ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Impact of persistent antigenic challenges and splenectomy on immune cells in β-Thalassemic patients.

Sonia Elia Ishaq[1], Taban Kamal Rasheed[1], Dara K. Mohammad[2,3]

[1]Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq.
[2]College of Agricultural engineering Science, Salahaddin University- Erbil, Kurdistan Region, Iraq.
[3]Department of Medicine Huddinge, Center for Hematology and Regenerative Medicine (HERM), Karolinska Institutet, Huddinge, Sweden.

A B S T R A C T:
Infection is common in thalassemia patients and is one of the leading causes of death. It's still unclear why these individuals are so sensitive to infection. There is strong evidence that a deficiency in the functioning of phagocytic cells plays a key role in the weakened resistance to pathogenic bacteria. The purpose of this study was to investigate the function of phagocytic cells by comparing the serum levels of granulocyte macrophage-colony stimulating factor (GM-CSF) and Neopterin in thalassemia patients to healthy people. The study included 50 thalassemia patients and 30 healthy controls. Enzyme-linked immunosorbent assay (ELISA) was applied to estimate the serum levels of GM-CSF and Neopterin. Serum levels of GM-CSF were significantly elevated in thalassemia patients when compared to healthy people ($p < 0.05$), while serum levels of Neopterin showed no significant change between thalassemia patients and healthy controls. Both serum levels of GM-CSF and Neopterin showed no significant difference between Splenectomized and healthy controls. Total leukocyte counts, lymphocytes, MID (monocytes), platelets, and RBC were all significantly higher in thalassemia patients compared to healthy controls. But, granulocyte counts showed no significant difference between the thalassemia patients and the healthy controls. On the other hand, total leukocytes, monocytes, lymphocytes and platelets counts were significantly raised in splenectomized patients when compared to healthy controls and non-splenectomized patients, respectively. We came to the conclusion that thalassemia patients have a high immune cell count, which is most likely due to the antigenic difficulties posed by blood transfusions. On the other hand, these patients have an impaired immune system.

KEY WORDS: Thalassemia, Macrophage, GM-CSF, Neopterin
DOI: http://dx.doi.org/10.21271/ZJPAS.34.4.13
ZJPAS (2022) , 34(4);116-122 .

1.INTRODUCTION:

Thalassemia is a genetic anemia caused by inadequate of hemoglobin synthesis (Higgs et al., 2001). β-thalassemia is a blood disorder that affects a variety of organs and has a high rate of morbidity and mortality. The cause is a decrease in the formation of Beta-globin chains (Cunningham et al., 2004). Beta-thalassemia is most commonly found in Mediterranean countries, the Middle East, Central Asia, India, Southern China, the Far East, and countries along Africa's north coast and in South America (Angastiniotis and Lobitz, 2019, Kattamis et al., 2020).

* **Corresponding Author:**
Sonia Elia Ishaq
E-mail: sonia.ishaq@su.edu.krd
Article History:
Received: 30/03/2022
Accepted: 10/06/2022
Published: 15/08 /2022

The elevated beta-thalassemia gene frequency in these areas is almost definitely owing to *Plasmodium falciparum* malaria-related selection pressure (Flint et al., 1998).
Infections are more common in β-thalassemia major, indicating that there is a basic deficiency in the host defense system. Infections can be caused by a variety of factors, including blood transfusions, splenectomy, iron overload in the body, and immune system malfunction. Patients with β-thalassemia have been found to have a wide range of immunological disorders (Asadov, 2014, Ghaffari et al., 2011, Ghaffari et al., 2008, Farmakis et al., 2003).
Blood transfusions are the most common treatment for thalassemia, although they have a

number of side effects, including hemosiderosis, transfusion reactions, alloimmunization, and infections. The immunologic consequences of alloimmunization have been explored from humoral allosensitization to the impact of transfusion on cellular immune function (Vichinsky et al., 2014, Dhawan et al., 2014).

Increased immunoglobulin production, insufficient complement system activity, reduced opsonization, and granulocyte phagocytosis have all been recorded as immunological abnormalities (Sinniah and Yadav, 1981, Tovo et al., 1981, Gluba-Brzózka et al., 2021). There is also evidence that thalassemia has an effect on the cell-mediated immune response and lymphocyte subsets (Javad et al., 2011).

Granulocyte-macrophage colony-stimulating factor is a multipotent cytokine that increases the growth of macrophages and granulocytes generated in the bone marrow. This cytokine is produced by various cells, including activated T lymphocytes, B lymphocytes, natural killer cells, monocytes/macrophages, endothelial, epithelial, and fibroblast cells (Zhan et al., 2012).

Antigen presentation, phagocytosis, chemotaxis, and cell adhesion are all affected by GM-CSF (Shapouri- Moghaddam et al., 2018, Li et al., 2013). In addition, GM-CSF defective animals are more susceptible to lung infection (Paine et al., 2000, LeVine et al., 1999), intestinal, and systemic infections, demonstrating its significance in immunological homeostasis (Hirata et al., 2010).

Neopterin is a cellular immune biomarker that appears early in immune response and monocyte/macrophage activation. When monocyte-derived macrophages and dendritic cells are stimulated with interferon-gamma (IFN-γ), they produce this low-molecular-weight molecule, which is a metabolite of guanosine triphosphate. It's also considered a reliable tool for assessing the rate of IFN- γ production. (Oxenkrug et al., 2011, Sucher et al., 2010). Neopterin has been used in clinical trials to evaluate bacterial and viral infections, autoimmune disorders, and cancer (Hoffmann et al., 2003). Obeid and his colleagues discovered that thalassemia patients had considerably greater serum levels of IFN-γ and Neopterin when compared to healthy controls (Obeid et al., 2018).

In this study, we aimed to determine the serum levels of GM-CSF and Neopterin in patients with thalassemia in Erbil city.

2. MATERIALS AND METHODS

2.1. Participants

Fifty patients were willing to participate in the study, with 26 men (52%) and 24 women (48%) with a mean age of 16.26±8.669 years, attended consultation agency of Thalassemia in Erbil city-Iraq. Sixteen (16%) of the patients had splenectomy. All of the patients were transfused on a regular basis to keep their hemoglobin levels over 9.5 g/dl with a mean transfusion interval of 17 days. The study was approved by the local ethics committee at the hospital, and legal guardians' informed consent was obtained in each case.

The control group consists of 30 volunteers, six men (20%) and 24 women (80%) with a mean age of 20.73± 0.907 years and from the same urban populations. Exclusion criteria for the healthy volunteer were a family history of thalassemia, smoking, medication, pregnancy, and any physical abnormalities.

2.2 Blood Sampling and Storage

Five milliliters of venous blood were drawn from patients and healthy controls using sterile disposable syringes. Then 2 ml was transferred to EDTA tube for complete blood count, and 3ml was put into a clot-activator tube for serum separation. The serum was collected after centrifugation at 3000 rounds per minute (rpm) for 15 minutes and put it in an Eppendrof tube and then stored at -20°C till the assay time.

2.3 Detection of GM-CSF and Neopterin

Serum levels of both Neopterin and GM-CSF were evaluated by ELISA technique. The assay was achieved according to the manufacturing company's instruction (Komabiotechinc Company Republic of Korea).

2.4 Hematological analysis

Total leukocytes, granulocytes, lymphocytes, platelets, and red blood cells were counted with a fully automated hematological analyzer (XT-

2000i sysmex, USA) directly after blood collection.

2.5 Statistical Analysis

Graphpad Prism™ software was used to collect and graph the data. The unpaired t-test and the ANOVA test were used to compare the groups. P values of less than 0.05 were considered statistically significant, and data were presented as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

Thalassemia is a major public health issue in more than sixty nations throughout the world, particularly in the eastern Mediterranean (Kattamis et al., 2020). In β-thalassemia patients, a routine blood transfusion program every 3–4 weeks was prescribed to correct anemia, which could lead to alloimmunization to erythrocyte antigens (Philip and Jain, 2014).

Infections are the second most prevalent cause of disability and possibly death in thalassemia patients, after cardiac failure (Farmakis et al., 2003). This increased risk may be due in part to compromised immune systems in thalassemia patients (Wiener, 2003). The spleen plays an important role in immunity, inflammation, and thrombosis regulation. Splenectomized persons are predicted to have systemic consequences and dysfunctions (O'Neal Jr et al., 2016). Splenectomized patients are more susceptible to infections, have a higher risk of septic complications, and have a higher mortality rate than non-splenectomized patients (Sinwar, 2014, Sari et al., 2016).

Serum levels of GM-CSF and Neopterin were analyzed from β-thalassemia patients and compared to the healthy people. Serum GM-CSF levels were significantly increased in thalassemia patients when compared to healthy volunteers (1.861 ± 0.072 vs. 1.487 ± 0.099). Serum Neopterin levels showed no significant difference between thalassemia patients and healthy volunteers (1.264 ± 0.038 vs. 1.259 ± 0.055) as shown in Table 1 and Figure 1.

Table (1) Immunological parameters in thalassemia patients and healthy controls

Parameters	Patients(n=50) Mean ± SD	Controls(n=30) Mean ± SD	P value
GM-CSF (pg/ml)	1.861 ± 0.072	1.487 ± 0.099	0.002
Neopterin (ng/ml)	1.264 ± 0.038	1.259 ± 0.055	0.943

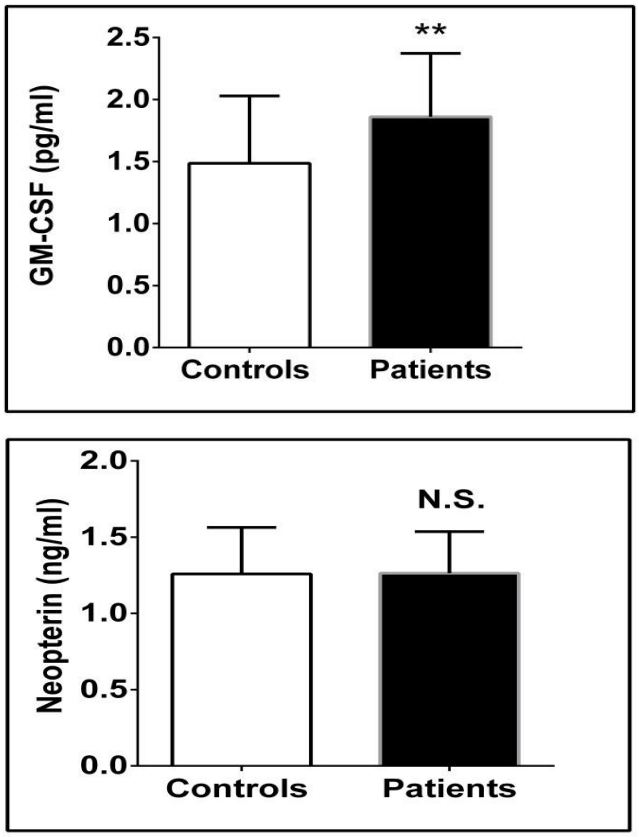

Figure 1; Immunological parameters in thalassemia patients and healthy controls.

Our results also showed that serum levels of GM-CSF and Neopterin were significantly elevated in non-splenectomized patients when compared to healthy volunteers. Furthermore, serum levels of GM-CSF and Neopterin were significantly lower in splenectomized individuals than in non-splenectomized patients as shown in Table 2 and Figure 2.

Table (2) Comparison of immunological and hematological parameters in non-splenectomized with splenectomized β-thalassemia patients

Parameters	Controls (N=30) Mean ± SD	Non-Splenectomized (N=26) Mean ± SD	Splenectomized (N=8) Mean ± SD	ANOVA P value
GM-CSF (pg/ml)	1.519 ± 0.599	1.911 ± 0.556	1.889 ± 0.645	0.038
Neopterin (ng/ml)	1.011 ± 0.012	1.015 ± 0.011	1.004 ± 0.006	0.058

WBC (109/L)	0.846 ± 0.107	0.889 ± 0.217	1.377 ± 0.418	< 0.0001
GRA (109/L)	0.624 ± 0.138	0.544 ± 0.223	0.762 ± 0.200	0.024
LYM (109/L)	0.322 ± 0.148	0.528 ± 0.286	1.048 ± 0.593	< 0.0001
MID (109/L)	0.609 ± 0.181	0.834 ± 0.307	1.156 ±0.572	0.0001
PLT (109/L)	2.385 ± 0.102	2.414 ± 0.294	2.768 ± 0.207	0.0002

Ud-Naen and his colleagues discovered that when non-thalassemia cells were stimulated with *P. insidiosum* zoospores, they produced more GM-CSF and IFN- γ than thalassemia cells. They also discovered that thalassemia patients' monocytes/macrophages produced significantly more GM-CSF than people who didn't take an iron chelator (Ud-Naen et al., 2019). Splenectomized β-thalassemia/hemoglobin E (Hb E) individuals are iron-overloaded due to iron absorption as a result of insufficient erythropoiesis (Pootrakul et al., 1988). Excess iron is toxic to all cells in the body and can cause serious and irreversible organic damage, such as heart disease, diabetes, bone fractures, cirrhosis, and hypogonadism (Ginzburg and Rivella, 2011). As a result, GM-CSF levels may be affected by iron overload, prolonged blood transfusions, iron chelator therapy, and splenectomy (Pootrakul et al., 1988, Leecharoenkiat et al., 2016, Ud-Naen et al., 2019).

In contrast to previous finding that indicated higher level of serum Neopterin in β-thalassemia patients (Gharagozloo et al., 2009, Obeid et al., 2018), our study showed that there was no significant difference between thalassemia patients and healthy controls. On the other hands, serum Neopterin level was significantly reduced in splenectomized patients when compared to non-splenoctimsed patients. Gharagozloo and his colleagues reported that the formation of IFN-γ and IL-2 was significantly reduced in thalassemia patients with high serum ferritin levels, suggesting that iron overload in β--thalassemia patients had an immunosuppressive effect (Gharagozloo et al., 2009). Furthermore, previous studies have demonstrated that iron overload can affect T helper 1 (Th1) growth in mice by lowering IFN- γ formation, which can be efficiently reversed with desferrioxamine therapy (Mencacci et al., 1997, Omara and Blakley, 1994). Iron overload affects T helper 1 and IFN-γ formation and subsequently affects monocytes/macrophages' function, and causes defective immune responses.

The current result showed that total leukocyte counts, lymphocytes, MID (monocytes), platelets and RBC were significantly elevated in thalassemia patients compared to healthy volunteers. While granulocyte counts showed no significant change between thalassemia patients and healthy volunteers as shown in table 3.

Table (3) Hematological parameters in thalassemia patients and healthy controls

parameters	**Controls N=30**	**Patients N=50**	**P value**
WBC (10^9/L)	0.849 ± 0.018	1.083 ± 0.049	0.0005
GRA (10^9/L)	0.624 ± 0.025	0.666 ± 0.032	0.360
LYM (10^9/L)	0.3222 ± 0.027	0.740 ± 0.065	< 0.0001
MID	0.612 ± 0.032	0.982 ± 0.055	< 0.0001
PLT	2.385 ± 0.018	2.549 ± 0.039	0.002
RBC	4.781 ± 0.091	3.171 ± 0.074	< 0.0001

Moreover, each of the total leukocytes, monocytes, lymphocytes and platelets was significantly elevated in splenectomized patients compared to healthy volunteers and non-splenectomized patients as shown in Table 2 and Figure 2.

Al- Awadhi and his colleague reported that thalassemia patients had significantly higher total leukocytes and lymphocyte counts than controls (Al- Awadhi et al., 2010). These results are consistent with our findings. In a previous study, it was discovered that removing the spleen after a traumatic rupture causes leukocytosis (neutrophils), which is followed by permanent lymphocytosis (McBride et al., 1968, Modell and Berdoukas, 1983).This may explain why

lymphocytosis persists in our thalassemia patients with splenectomies.

4. CONCLUSIONS

We concluded that thalassemia patients have high immune cell counts, but their immune function is impaired, most likely due to antigenic challenges from blood transfusions, iron overload, and splenectomy.

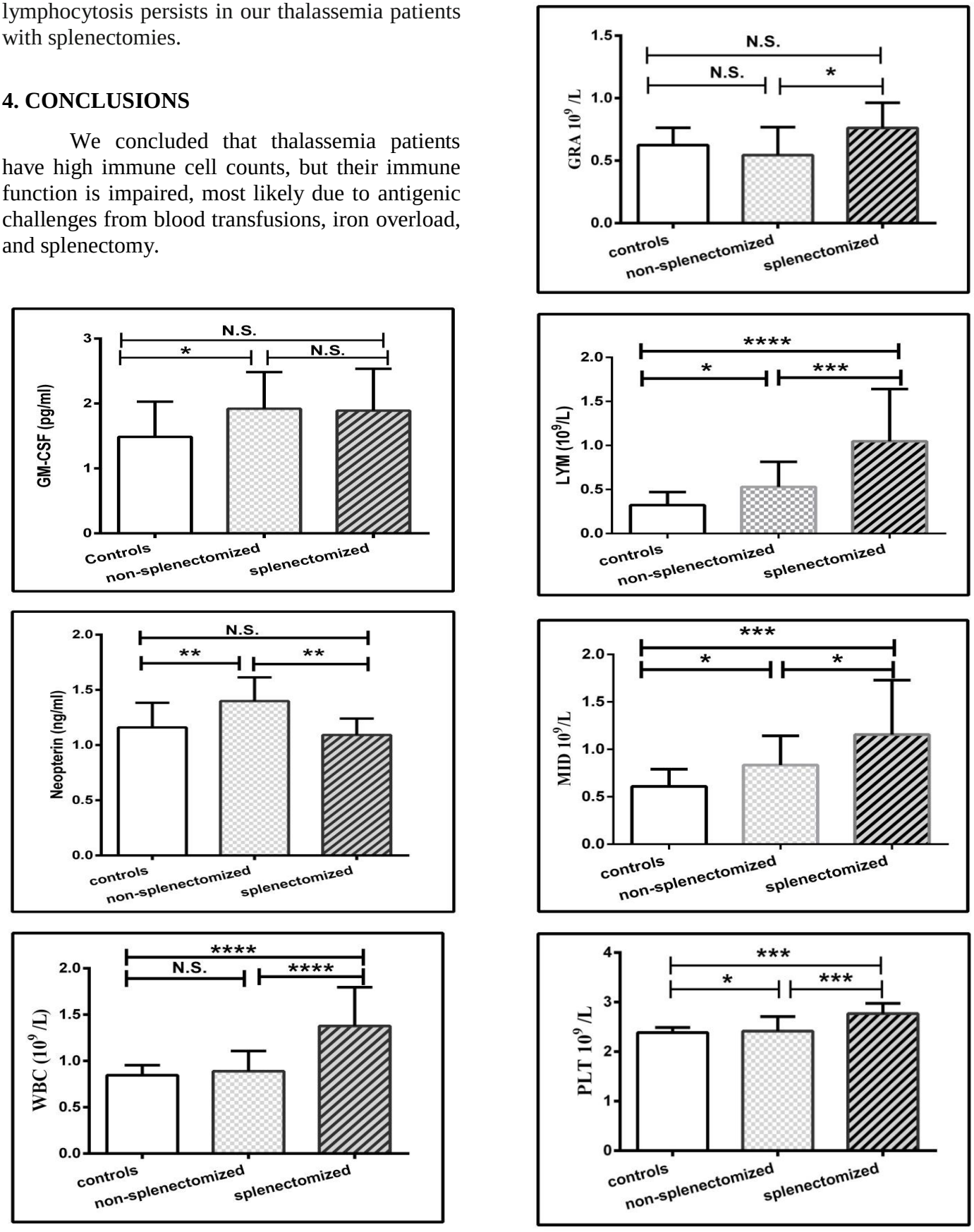

Figure 2: Immunological and hematological parameters in healthy controls, non-splenectomized and splenectomized thalassemia patients

References

AL- AWADHI, A., ALFADHLI, S., AL- KHALDI, D., BORHAMA, M. & BORUSLY, M. 2010. Investigation of the distribution of lymphocyte subsets and zinc levels in multitransfused β-thalassemia major patients. *International journal of laboratory hematology,* 32**,** 191-196.

ANGASTINIOTIS, M. & LOBITZ, S. 2019. Thalassemias: an overview. *International Journal of Neonatal Screening,* 5**,** 16.

ASADOV, C. D. 2014. Immunologic abnormalities in β-thalassemia. *Journal of Blood Disorders Transfusion,* 5**,** 1-5.

CUNNINGHAM, M. J., MACKLIN, E. A., NEUFELD, E. J., COHEN, A. R. & NETWORK, T. C. R. 2004. Complications of β-thalassemia major in North America. *Blood,* 104**,** 34-39.

DHAWAN, H. K., KUMAWAT, V., MARWAHA, N., SHARMA, R. R., SACHDEV, S., BANSAL, D., MARWAHA, R. K. & ARORA, S. 2014. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. *Asian journal of transfusion science,* 8**,** 84.

FARMAKIS, D., GIAKOUMIS, A., AESSOPOS, A. & POLYMEROPOULOS, E. 2003. Pathogenetic aspects of immune deficiency associated with ß thalassemia. *Medical Science Monitor,* 9**,** RA19-RA22.

FLINT, J., HARDING, R., BOYCE, A. & CLEGG, J. 1998. The population genetics of the hemoglobinopathies, Bailliere's Clin. Hematol.

GHAFFARI, J., VAHIDSHAHI, K., KOSARYAN, M., PARVINNEJAD, N., MAHDAVI, M. & KARAMI, H. 2008. Nitroblue tetrazolium test in patients with beta-thalassemia major. *Saudi Med J,* 29**,** 1601-5.

GHAFFARI, J., VAHIDSHAHI, K., KOSARYAN, M., SOLTANTOOYEH, Z. & MOHAMADI, M. 2011. Humoral immune system state in ß thalassemia major. *Med Glas (Zenica),* 8**,** 192-6.

GHARAGOZLOO, M., KARIMI, M. & AMIRGHOFRAN, Z. 2009. Double-faced cell-mediated immunity in β-thalassemia major: stimulated phenotype versus suppressed activity. *Annals of hematology,* 88**,** 21-27.

GINZBURG, Y. & RIVELLA, S. 2011. β-thalassemia: a model for elucidating the dynamic regulation of ineffective erythropoiesis and iron metabolism. *Blood, The Journal of the American Society of Hematology,* 118**,** 4321-4330.

GLUBA-BRZÓZKA, A., FRANCZYK, B., RYSZ-GÓRZYŃSKA, M., ROKICKI, R., KOZIARSKA-ROŚCISZEWSKA, M. & RYSZ, J. 2021. Pathomechanisms of Immunological Disturbances in β-Thalassemia. *International Journal of Molecular Sciences,* 22**,** 9677.

HIGGS, D., THEIN, S. & WOODS, W. 2001. The molecular pathology of the thalassaemias. *The thalassaemia syndromes. 4th ed. Oxford, England: Blackwell Science***,** 133-91.

HIRATA, Y., EGEA, L., DANN, S. M., ECKMANN, L. & KAGNOFF, M. F. 2010. GM-CSF-facilitated dendritic cell recruitment and survival govern the intestinal mucosal response to a mouse enteric bacterial pathogen. *Cell host & microbe,* 7**,** 151-163.

HOFFMANN, G., WIRLEITNER, B. & FUCHS, D. 2003. Potential role of immune system activation-associated production of neopterin derivatives in humans. *Inflammation Research,* 52**,** 313-321.

JAVAD, G., SAEID, A. & MOHAMMADMEHDI, N. 2011. Thalassemia and immune system dysfunction-review article. *Int J Curr Res,* 3**,** 105-108.

KATTAMIS, A., FORNI, G. L., AYDINOK, Y. & VIPRAKASIT, V. 2020. Changing patterns in the epidemiology of β- thalassemia. *European Journal of Haematology,* 105**,** 692-703.

LEECHAROENKIAT, K., LITHANATUDOM, P., SORNJAI, W. & SMITH, D. R. 2016. Iron dysregulation in beta-thalassemia. *Asian Pacific journal of tropical medicine,* 9**,** 1035-1043.

LEVINE, A. M., REED, J. A., KURAK, K. E., CIANCIOLO, E. & WHITSETT, J. A. 1999. GM-CSF–deficient mice are susceptible to pulmonary group B streptococcal infection. *The Journal of clinical investigation,* 103**,** 563-569.

LI, B.-Z., YE, Q.-L., XU, W.-D., LI, J.-H., YE, D.-Q. & XU, Y. 2013. GM-CSF alters dendritic cells in autoimmune diseases. *Autoimmunity,* 46**,** 409-418.

MCBRIDE, J., DACIE, J. & SHAPLEY, R. 1968. The effect of splenectomy on the leucocyte count. *British journal of haematology,* 14**,** 225-231.

MENCACCI, A., CENCI, E., BOELAERT, J. R., BUCCI, P., MOSCI, P., D'OSTIANI, C. F., BISTONI, F. & ROMANI, L. 1997. Iron overload alters innate and T helper cell responses to Candida albicans in mice. *The Journal of infectious diseases,* 175**,** 1467-1476.

MODELL, B. & BERDOUKAS, V. 1983. The clinical approach to thalassaemia. *GRUNE AND STRATTON, NEW YORK, NY(USA). 1983.*

O'NEAL JR, H. R., NIVEN, A. S. & KARAM, G. H. 2016. Critical illness in patients with asplenia. *Chest,* 150**,** 1394-1402.

OBEID, S. F., AL-A'ARAJI, S. B., MATTI, B. F. & FAWZI, H. A. 2018. Neopterin And Interferon-Gamma as Immune Response Markers In Beta-Thalassemia Major Patients. *Asian J Pharm Clin Res,* 11**,** 192-194.

OMARA, F. O. & BLAKLEY, B. R. 1994. The effects of iron deficiency and iron overload on cell-mediated immunity in the mouse. *British Journal of Nutrition,* 72**,** 899-909.

OXENKRUG, G., TUCKER, K., REQUINTINA, P. & SUMMERGRAD, P. 2011. Neopterin, a marker of interferon-gamma-inducible inflammation, correlates with pyridoxal-5′-phosphate, waist circumference, HDL-cholesterol, insulin resistance and mortality risk in adult Boston community

dwellers of Puerto Rican origin. *American journal of neuroprotection and neuroregeneration*, 3**,** 48-52.

PAINE, R., PRESTON, A. M., WILCOXEN, S., JIN, H., SIU, B. B., MORRIS, S. B., REED, J. A., ROSS, G., WHITSETT, J. A. & BECK, J. M. 2000. Granulocyte-macrophage colony-stimulating factor in the innate immune response to Pneumocystis carinii pneumonia in mice. *The Journal of Immunology*, 164**,** 2602-2609.

PHILIP, J. & JAIN, N. 2014. Resolution of alloimmunization and refractory autoimmune hemolytic anemia in a multi-transfused beta-thalassemia major patient. *Asian Journal of Transfusion Science*, 8**,** 128.

POOTRAKUL, P., KITCHAROEN, K., YANSUKON, P., WASI, P., FUCHAROEN, S., CHAROENLARP, P., BRITTENHAM, G., PIPPARD, M. J. & FINCH, C. A. 1988. The effect of erythroid hyperplasia on iron balance.

SARI, T. T., GATOT, D., AKIB, A. A., BARDOSONO, S., HADINEGORO, S. R., HARAHAP, A. R. & IDJRADINATA, P. S. 2016. Immune response of thalassemia major patients in Indonesia with and without splenectomy. *Acta Medica Indonesiana*, 46.

SHAPOURI- MOGHADDAM, A., MOHAMMADIAN, S., VAZINI, H., TAGHADOSI, M., ESMAEILI, S. A., MARDANI, F., SEIFI, B., MOHAMMADI, A., AFSHARI, J. T. & SAHEBKAR, A. 2018. Macrophage plasticity, polarization, and function in health and disease. *Journal of cellular physiology*, 233**,** 6425-6440.

SINNIAH, D. & YADAV, M. 1981. Elevated IgG and decreased complement component C3 and factor B in B- thalassaemia major. *Acta Paediatrica*, 70**,** 547-550.

SINWAR, P. D. 2014. Overwhelming post splenectomy infection syndrome–review study. *International journal of surgery*, 12**,** 1314-1316.

SUCHER, R., SCHROECKSNADEL, K., WEISS, G., MARGREITER, R., FUCHS, D. & BRANDACHER, G. 2010. Neopterin, a prognostic marker in human malignancies. *Cancer letters*, 287**,** 13-22.

TOVO, P. A., MINIERO, R., BARBERA, C., SACCHETTI, L. & SAITTA, M. 1981. Serum immunoglobulins in homozygous β-thalassemia. *Acta haematologica*, 65**,** 21-25.

UD-NAEN, S., TANSIT, T., KANISTANON, D., CHAIPRASERT, A., WANACHIWANAWIN, W. & SRINOULPRASERT, Y. 2019. Defective cytokine production from monocytes/macrophages of E-beta thalassemia patients in response to Pythium insidiosum infection. *Immunobiology*, 224**,** 427-432.

VICHINSKY, E., NEUMAYR, L., TRIMBLE, S., GIARDINA, P. J., COHEN, A. R., COATES, T., BOUDREAUX, J., NEUFELD, E. J., KENNEY, K. & GRANT, A. 2014. Transfusion complications in thalassemia patients: a report from the C enters for D isease C ontrol and P revention (CME). *Transfusion*, 54**,** 972-981.

WIENER, E. 2003. Impaired phagocyte antibacterial effector functions in β-thalassemia: A likely factor in the increased susceptibility to bacterial infections. *Hematology*, 8**,** 35-40.

ZHAN, Y., XU, Y. & LEW, A. M. 2012. The regulation of the development and function of dendritic cell subsets by GM-CSF: more than a hematopoietic growth factor. *Molecular immunology*, 52**,** 30-37.