of 73-76% and 5-year overall survival (OS) of up to 90% in intermediate and advanced stage HL (Gordon et al., 2013). A gradual lower of sCD163 during and after treatment in serum samples of cHL patients than that in plasma samples, while some times showed a slight post-treatment increase in sCD163 levels. This increase might reflect treatment-induced inflammatory responses as showed by (Jones et al, 2013). In our study the ROC curve shows that CD163 had diagnostic performance with AUC=0.77, sensitivity (76.7%), specificity (70.0%). similar to results which recorded by (Jones et al, 2013) which indicated that the cut off level of 500 ng/ml for sCD163 resulted in AUC = 0.7333, 62% sensitivity and 75% specificity. In the present study the ROC curve shows that B2M had diagnostic performance with AUC=0.68, sensitivity (66.7%), specificity (72.4%), which is in accordance with (Nakajima et al., 2014). Who demonstrated that the the ROC curve analysis showed the appropriate cut off of serum B2M levels to be 2.5 mg/L for predicting OS (area under the curve= 0.81, sensitivity, 0.667, specificity) elevated levels of serum B2M had a tendency to be associated with age more than 45 years (P =0.001), Ann Arbor stages III–IV (P = 0.004). Finally, the relation between markers and the clinico-pathological behavior in this study indicated that CD163 (p value < 0.001**) in stages, (p value: <0.01*) in symptoms and a less significant (p value < 0.05) in response for traetment and B2M significantly correlated to stage (p value < 0.001**), related to response for treatment (p value < 0.01) and a less significant to symptoms (p value < 0.05), there is no effect in pathology and parameters (CD163 and B2M) were showed significant (p value < 0.05).

In conclusion, this study showed that the serum CD163 may serve as a good prognostic biomarkers for the follow up of Hodgkin lymphoma Particularly at in presence of symptoms, also CD163 alone or in combination with B2M significantly improve the prognostic of Hodgkin lymphoma with a sensitivity, specificity higher than that of B2M alone, furthermore the combination of these markers has the largest AUC, sensitivity and specificity and may improve the prognostic value in distinguishing patients with Hodgkin lymphoma.

In conclusion, an elevated level of sCD163 was found in cHL patients, sCD163 are sensitive and specific marker of disease resolution post-therapy. Therefore, this marker

might be used as a cancer-specific serum biomarker for various human cancers, including Hodgkin lymphoma.

CONCLUSIONS

CD163 was found to be decreased or normal during or post therapy in Hodgkin lymphoma group can be used as tumor marker for the prognosis of Hodgkin lymphoma, where they are promising prognostic markers for follow up of Hodgkin lymphoma and may serve as useful tumor markers with good sensitivity and good specificity. Addition of CD163 and B2M to give a significant improvement in detection of response in patients with Hodgkin lymphoma. Therefore, combination of multiple markers may be more valuable in the prognosis of Hodgkin lymphoma. Despite of being sometimes used in Hodgkin lymphoma as markers, B2M is a marker for Hodgkin lymphoma, where its serum concentration was found to be elevated in many other conditions other than Hodgkin lymphoma.

REFERENCES

1. Armitage, J. O., Gascoyne, R. D., Lunning, M. A. and Cavalli, F. (2017): Non- Hodgkin lymphoma. Lancet, 6736: 32402-32407.
2. Diehl, V., Stein, H., Hummel, M., Zollinger, R. and Connors, J. M. (2003): Hodgkin's lymphoma: biology and treatment strategies for primary, refractory, and relapsed disease. Hematology Am Soc Hematol Educ Program, 2003; 225-247.
3. Dimopoulos, M.A., Cabanillas, F., Lee, J.J., Swan, F., Fuller, L., Allen, P.K., et al. (1993): Prognostic role of serum beta 2-microglobulin in Hodgkin's disease. J Clin Oncol, 11: 1108–1111.
4. ElBolkainy, M.N., Gad El-Mawla, N.S., Tawfik, H.N., Aboul-Enein, M. (1984): Epidemiology of lymphoma andleukaemia in Egypt. J Egypt Nat Cancer Instit, 1: 19- 16.
5. Gandhi, M.K., Tellam, J.T. and Khanna, R. (2004): Epstein-Barr virus-associated Hodgkin's lymphoma." Br J Haematol, 125: 267–281.
6. Gandhi, M.K., Lambley, E., Duraiswamy, J., Dua, U., Smith, C., Elliott, S., Gill D., Marlton, P., Seymour, J. and Khanna, R. (2006): Expression of LAG-3 by tumor-infiltrating lymphocytes is coincident with the suppression of latent membrane antigen-specific CD8+ T-cell function in Hodgkin lymphoma patients. Blood, 108: 2280–2289.
7. Gupta, G., Lokanathan, V., Ghalaut, M.S., Sarker, M., Sharma, T. and Dokwal, S. (2016): Prediction of Disease Severity by Beta 2 Microglobulin Levels In HodgkinLymphoma.. Acad. J. Biosci, 4: 230-232.
8. Glimelius, I and Diepstra, A. (2017): Novel treatment concepts in Hodgkin lymphoma. J Intern Med, 281: 247-260.
9. Gocheva, L.; Koleva, Klin and Onkol, I. (2010): Long-term outcome of treatment for Hodgkin's disease: the University Hospital Sofia experience, 23: 34-42.
10. Goodman, C.C and Fuller, K.S. (2009): Implications for the Physical Therapist. 3rd edition. St. Louis, Missouri: Saunders Elsevier.
11. Goodman, C.C. and Snyder, T.K. (2007): Differential Diagnosis for Physical Therapists: Screening for Referral. 4th edition. St. Louis, Missouri: Saunders Elsevier.
12. Gordon, L.I., Hong, F., Fisher, R.I., et al. (2013): Randomized phase III trial of ABVD versus Stanford V with or without radiation therapy in locally extensive and advanced-stage Hodgkin lymphoma: an intergroup study coordinated by the Eastern Cooperative Oncology Group (E2496). J Clin Oncol, 31: 684-91.
13. Hodgson, D. C., E. S. Gilbert, G. M. Dores, et al. (2007): Long-term solid cancer risk among 5-year survivors of Hodgkin's lymphoma. J. Clin. Oncol, 25: 1489–1497.
14. Jensen, T.O., Schmidt, H., Moller, H.J., Hoyer, M., Maniecki, M.B., Sjoegren, P., Christensen, I.J and Steiniche, T. (2009): Macrophage markers in serum and tumor have prognostic impact in American Joint Committee on Cancer stageI/IImelanoma. J Clin Oncol, 27: 3330–3337.
15. Jones, K., Vari, F., Keane, C., Crooks, P., Nourse, J.P., Seymour, L.A., Gottlieb, D., Ritchie, D., Gill, D. and Gandhi, M.K. (2013): Serum CD163 and TARC as disease response biomarkers in classical Hodgkin lymphoma. Clinical Cancer
16. Research: An Official Journal of the American Association for Cancer Research, 19: 731–742.
17. Kanno, N. H.; Wang, L.; Yuzawa, S.; Kobayashi, H.; Tsuda, M.; et al. (2013): Expression of CD163 prevents apoptosis through the production of granulocyte colony-stimulating factor in meningioma. Neuro-Oncology, 15: 853–864.
18. Komohara, Y., Niino, D., Saito, Y., Ohnishi, K., Horlad, H., Ohshima, K., et al. (2013): Clinical significance of CD163 (+) tumor-associated macrophages in patients with adult T-cell leukemia/lymphoma. Cancer Science, 104: 945–951.
19. Kuppers, R., Engert, A., Hansmann, M.L. (2012): Hodgkin lymphoma. J Clin Investig, 122: 3439–3447.
20. Kuruvilla, J. (2009): Standard therapy of advanced Hodgkin lymphoma. Hematology Am Soc Hematol Educ Program, 4: 497–506.
21. Liu, Y., Sattarzadeh, A., Diepstra, A., Visser, L., Van den Berg, A. (2014): The microenvironment in classical Hodgkin Lymphoma an actively shaped and essential tumor component Seminars in Cancer Biology, 24: 15-22.
22. Mokhtar, N. and Khaled, H. (2002): Lymphoma. NCI (publisher) Cairo., 185-204.
23. Moller, H.J. (2012): Soluble CD163. Scand J Clin Lab Invest, 72: 1–13.
24. Nakajima Y, Tomita N, Watanabe R, Ishiyama Y, Yamamoto E, Ishibashi D et al (2014): Prognostic significance of serum beta-2 microglobulin level in Hodgkin lymphoma treated with ABVD-basedtherapy. Medical Oncology, 31: 185.
25. Ouyang, J., Plutschow, A., Pogge von Strandmann,

E., Reiners, K.S., Ponader, S., Rabinovich, G.A., Neuberg, D., Engert, A. and Shipp, M.A. (2013): Galectin-1 serum levels reflect tumor burdenand adverse clinical features in classical Hodgkinp18INK4c gene by promoter hypermethylation in Reed-Sternberg cells in Hodgkin lymphomas. Blood, 103: 2351-2357.

26. Sauer, M., Plutschow, A., Jachimowicz, R.D., Kleefisch, D., Reiners, K.S., Ponader, S., Engert, A. and von Strandmann, E.P. (2013): Baseline serum TARC levels predict therapy outcome in patientswith Hodgkin lymphoma. American Journal of Hematology, 88: 113–115.
27. Shankland, K.R., Armitage, J.O and Hancock, B.W. (2012): Non Hodgkin lymphoma. The Lancet, 380(9,844): 848-857.
28. Shim, H.K., Lee, W.W., et al. (2009): Relationship between FDG uptake and expressions of glucose transporter type 1, type 3, and hexokinase-II in Reed-Sternberg cells of Hodgkin lymphoma. Oncol Res, 17: 331–337.
29. Steidl, C., Lee, T., Shah, S.P., Farinha, P., Han, G., Nayar, T., et al. (2010): Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. The New England journal of medicine, 362: 8758-8785.
30. Terasawa, T., Lau, J., Bardet, S., et al. (2009): Fluorine-18-fluorodeoxyglucose positron emission tomography for interim response assessment of advanced-stage Hodgkin's lymphoma and diffuse large B-cell lymphoma: a systematic review. Journal of Clinical Oncology, 27: 1906–1914.
31. Vassilakopoulos, T.P., Nadali, G., Angelopoulou, M.K., Siakantaris, M.P., Dimopoulou, M.N., Kontopidou, F.N., Karkantaris, C., Kokoris, S.I., Kyrtsonis M.C., Tsaftaridis, P., Pizzolo, G and Pangalis, G., A.(2002): The prognostic significance of beta(2)-microglobulin in patients with Hodgkin's lymphoma. Haematologica, 87: 701–708.
32. Viviani, S., Tabanelli, V., Stefano, A and Pileri. (2017): The pathobiology and treatment of Hodgkin Lymphoma. Where do we go from GianniBonadonna's esson? Tumori, 103: 101-113.
33. Wouter, J.P., Zainab, N. D., Gustaaf, A. W., Diepstra, I.V., van den Berg, A and Visser, L. (2016): Biomarkers for evaluation of treatment response in classical Hodgkin lymphoma: comparison of sGalectin-1, sCD163 andsCD30 with TARC2 British Journal of Haematology, 175: 868–875.
34. Yoon, D.H., Koh, Y.W., Kang, H.J., Kim, S., Park, C.S., Lee, S.W., et al. (2012): CD68 and CD163 as prognostic factors for Korean patients with Hodgkin lymphoma. European journal of haematology, 88: 292-305.