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The Detection of antibody titer in Thalassemia patients in Al-Najaf, Iraq

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Abstract : The present study included **60** samples collected from patients suffering of thalassemia who attended to Thalassemia Center in Al-Zahraa hospital for birth and children in Al-Najaf governorate during the period from February to august-2014.
All Subjects employed in this study were selected to confirm the presence or absence of antibodies, both alloantibodies or autoantibodies include coomb's test in case of Positive DAT (direct anti globin test) and, IDAT(In direct anti globin test) for antibodies detection. Samples of blood were treated to tests of blood bank procedure for observed of antibodies.
The study discovered infect in 32 male (53.3%) with was in28 female as (46.4) and 14 patients had autoantibodies as (23.3%) while patients have alloantibodies 24(40%).
the goal of this research was to limit the prevalence of R.B.C autoantibodies and alloantibodies between frequency transfused thalassaemic patients and factors that contributed to its development.
Keywords : Autoantibodies , epitopes , DAT, EDTA, Alloantibodies

Introduction:

Thalassemia , a major public health problem in Iraq , is a heterogeneous clan of autosomal recessive troublewhich haemoglobin make . It is characterized by the lack or decrease of one or more globin chains of haemoglobin[1]. The treatment recommendation for Thalassemia mainly contains orderly backed cell of R.B.C transfusion oftencirculation every fourteen days to thirty- five days, to maintain a pre-transfusion hemoglobin scaleover**9-10.5g/dl** .

The serious problems in blood transfusion is the accumulation of alloantibodies and autoantibodies opposite to RBC antigens. Result of some studies demonstratesevident different recurrence and proportions of alloantibodies and autoantibodies accumulation in multi-transfused persons[2,3,4]. Alloantibodies aresometimes responsible for the production of hemolytic transfusion reactions and it reduce the incidence of safe transfusion of blood and some blood product for thalassemia patients , but in some clinical cases may in few cases result this become ineffective. Theautoantibodiesarelinkedwith the R.B.C have the resulting effectappear frequently less , however, can consequences in hemolysis and problems in blood cross-matching when blood is transfused from the donor to the recipient[5].

Autoantibodies and alloantibodies should be determined in the recipients serum before any blood transfusion or blood matching is done. The reasons of alloimmunization& autoimmunization in Thalassemia patients are not easy tocomprehend. In addition, studies suggest that the immune status of recipients, lack of spleen and various in the R.B.Cs phenotype among donor and beneficiary are probable to redound further to the phenomena[6].

This paper aim to report the consequences the prevalence of red blood cellsautoantibodies and alloantibodies and the other factors that mayredound to their development.

Materials and Methods:

The study involved**60** patients with thalassemia major who had received orderly transfusion atAL-Zahra Hospitalfor Birth and Children during the period from February-August ,**2014.** Agreements from all patients were gotten from case sheet of all patients. Clinical and transfusion records of these patients of a mean age **8.35** years (range **2-15** years) were analysed. All patients were tested for the presence of autoimmunization or alloimmunization include coomb's test in case of Positive DAT(direct anti globin test) and,IDAT(In direct anti globin test) for antibodies detection. Blood samples were treated according to standard blood bank procedure for the investigation of antibodies.

Collection and Storage of Samples.

A specific preparation of the patient is required before the collection of samples by approved techniques. hemolyzed samples were never used .

❖ **For Direct Antiglobulin Test :** drawn into blood EDTA is favourite but, citrated and oxalate or clotted whole blood may be used to. The sample of blood should be tested as soon as possible after the collection and must not be stored.

❖ **For Indirect Antiglobulin Test :** Serum , at least 48 hours old, should be used. Beneficiary units must be examinedby to the end of their dating.

Results:.

Demographic data:

A total of **60**frequently- transfusingthalassemia patients were included in this research.

Demographical data are shown in Table-**1**. 32 were males and 28 females with a male: females

53.3:46.7 % .Patients (16.6) with splenectomy were shown in Table-1- .

Table (1)Demographic data of Thalassemia patients who received a regular blood transfusion .

Demographic data	*Number of patients*	*%*
Total Patients	60	
Gender		
Male	32	53.3%
Females	28	46.4%
Splenectomy		
Yes	10	16.6%
No	40	83.4%

Direct coombs test:

The distribution of Autoantibodies with AB group system and Rhesus system was shown in Table -2- .The majority of DAT belonged to blood group **O**(6 patients)4 patients belonged to blood group A; 3patients belonged to blood group B and one patient belonged to blood group AB.All the 60 patients were Rhesus positive .

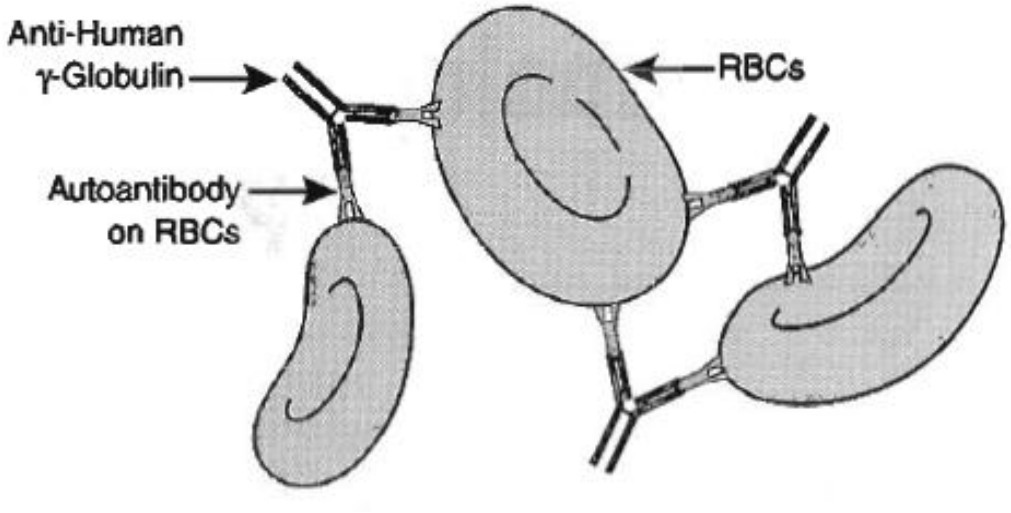

Fig .(1) : Autoantibody on the surface of R.B.C [11]

Table -2- DAT (Autoantibody) with its frequenciesaccording to blood groups.

DAT group	*O*	*A*	*B*	*AB*	*Total*
DAT **+**	6	4	3	1	14
DAT **-**	14	11	15	6	46
	20	15	18	7	60

Indirect coombs test :

The distribution of alloantibodies with ABO group system and Rhesus system was shown in table-3- . The majority IDAT was blood group 9 patients belonged to O blood group ,7 patients were with blood group A, 5patients were with blood group B and 3patients were with blood group AB.All the 60 patients were Rhesus positive .

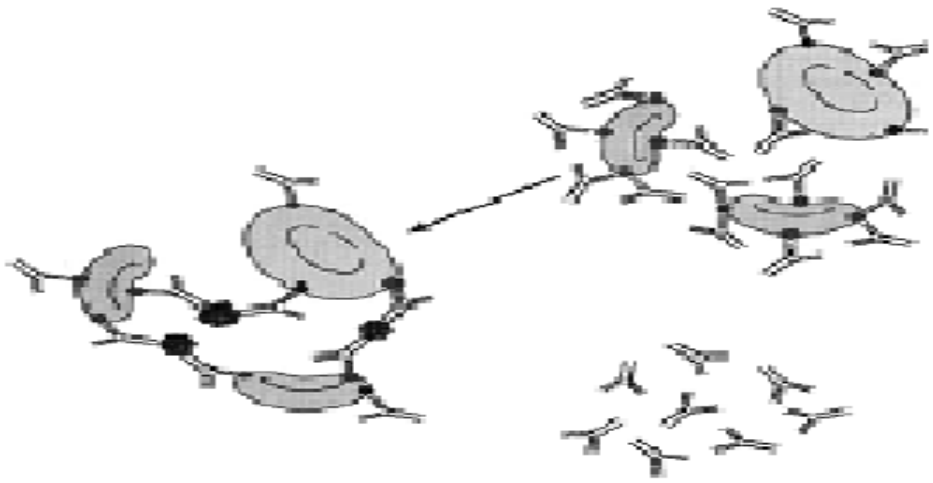

Fig .(2) : Alloantibody on the surface of R.B.C [11]

Table (3): IDAT (Alloantibody) with its frequenciesaccording to blood groups .

IDAT group	*O*	*A*	*B*	*AB*	*Total*
IDAT +	9	7	5	3	24
IDAT -	11	8	13	4	36
	20	15	18	7	60

Discussion:

Thalassemia in Iraq can be disturbing for both patients and doctorsit is a public health problemdue to its phenotype different from one patient to another .

A transfusion needs in excess of 180-200 ml/kg of a packed cell according toyear which is taken as an evidence of hypersplenism and is an indication for splenectomy[7]. The pathogenesis of erythrocyte autoantibody accumulation following transfusions is not fully understood. However, clinical proof of thalassemia has been correlated with large amounts of RBCs associated toIgG[8].It was also suggested thatthelinkof RBCs with alloantibodies could lead to conformational alteration epitope of the antigenic that ultimately activates autoantibodies production. This study observed that there was no relation between gender and age witha production of autoantibodies at the begins of transfusions, the volume of packed cells transfused and splenectomy in our patients. It is possible that certain patients are genetic "responders" who have a boost to develop red blood cellsautoantibodies with a distribution of blood group and the tendency towardsautoantibodies productionwhich could opposetheoveralllack of the function of the immune system[9]. In this research, we found that theresults of our studywereidenticalto the resultscarried out in Hong Kong where 10% patients with transfusions –dependent Thalassemia developed RBCs autoantibodies was found[10]. In similar, it is to a study conducted in Greece where it was found that 16 of their transfusion-reliancethalassemia patients developed autoantibodies which were in relation with the existing alloantibodies[3]. The study conducted in a state of Kuwait found that 11% of their patients developed autoantibodies with latent alloantibodies[4]. Our resultswere supported by [11]who discover that an important elevation in RBCs-linked IgG was seen in severing thalassemia patients and it was available more in splenectomized subject than inthe non-splenectomized subjects[11].

Our consequences showed that there wasno significant correlation between alloimmunization and gender. The factors for alloimmunization were complex and included at least 3 central contributing elements:

1-The RBCs antigenic variation between the donor of blood and recipients.

2-The recipient's status of immune and to the immunodulatory effect of the allogeneic R.B.Cs transfusions [3].

Our data in Table-3- were identical with a study done by (**3,6**) . Our resultswere supported by in a study in Kuwait with showed a higher frequency of alloantibodies (28%)[4] **.**

Alloimmunization and autoimmunization in patients with frequent transfusion are equally significant both in the laboratory and clinicalpractice and from a social point of view. Our data showed a low alloimmunization and autoimmunization rate in multiply transfused Thalassemia patients. In our study, the statistical difference was not significant between splenectomy and age at beginning of transfusion and alloimmunization rate. However, we observed in this study that there were some factors that contributed to the alloantibody formation such as age at the beginning of transfusion ,the difference in an ethnic race of donor and recipient and the blood that were matched only for ABO and RhD antigen.

We recommended that blood bank procedure RBC antigen phenotyping be utilized for all transfusion dependent thalassemia patients before any toblood transfusion and processingthe blood cross-match for blood group(ABO) and rhesus factor (Rh). The transfusion of matched R.B.Csisnecessary for chronically multi-transfused patients who started transfusion at or after the age of 1 year. In order to underestimate formation of Alloimmunization and autoimmunization[12] .

Conclusion

Autoimmunization & alloimmunization due to foreign RBC is an important adverse effect of blood transfusion in thalassaemic patients. Guidelines should be considered for local blood banks with limited technical facilities and donor resources and phenotypically matched blood cannot always be made available.

Ethics Statement

All procedures conducted in this study (including human participants) were consistent with the ethical standards of Iraqi IRP.

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