World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

HEMATOLOGICAL AND SAME BACTERIAL INFECTIONS AS PARAMETERS IN THALASSEMIA PATIENTS

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Article Received on 06/10/2019 **Article Revised on 26/10/2019** **Article Accepted on 16/11/2019**

ABSTRACT

Background: Thalassemia is a genetic disorder of hemoglobin production, patient with thalassemia major and some intermediate needed regular blood transfusion for correction of anemia to keep hemoglobin level at lower limit enough for oxygenating tissues, Those patients have many complication from those anemia that threating their life. **Objective**: To evaluate some hematological and bacterial infection parameters such as urinary tract infection in splenectomized and non-splenectomized thalassemia patient. **Materials and Methods:** This clinical study had been done at Hematology center/ Baqubah Teaching Hospital from the period of 15 July 2018 to 19 April 2019. The total number of patient is sixty, thirty of them lifting the spleen and thirty others were not lifting the spleen as a study group was chosen according to the study criteria, and patients with viral hepatitis were excluded. All patients had been referred to hematology center after specialist physician requests. The main compliant was anemia. Detailed history of each patient was obtained concerning name, age, gender, address, degree of kinship, diagnosis, number of transfusion in month, date of spleen lift. **Results:** In our clinical study we compare between two groups of thalassemic patients. Regarding the age group we found the patients were aged between 1 to 39 years, with an average of 20 years. Decrease neutrophils in splenectomized group (26.95% ± 1.80) compared to control group (64.23% ± 1.01). Appearance different statically significant more prevalent in patients with splenectomized than those who non- splenectomized. Opsonized bacteria are removed efficiently by macrophages in the spleen and liver, poorly opsonized bacteria, such as encapsulated bacteria, are only cleared by the spleen. *E. coli* isolate (58%) in UTIs more distribution than other causative pathogenic bacteria. **Conclusion**: There are many parameters related with thalassemia appears between Iraqi people, from these, related with hematological, and physiological characteristics of patients.

KEY WORDS: Thalassemia, urinary tract infection, bacterial infections, splenectomized, hematology parameters.

INTRODUCTION

In the physiological state, the hemoglobin molecule is a heterotetramer consisting of two α and two non-α globin chains, each carrying a heme molecule with a central iron(Taher.*et al*, 2018). The oxygen-carrying capacity of the molecule is maximal. The non-α globin chains can be β chains which coupled with αchains form adult hemoglobin (HbA), while α and δ chains form a minor fraction of adult hemoglobin (HbA2). Finally, α and γ chains form the fetal hemoglobin (HbF) (Weatherall, 2010). The production of the globin chains is regulated by the α globin cluster on chromosome 16 with the two α globin genes (HBA1 and HBA2), the β globin cluster on chromosome 11 with the genes for the γ, δ, and β globin chains. In the thalassemia, this equilibrium is disrupted by the defective production of one of the globin chains. Any reduced production of one of the globin chains within the developing red cell will cause an accumulation of the normally produced chain, If α globin chains are not produced in adequate amounts there will be an accumulation of β globin chains (Clegg *et al.*, 1965). The excess unpaired and insoluble globin chains in thalassemia cause apoptosis of red cell precursors, resulting in ineffective erythropoiesis. The excess non-globin chains in thalassemia assemble as 4 tetramers (Hb Bart's) in intrauterine life, and 4 tetramers (HbH) after birth.(Songdej *et al.*, 2017). The excess chains have further devastating effects on the function of erythrocytes and their ability to deliver oxygen, (Chiappe, 2015). Some of the immature red cells pass into the circulation. Because of their membrane defect, they are fragile and prone to hemolysis. An altered deformability and are trapped by the spleen where they are destroyed by macrophages, leads to an enlargement of the spleen

which can become massive, and development of functional hypersplenism with removal of platelets and white cells as well as red cells. Ineffective erythropoiesis, removal of abnormal cells by the spleen, and hemolysis all contribute to an anemia of variable severity(Angastiniotis and Lobitz, 2019). The degree of anemia and the severity of the clinical effect can be modified by other mitigating factors. The most common of these is the co-inheritance of factors that reduce globin chain imbalance such as when α-thalassemia is co-inherited in β-thalassemia homozygotes, resulting in a milder β-thalassemia syndrome(Chiappe, 2015).

MATERIALS AND METHODS

This clinical study had been done at Hematology center/ Baqubah Teaching Hospital from the period of 15 July 2018 to 19 April 2019. The total number of patient is sixty, thirty of them lifting the spleen and thirty others were not lifting the spleen as a study group was chosen according to the study criteria, and patients with viral hepatitis were excluded. All patients had been referred to hematology center after specialist physician requests. The main compliant was anemia. Detailed history of each patient was obtained concerning name, age, gender, address, degree of kinship, diagnosis, take benzethin penicillin or not, number of transfusion in month , date of spleen lift. Thirty blood samples from healthy person were also taken as a control group from blood bank. Took a small amount of urine (usually 10 microliters) by loop is cultured on blood agar as enrichment media and MacConky agar, and placed in an incubator at 37c for 24 hours, all isolates were diagnosed by biochemical tests and confirmed by vitek2, Cassette AST used to sensitivity test.

RESULTS AND DISSCUSION

In our clinical study we tried to compare between two groups of thalassemic patients, first group who had been removed their spleen ,second group with their spleen intact and then we compared these two groups with control group who are healthy people .Regarding the age group we found the patients were aged between 1 to 39 years, with an average of 20 years, this finding is identical to study conducted in Iran by Ghaffari (Ghaffari *et al.*, 2011). The female to male ratio was(~ 1:1), which is not going with Pecorari et al study (Pecorari *et al.*, 2008) who had a ratio of (7: 3), other studies shows the ratio (~6: 4) (Saleh, *et al*., 2018). The major indication for splenectomy in our patients is to decrease blood transfusion requirement, the interval of blood transfusion in splenectomized patients in our study was 1 U every 15 days 54%(16), every 20days 13%(4) and every 30days 33%(10). In non-splenectomized patient 57%(17) blood transfusion every 15days, 7%(2) every 20days and 36%(11) every 30days, the minimum blood transfusion requirement was once every 15 days for most patients with splenectomy and patients with intact spleen,

Table (1) illustrated characterization of patients with thalassemia including patient age, gender, kinship, Prophylactic antibiotic used, associated disease, type of thalassemia, and interval of blood transfusion.

Table 1:- **Characterization of study patients.**

Characteristics of thalassemia patients (N=60)				
Age (year)	Range (1-39)		Mean (20)	
Gender	Female		(N=28)	(47%)
	Male		(N=32)	(53%)
Kinship	Relative		(N=46)	(77%)
	Non-relative		(N=14)	(23%)
Prophylactic antibiotic	Received penzathin pencillen		(N=12)	(20%)
	Non-received penzathin pencillen		(N=48)	(80%)
Associated disease	Chest infection		(N=2)	(3%)
	Diabetes		(N=1)	(1%)
	Cholangitis		(N=2)	(3%)
	Hypertension		(N=2)	(3%)
	Tonsillitis		(N=2)	(3%)
	H.pylori		(N=2)	(3%)
Type of thalassemia	Major		(N=44)	(73%)
	Intermediate		(N=16)	(27%)
Interval blood transfusion	Every15day Every 20day Every 30day Every15day Every 20day Every 30day	 (splenectomized) N=30 (non-splenectomized) N=30	(N=16) (N=4) (N=10) (N=17) (N=2) (N=11)	(54%) (13%) (33%) (57%) (7%) (36%)

Table (2) illustrated WBC (lymphocytes%, neutrophils%, Monocytes %) and platelets count. The results showed increased WBC count significantly in splenectomized group (38.91×10^9/L ± 3.90) incomparism with control group (7.71×10^9/ L± 0.20) p=0.000, and the same result obtained incomparism between the two study groups (splenectomized and non-splenectomized) (38.91×10^9/L ± 3.90)(4.24×10^9/ L ± 0.33) p=0.000, but the results showed no significant difference in Non-splenectomized group compared with control group p=0.281. There is a sharp increase in the total white cell count which is obvious within several hours and often maximal in a day or two; it then gradually fall over a period of weeks or months to normal values; in some cases a mild increase persist for many years. The sharp rise in the count is due mainly to an increase in neutrophils; however, after a few weeks to months, the neutrophil count falls to near normal levels and the number of circulating lymphocytes and monocytes rise and remain increased. The changes which arise in the peripheral blood following splenectomy used to be interpreted as evidence that the spleen regulated release of cells from the bone marrow, present evidence points strongly to these changes reflecting the capacity of spleen to sequent large numbers of cells newly released from the bone marrow. In splenectomized patient leukocytosis in response to infection is characterized by a greater than normal (Ayyash and Sirdah, 2018) .our results compatible this finding that showed significant increase lymphocytes in splenectomized group (66.14% ± 1.89) compared to control group (28.82% ± 1.03) p=0.000, as well as rise significant in splenectomized group when compared with Non-splenectomized group (40.46% ± 1.32) p=0.000, and the results showed significant increase in Non-splenectomized group when compared with control group p=0.000 (Table 2). The results showed decrease neutrophils in splenectomized group (26.95% ± 1.80) compared to control group (64.23% ± 1.01) p=0.000, as well as significant decrease in splenectomized group compared to Non-splenectomized (52.26% ± 1.33) p=0.000, decrease significantly in Non-splenectomized group when compared with control group p=0.000 (Table 2). Normally, red blood cells at the end of their life span are removed from the circulation by phagocytic cells . The spleen, because it contains a large number of these cells which by reason of their special anatomical arrangement are in intimate contact with the circulating blood, is an important and possibly the main site of destruction of old red cells, red cell life span is not prolonged following splenectomy .It also removes imperfectly formed, fragmented and damaged cells from the blood, this process is called 'culling' function. It is probable; spleen plays apart in the removal of leucocytes and platelets from the blood at the end of their life span. The platelet results showed significant decrease in non-splenectomized group (111.83×10^9/L ± 5.33) when compared with splenectomized and control group (281.07×10^9/ L ± 57.18 and 245.26×10^9 /L ± 10.19) p=0.001 and p=0.006 respectively, but no significant different between splenectomized and control group p=0.454. The spleen contain a pool of platelets, and dynamic exchange, exists between splenic and circulating platelets; the exchangeable pool in the normal spleen represents approximately 30%of total circulating mass of platelets, the raise platelets count might be contribute to the severe and occasionally fatal event of thrombosis in deep vein, embolism of pulmonary and that has occurred with splenectomy in some of our thalassemia patients.

Table 2:- Hematological parameters results.

Parameters	Control (N=30) M±SE	N-Splenectomized (N=30) M±SE	Splenectomized (N=30) M±SE	P-Value
WBC cell/L	7.71×10^9 ± 0.20	4.24×10^9 ± 0.33	38.81×10^9 ± 3.09	Con vas non 0. 281 Con vas sple 0.000 Non vas sple 0.000
Lymphocytes%	28.82×10^9 ± 1.03	40.46×10^9 ± 1.32	66.14×10^9 ± 1,89	Con vas non 0.000 Con vas sple 0.000 Non vas sple 0.000
Neutrophils%	64.23×10^9 ± 1.01	52.26×10^9 ± 1.33	26.95×10^9 ± 1.80	Con vas non 0.000 Con vas sple 0.000 Non vas sple 0.000
Monocytes%	6.1933×10^9 ± 0.276	6.183×10^9 ± 0.253	6.64×10^9 ± 0.30	Con vas non 0.980 Con vas sple 0.256 Non vas sple 0.246
Platelets cell/L	245.26×10^9 ± 10.19	111.83×10^9 ± 5.33	281.07×10^9 ± 57.18	Con vas non 0.006 Con vas sple 0.454 Non vas sple 0.001
Con. Control, Non. Non -splenectomized, sple. splenectomized, WBC white blood cells, p value < 0.05				

Appearance different statically significant more prevalent in patients with splenectomized than those who non- splenectomized. Opsonized bacteria are removed efficiently by macrophages in the spleen and liver, poorly opsonized bacteria, such as encapsulated bacteria, are only cleared by the spleen. For removal of these

bacteria during the course of initial infection, natural antibodies are needed, which are pentameric IgM that can facilitate phagocytosis either directly or through complement deposition on the capsule (Allen *et al.*, 2012).

Table 3:- Urinary tract infection in study groups.

Group	Urinary tract infection		
Splenectomizd	Positive (N=14) Negative (N=16)	(47%) (53%)	Total =30
Non- splenectomized	Positive (N=3) Negative (N=27)	(10%) (90%)	Total=30
X^2 = 9.932 , `P value = 0.002, df =1			

Diagnosis result by VITSK 2 show as in Table (4), *E. coli* isolate (58%) in UTIs more distribution than other causative pathogenic bacteria. The *E.coli* was the most common pathogen of UTI mentioned by other studies:

Table 4:- Types of isolated bacteria in study groups.

Type of isolated bacteria N=(17)	Number	Parentage
E. coli	10	58%
Enterbacter caecloa	2	12%
Proteus mirabilis	2	12%
Pseudomonas aeruginosa	1	6%
Klebsiella pneumonia	2	12%

E. coli was the most common pathogen of urinary tract. The distribution of *E. coli* isolate the high incidence of *E. coli* is explained by they have many virulence factors that facilitate them to get infected with urinary tract infections, secreted to the site of action and surface bacterial cell virulence factors. The secreted virulence factors such as α-haemolysin, is pore forming toxin gain enhance access to host nutrient and iron stores, and damage effector immune cells (Raksha *et al.*, 2003). Surface virulence factors such as fimbria , these type of organelles different way to in virulence like promoting bacterial invasion, directly triggering host and bacterial cell signaling pathways, and facilitating the delivery of other bacterial products (Müller *et al.*, 2007). Antibiotics are most frequently prescribed on the basis of general guidelines and knowledge about sensitivity: e.g. uncomplicated urinary tract infections can be treated with a first generation quinolone, etc. This is because *Escherichia coli* is the most likely causative pathogen, and it is known to be sensitive to quinolone treatment. The result of susceptibility against bacterial isolates shown in table (5). The transmission phenomenon of antibiotic resistance from bacteria to other spread around the world and the results are dangers, and the selection of optimum antibiotics for treatment is important to limit this phenomenon.

Table 5:- Sensitivity and resistance to antibiotic.

Antibiotic		Sensitive %	Resists %
Penicillin	Pipracillin	3(18%)	14(82%)
	Pipracillin\Tazobactam	6(35%)	11(65%)
	Tricarcillin	3(18%)	14(82%)
B-lactam Cephalosporin	Cefazidime (3^{ed} generation)	7(41%)	10(59%)
	Cefipime (4th generation)	9(53%)	8(47%)
Monobactams	Aztreonam	13(76%)	4(24%)
Carbapenems	Imipenem	17(100%)	0(0%)
	Meropenem	17(100%)	0(0%)
Quinolones	Ciprofloxacin (1st generation)	11(65%)	6(35%)
Sulfa drugs	Trimethoprim	10(59%)	7(41%)
Aminoglycosides	Gentamycin	10(59%)	7(41%)
	Amikacin	12(71%)	5(29%)
	Tobromycin	9(53%)	8(47%)

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