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Mohamed Ali

*Internal Medicine , Faculty of medicine MBBch Cairo university M.Sc Al-Azhar university,*  
dr.m.samy.temraz@gmail.com

Mahmoud Bazeed

*Internal Medicine Department, Faculty of Medicine, Al-Azhar University Cairo, Egypt,*  
dr.mahmoudbazed@yahoo.com

Mohamed elshourbagy

*professor of clinical pathology , Faculty of medicine , Al-Azhar University, karim-elshourbagy@hotmail.com*

Mahmoud Allam

*Assistant professor of Internal Medicine , Faculty of Medicine , Al-Azhar University,*  
dr.mahmoudallam75@gmail.com

Mohamed bakeer

*Assistant professor of Internal medicine , Faculty of medicine , Al-Azhar University,*  
bakeerthismailonly@gmail.com at: <https://aimj.researchcommons.org/journal>



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## Study of High Mobility Group Box 1 Protein Level in Patients with Diffuse Large B-Cell Lymphoma

Mohamed Samy Abdelaziz Ali,<sup>1</sup> M.Sc, Mahmoud Mohamed Moussa Bazeed,<sup>1</sup> PhD, Mohamed Saied El Shorbagy,<sup>2</sup> PhD, Mahmoud Abd El Rasheed Abd El Khalek Allam,<sup>1</sup> MD, Mohamed Saied Mohammed Baker,<sup>1</sup> MD.

### \* Corresponding Author:

Mohamed Samy Abdelaziz Ali  
[dr.m.samy.temraz@gmail.com](mailto:dr.m.samy.temraz@gmail.com)

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<sup>1</sup>Internal Medicine Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

<sup>2</sup>Clinical pathology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

### ABSTRACT

**Background:** Diffuse large B-cell lymphoma (DLBCL) has been the most common kind of non-Hodgkin lymphoma (NHL), representing for roughly 24% of new instances per year. The HMG box 1 protein is a nuclear DNA-binding protein that was identified more than thirty years ago. It binds to DNA and functions as a cofactor in the transcription of genes. Overexpression of HMGB1 was being discovered in many cancers, including breast cancer, gastric cancer, and non-Hodgkin lymphoma.

**Aim of the study:** to see how high mobility group box 1 protein levels are affected by DLBCL and how chemotherapy affects HMGB1 levels in DLBCL patients.

**Patients and Methods:** This study was conducted out in oncology department of Al-Hussein University hospital. Two groups of patients were formed. Group (A): composed of 35 patients with DLBCL for whom pre-treatment and post-treatment level of HMGB1 protein were measured. Group (B): composed of 35 normal subjects act as a control group. All procedures followed Al-Azhar University Ethical Committee regulations.

**Results:** there is statistically significant improvement after chemotherapy as t value equaled (8.632) with P-value (0.000). Also, when comparing pre-value with normal, paired t test equaled (10.16) with P-value (0.000). Using ROC curve, it was stated that area under the curve equaled 1 with standard error 0 and P=0.017.

**Conclusion:** The HMG box 1 protein is a significant indicator in patients with DLBCL.

**Keywords:** Diffused Large B Cell Lymphoma; Chemotherapy; High Mobility Group Box 1 Protein.

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### INTRODUCTION

More than thirty years ago, HMGB1 was found as a nuclear DNA-binding protein. HMGB1 is a nuclear protein which functions as a cofactor for gene transcription by binding to the DNA<sup>1</sup>, it activates several cell surface receptors, including the receptor for advanced glycation end-products (RAGE) and toll-like receptors (TLRs), TLR2, TLR4, and TLR9, when a damage-associated molecular pattern molecule (DAMP) (also recognized as an alarmin) is detected in the extracellular fluid, triggering innate immune responses<sup>2</sup>.

Sepsis, ischemic-reperfusion injury, neurodegeneration, arthritis, and cancer have all been linked to HMGB1<sup>3</sup>. Overexpression of HMGB1 was being discovered in a variety of cancers, including breast

cancer and melanoma<sup>4</sup>, gastric cancer<sup>5</sup>, and non-Hodgkin lymphoma<sup>6</sup>.

Furthermore, it has been shown that serum HMGB1 levels are higher in late-stage pancreatic ductal adenocarcinoma than in early-stage PDAC<sup>7</sup>. When compared to healthy controls, patients with chronic lymphocytic leukemia had higher HMGB1 levels in their plasma, and the absolute number of lymphocytes is thought to be linked to HMGB1 concentration<sup>8</sup>.

Diffuse large B-cell lymphoma is the most frequent aggressive kind of non-Hodgkin lymphoma (DLBCL)<sup>9</sup>. It was found that DLBCL is expressing a high level of HMGB1 protein<sup>6</sup>. Not only this, but it was found that one mechanism of anti-lymphoma effect of Rituximab, is that it induces HMGB1 protein<sup>10</sup>.

Considering the results, we hypothesize that serum HMGB1 levels are linked to the diagnosis and prognosis of DLBCL.

So, the purpose of this research was to see how DLBCL impacts HMGB1 protein levels and how chemotherapy affects HMGB1 levels in DLBCL patients.

## PATIENTS AND METHODS

This research was conducted at the period between December 2017 and December 2020 on patients attending to Al-Hussein University hospital during the time of the study. Randomized controlled study design was used.

The work included 2 groups, Study Group (A): composed of 35 patients who had recently been diagnosed with Diffuse Large B-cell lymphoma (DLBCL) and for whom HMGB1 protein levels were measured pre and post treatment. Control Group (B): composed of 35 normal subjects. Two groups were matched, as regard to age, sex and performance status.

The patients were chosen under the following criteria: Both genders were included, Age above 16 years, Stage I to IV of an Arbor stages The current study excluded patients with Anemia, Age less than 16 years, severe renal impairment patients, history of end-stage liver disease, history of autoimmune disease and other malignancies.

Patients who took part in the research were subject to the following procedures: complete medical history, clinical examination and dental examination. Lymph node biopsy with histopathological assessment. Laboratory investigations: Blood sampling was performed for measuring complete blood picture, Liver profile, lipid profile, Renal profile, Random blood sugar and Coagulation profile.

### Evaluation of serum HMGB1 level:

Four milliliters of venous blood were taken from patients and controls, and then collected in clot activator and gel containing tubes for serum separation. Before assaying, blood specimens have been centrifuged at 3000 rpm for 15 min to separate serum and then kept at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ . The manufacturer's protocol was followed to detect HMGB1 levels in the serum employing an enzyme linked immunosorbent assay (ELISA) kit (MyBioSource, San Diego, CA). The kit assays HMGB1 using an ELISA relying on biotin double antibody sandwich technique.

Principle of the test: The color change of the tetramethylbenzidine (TMB) substrate by the addition of a stop solution causes a color shift, which is detected at 450 nm. The optical density (OD) of the specimens is then compared to the standard curve to determine the HMGB1 concentration in the

specimens. Add HMGB1 to the wells that were pre-coated with a purified Human HMGB1 and then incubated at  $37^{\circ}\text{C}$  for 30 min. Following incubation and washing, add biotin-labeled anti-HMGB1 antibodies to form an immune complex with streptavidin-HRP conjugate reagent. Following incubation and washing, eliminate any unbound enzymes and add solution A and B (preservation for 15 minutes at  $37^{\circ}\text{C}$ ). Add the halting solution (the blue change to yellow). After 15 minutes, the concentration of HMGB1 and the shade of the solution are positively associated.

### Chemotherapy:

R-CHOP regimen was used in this study. Concurrent Medication: According to local policy, antihistamines, corticosteroids, and paracetamol should be taken before rituximab. With at least the first cycle, allopurinol should be used. Anti-emetics, PPI, antiviral, antifungal, PCP prophylaxis, and mouthwashes in accordance with local policies.

### Ethical considerations:

All procedures were followed Al-Azhar University Ethical committee regulations and patient consent was taken from all patients.

### Statistical analysis:

Statistical Program for Social Science (SPSS) version 24 was used to conduct the concurrent study analysis. For each subject in the study, descriptive statistics (mean, standard deviation, maximum, minimum, and range) were computed.

Correlation analysis will be performed with the Pearson correlation test. The difference between the pre and post treatment outcomes in the experimental group for HMGB1 will be compared using a paired sample t-test. Unpaired sample t-test will be utilized to start comparing before treatment results in the study group and results of control group for HMGB1. P values  $< 0.05$  will be regarded statistically significant, and P-value  $> 0.05$  regarded statistically non-significant. P-value  $< 0.001$  to be highly statistically significant.

## RESULTS

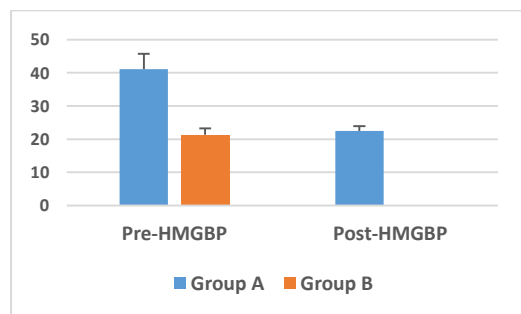
Before final analysis, the data have been checked for normality, variance homogeneity, and the presence of extreme scores. This investigation was carried out as a prerequisite for the analysis of difference's parametric calculations. Preliminary assumption checking revealed that data for all variables are normally distributed.

The Mean  $\pm$  SD age of group (A) became  $47.86 \pm 11.27$ , while in group (B) was  $32 \pm 12.64$  and unpaired t test statistics showed no statistically significant difference between both groups as t equaled 1.866 and P-value (0.099). Lymphoma group include 25 males (71%) and 10 females (29%) and included 10 smokers (29%) and 25 non-smokers

(71%) and also, included 15(42.86%) patients with peripheral lymph nodes involvement and 15(42.86%) patients with Extra nodal involvement.

According to Ann Arbor staging system Lymphoma group included 10(28.57%) patients diagnosed as stage 3 and 25(71.43%) patients diagnosed as stage 4. But when looking to age-adjusted international prognostic index it included 10(28.57%) patients that are at low risk and 25(71.43%) patients that are at high risk. Regarding comorbidities, 25(71.43%) patients have Diabetes mellitus, 20(57.14%) patients have hypertension, 25(71.43%) patients have Hepatosplenomegaly and 10(28.57%) have B symptoms. Regarding LDH level, only 20(57.14%) patients showed high LDH level, on the response to treatment, 20(57.14%) patients showed good response while the rest showed partial response.

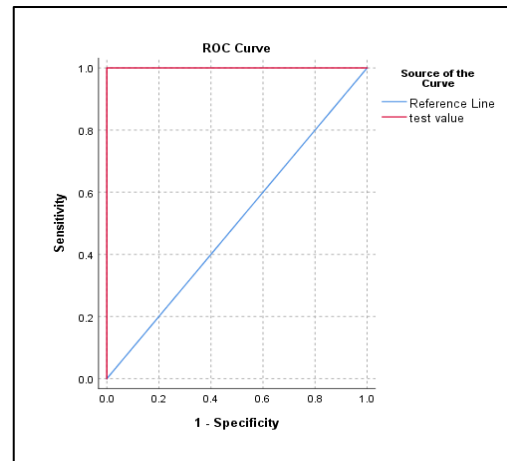
The Mean  $\pm$  SD HMGB1 level of group (A) was  $41.14 \pm 4.6$  before treatment and decreased by 45.48% to be  $22.43 \pm 1.9$  after treatment with statistically significant improvement as t value equaled 8.632 with p-value (0.000). Also, when comparing pre-value with normal ( $21.33 \pm 1.53$ ), paired t test equaled 10.16 and p value equaled 0.000. Using ROC curve, it was stated that area under the curve equaled 1 with standard error 0 and  $P=0.017$ . and Eta square equaled .929. PRC curve shows good Precision /recall (sensitivity) of the HMGB1 test and reveals that the test is perfect for diagnosis of DLBCL patients (Figure 1, 2 &3; Table 1).



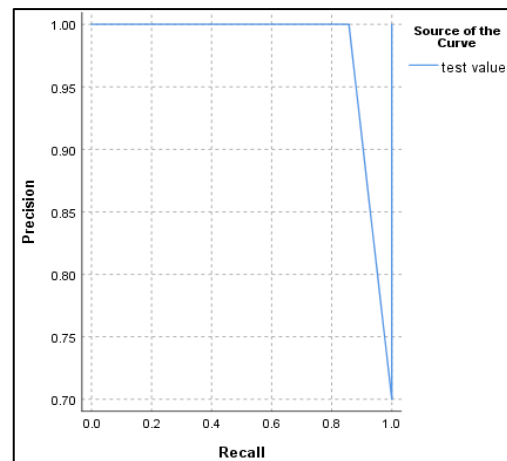
	Group A	Group B	T	P – Value
Pre-treatment level	41.14 ± 4.6	21.33 ± 1.53	10.16	0.000 HS
Post-treatment level	22.43 ± 1.9			
Change	DE-45.48%			
T	8.632			
P-value	0.000 HS			

**Table 1:** HMGB1 distribution among groups. pre and post treatment level in study group (A). control group (B) level of HMGB1 (NS: p-value > 0.05 is regarded non-significant, HS: p-value < 0.001 is regarded highly significant).

**Fig 1:** HMGB1 distribution among groups



**Fig 2:** ROC curve showed very high sensitivity and specificity.



**Fig 3:** Precision- recall curve

## DISCUSSION

This current study is designed to investigate at the levels of High mobility group box 1 (HMGB1) protein in DLBCL patients, as well as the effects of chemotherapy on HMGB1 protein level on those patients. Thirty-five patients with DLBCL and thirty-five healthy subjects participated in the study and each participant read and signed the consent form.

HMGB1 was analyzed before and after treatment. The mean value of HMGB1 was  $41.14 \pm 4.6$  while the normal was  $21.33 \pm 1.53$  with P-value (0.000) and after treatment the value improved by 45.48% to be  $22.43 \pm 1.9$  (P-value 0.000) while the ROC curve revealed very high sensitivity and specificity of HMGB1 to diagnose diffuse large B cell lymphoma (P=0.017) and predict prognosis on those patients

This came in agreement with studies that show HMGB1 overexpression and/or elevated serum HMGB1 levels in a variety of cancers, such as non-Hodgkin lymphoma, gastric cancer, melanoma, liver cancer, breast cancer, and cervical cancer.<sup>11</sup> Also, Wu T., et al., (2016)<sup>12</sup> stated that HMGB1 overexpression is related with poor prognosis in a systematic literature review of 18 studies encompassing 11 forms of cancer<sup>12</sup>. Matching with our study, further meta-analyses by Li P., et al (2019)<sup>14</sup> reveal that high HMGB1 levels have been correlated with negative clinical results in non-small cell lung cancer and cervical cancer<sup>13</sup>, confirming HMGB1's as predictive value<sup>14</sup>.

One explanation of this association is mentioned by Van Beijnum et al (2013)<sup>15</sup> that cancer cells actively secrete HMGB1 in addition to passive release during necrosis. Extracellular HMGB1 represents a dynamic driver of cancer progression, proliferation, migration, and angiogenesis, and it can act as a paracrine/autocrine cancer factor<sup>15</sup>.

Nearly all recent studies agree with the findings of the current study, HMGB1 is a non-histone chromatin-associated protein which is being implicated throughout the pathogenesis of hematopoietic malignancies. HMGB1 is a damage-associated molecular pattern that is normally found in cells (DAMP), but it could be released passively or actively in the extracellular environment. Hematopoietic stem cells' proliferation, differentiation, mobilization, and senescence are mediated by extracellular HMGB1, which binds to a variety of receptors and interactors. Furthermore, Yuan S. et al (2020)<sup>16</sup> systematically summarizes in his study the evolving role of HMGB1 in carcinogenesis, development, diagnosis, and possible medical application in various hematopoietic malignancies.<sup>16</sup>

During the period of our study, we observe that HMGB1 pre-treatment level of DLBCL patient

( $41.14 \pm 4.6$ ) is higher when comparing with normal subjects ( $21.33 \pm 1.53$ ), Meyer A et al (2008)<sup>6</sup> reported that Many primary lymphomas have higher HMGB1 expression than normal lymph nodes, and HMGB1 has been found only in lymphoma cells. The expression of HMGB1 and classification has a correlation<sup>6</sup>.

Our study shows the mean value of HMGB1 was  $41.14 \pm 4.6$  while the normal was  $21.33 \pm 1.53$  with P-value (0.000) and after treatment the value improved by 45.48% to be  $22.43 \pm 1.9$  (P-value 0.000). This was consistent with He SJ et al (2017)<sup>17</sup> who reported that in DLBCL, HMGB1 functions as both an inflammatory factor which encourages tumorigenesis and a cytokine which stimulates immune responses, implying that HMGB1 may be useful in the diagnosis and pathogenesis<sup>17</sup>. HMGB1 promotes the proliferation of DLBCL cells by stimulating the Src/ERK pathway that would be blocked by EP, Zhang T. et al (2019)<sup>18</sup> proposed that blocking the HMGB1-mediated signalling pathway via EP will effectively prevent the incidence of DLBCL and the progression of disease<sup>18</sup>.

In disparity, Zhao T et al., (2015)<sup>19</sup> showed a rise in HMGB1 release and a decline in IL-10 release, inducing immunological responses and significantly enhancing the clinical results of sufferers with (DLBCL). This implies that instead of directly destroying cells, eliminating DLBCL by indirectly influencing the immune system may be more efficient<sup>19</sup>.

During the period of the study, the HMGB1 pre-treatment level  $41.14 \pm 4.6$  was significantly higher in DLBCL patient than the normal subjects  $21.33 \pm 1.53$  with P-value equaled 0.000. Another agreement study by Jia L et al (2014)<sup>8</sup> stated that in chronic lymphocytic leukaemia plasma HMGB1 levels have been substantially higher than in healthy controls, and that the HMGB1 level has been linked to the absolute lymphocyte count<sup>8</sup>.

The mean value of HMGB1 in our study was  $41.14 \pm 4.6$  and after treatment the value improved by 45.48% to be  $22.43 \pm 1.9$  (P-value =0.000). Dejean et al (2012)<sup>20</sup> discovered that HMGB1 in anaplastic large-cell lymphomas (ALCLs), promoting lymphoid cell proliferation and metastasis. Following therapy with the HMGB1 inhibitor, lymphoma cells' invasion and metastatic abilities have been greatly reduced<sup>20</sup>.

HMGB1 is a nuclear protein that's been extensively investigated in both physiological and pathological processes, which include leukaemia. Liu L. et al (2021)<sup>21</sup> also found that HMGB1 has a higher expression level in the bone marrow cells of acute myeloid leukaemia AML patients than in normal controls, and that it leads to AML pathogenesis and progression via hindering apoptosis promoting proliferation, and causing myeloid differentiation blockade in AML cells. To summarize, our findings contribute to the expanding body of evidence which

HMGB1 is an efficient treatment goal for AML, and they offer experimental support for the clinical use of HMGB1 as a new agent in the treatment of acute myeloid leukaemia (AML)<sup>21</sup>.

According to Mao XJ et al (2012)<sup>22</sup> research, HMGB1 expression was considerably higher in 120 instances of T cell lymphoma compared to 40 instances of reactive lymphoid hyperplasia, which was used to assess the importance of HMGB1. Moreover, in lymph node biopsy, the HMGB1 positivity rate has been used as a diagnostic indicator for T cell lymphoma. This result had 63.7 % specificity, and it was linked to malignancy and clinical stage however not to sex, age, or tumour position. This agreed with the result of our study on DLBCL patients The Mean  $\pm$  SD HMGB1 level of 35 cases was  $41.14 \pm 4.6$  before treatment when comparing pre-value with normal ( $21.33 \pm 1.53$ ), paired t test equaled 10.16 with P-value (0.000) and ROC curve showed very high sensitivity and specificity. On the other hand, Increased HMGB1 expression could be a possible diagnostic indicator for T cell lymphoma development and progression<sup>22</sup>.

### CONCLUSION

The results obtained from the current study and the discussion that followed it was concluded that: In patients with (DLBCL), the high mobility group box 1 protein is a significant indicator. Also, it could be used as a sign for improvement and deterioration in lymphoma cases.

Limitations of our study include the lack of control group regarding post values of DLBCL patients. In addition, no follow up has been done for these patients.

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