Summary

Thalassemia syndromes are genetic disorders in globin chain production, characterized by varying degree of ineffective hematopoiesis and increase hemolysis. Their clinical severity varies widely, ranging from asymptomatic forms to severe or even fatal entities. Genetically Thalassemia represents the most common hereditary hemolytic anemia. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of beta-thalassemia gene, with about 60,000 symptomatic individuals born annually, the great majority in the developing world.βTM has been the most common chronic hemolytic anemia in Egypt with a carrier rate ranging from 5.3% to 10.

Life-long blood transfusion is a standard protocol used for the treatment and care of patients with β-thalassemia major. Although lifesaving , chronic blood transfusion however, results in iron overload with subsequent organ and tissue damages.

Oxidative stress is an important mechanism in the progression of β-TM, whose contribution to β-thalassemic anemia is only partially understood. Oxygen free radicals generation process gains importance because of its role in the pathogenesis of a lot of pathological processes and its effect on mortality and morbidity. Oxygen free radicals cause lipid peroxidation, the end product of lipid peroxidation is MDA. The iron chelator DFO has been used for several decades to prevent iron overload in patients with thalassemia, due to its ability to inhibit the redox activity of transition metals through chelation.

Haptoglobin is an acute phase protein that scavenges hemoglobin in the event of intravascular or extravascular hemolysis and reduces the oxidative and peroxidative potential of free Hb. In human populations three common phenotypes are represented: Hp1-1, Hp2-2 and the heterozygous Hp2-1, which is determined by two alleles Hp1 and Hp2 .The unique and peculiar functional activities defined by the phenotypes make consideration of Hp gene polymorphism.

More recently, several functional differences between haptoglobin phenotypes have been demonstrated that appear to have important biological and clinical consequences. Haptoglobin polymorphism is associated with the prevalence and clinical evolution of many inflammatory diseases, including infections, atherosclerosis, and autoimmune disorders. These effects are explained by a phenotype-dependent modulation of oxidative stress and prostaglandin synthesis. The antioxidant role of haptoglobin and the phenotype dependence were confirmed for preventing possible oxidative damage induced by free hemoglobin and iron release during its catabolism.

The aim of the work is to study the impact of haptoglobin gene polymorphism on phenotypic variability in patients with β thalassemia major in relation to iron overload and oxidative stress.

The study was conducted among fifty patients with established βTM, they were on regular RBCs transfusion and all patients were adherent to iron chelation therapy using DFO. Blood samples were collected before blood transfusion, Cases with apparent acute infection were excluded. Twenty five normal apparently healthy individuals of matching age and sex with previous group were served as control. All patients as well control subjects included in the study were subjected to the following:

1. Thorough history taking with special emphasis on patient's age at first presentation, frequency of blood transfusion and chelation therapy and history of operative procedures(splenectomy).)
2. Complete clinical examination with special emphasis on thalassemic manifestations, hepatomegaly, splenomegaly.
3. Laboratory investigations including:

a) Routine investigation including

- Complete Blood Count (CBC), Blood film, Reticulocytes count, Hb electrophoresis.
- Iron profile (serum iron, total iron binding capacity, transferrin saturation)
- Serum ferritin, C- reactive protein (CRP) and Coombs' test.

b) Special laboratory tests

- Serum haptoglobin (Hp).
- Serum malondialdehyde (MDA).
- Haptoglobin gene polymorphism by PCR.

Data from our study confirm that the decrease of the hemoglobin level in thalassemic group is accompanied by a decrease in the erythrocytes number and by diminished values of their specific indexes (MCV, MCH, PCV,etc.).

The content of mean serum iron (42.30µmol/l) and mean serum ferritin (3287.22 µg/l) were significantly increased above that of the controls in all the patients examined. The study revealed that 32% of thalassemic patients are HCV positive which is higher than the reported prevalence of HCV in Egypt (15-20%). 16% of our patients had a positive antiglobulin test, this is partly attributed to lack of extended phenotyping as a standard routine in our blood banks. In the present study, a significant correlation was found between a positive DAT and a lower serum haptoglobin level (P=0.017) being consumed in the trapping free Hb from circulation.

On the other hand, the lower levels of serum haptoglobin in thalassemic patients could be attributed to the presence of different polymorphisms of the haptoglobin gene. Consequently, the genotype Hp1-1results in the highest level of serum haptoglobin, while Hp2-1 and Hp2-2 are associated with lower serum haptoglobin levels approaching statistical significance (P=0.067).

In the present study, 56% of patients had the Hp2-2 genotype, followed by Hp 2—1 genotype, while in the control, Hp2-1 predominated, the study also revealed that thalassemic patients with Hp2-2 have the highest serum ferritin.

In the present study, a significantly higher level of MDA, a marker of lipid peroxidation was found in patients versus the control,(P <0.001)reflecting a state of significant oxidative stress in patients group. The highest level was found in patients with the Hp2-2 phenotype (P=0.056) as compared with the other phenotypes.

Therefore, we hypothesize that oxidative stress is a major factor of morbidity in β-thalassemic and is correlated with iron overload and metabolic dysfunctions.We have demonstrated that there are functional differences in the antioxidant capacity of the different haptoglobin proteins toward hemoglobin, suggesting that those with haptoglobin 1-1 protein may have superior antioxidant protection than those with Hp 2-2 protein .

Conclusions

From the above findings we could extrapolate several conclusions:

- Thalassemia patients are at a great risk of oxidative stress.
- Haptoglobin polymorphism and phenotypic variability have a great impact on oxidative stress in thalassemia patients.
- Thalassemia patients with Hp 2-2 phenotype are under greater iron-driven oxidative stress than patients with other phenotypes.
- Despite chelation, they could not achieve the desired threshold of serum ferritin levels possibly due to non-compliance.
- Haptoglobin served as an antioxidant by virtue of its ability to prevent hemoglobin driven oxidative tissue damage.
- The frequency of blood transfusions given per month appears to be inadequate. In terms of pre transfusion hemoglobin levels (taking 9.5 gm% as cut off), 98% of patients are undertransfused.
- In terms of serum ferritin levels (taking 1000 ng/ml as cut off), nearly 94% of patients need their chelation regime to be reviewed.
- To prevent anaphylactic transfusion reactions, distinguishing the *Hp del* allele from the non-deficient allele is most important.

Recommendations

From the findings of the present study, we may recommend the following:

- Determination of haptoglobin phenotype is mandatory to these patients in order to tailor iron chelation therapy, and to detect Hp0 allele.
- MDA should be estimated at least once per year to guide the proper antioxidant therapy.
- Thalassemia patients should receive filtered blood to dampen activated neutrophils and ROS.
- Extended blood group phenotyping is highly warranted to prevent alloimmunization and secondary autoimmunization.
- The study suggests the need to step up the transfusions to achieve pre-transfusion hemoglobin goal of 9.5 gm/dl and also to institute urgent and effective chelation measures with the aim of keeping serum ferritin levels below 1000 ng/ml.

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الملخص العربى

تعتبر متلازمة الثلاسيميا خللا موروثا في الهيموجلوبين و ناتجا عن الانتاج غير المتوازن لسلاسل الجلوبين المكونة لجزئى الهيموجلوبين مما يؤدي الي الزيادة النسبية لاحداها و ينتج عن ذلك انيميا تكسيرية مزمنة تتراوح شدتها على نطاق واسع بين اعراض طفيفة و اشكال قاتلة. وراثيا يعتبر مرض الثلاسيميا المرض الوراثي الاكثر شيوعا في العالم لفقر الدم التكسيرى الوراثي حيث يقدر أن حوالي ١,٥٪ من سكان العالم (٨٠ إلى٠ ٩ مليون نسمة) حاملين للبيتا ثلاسيميا ،و يولد سنويا حوالي ٦٠٠٠٠فرد تظهر عليهم الأعراض ، الغالبية العظمى في العالم النامي و في مصر تتراوح نسبة الحاملين له بين ٥,٣ الى ١٠٪٠

يعتمد مرضي الثلاسيميا الكبرى بيتا على النقل المنتظم لكرات الدم الحمراء مدي الحياة ، وبالرغم من ان هذا البروتوكول القياسي لنقل الدم ساهم بنسبة كبيرة في اطالة و تحسين نمط الحياة لمرضي الثلاسيميا الكبري، الا انه يؤدي الي زيادة نسبة الحديد بالدم مما يؤثر علي معظم اعضاء الجسم و يؤدي الى مضاعفات خطيرة قد تنتهي بالوفاة.

يعتبر الجهد التاكسدى الية هامة في تقدم و تفاقم الثلاسيميا الكبرى و إسهاماته في تقدم المرض مفهوم جزئيا فقط

كما اكتسبت عملية توليد الأكسجين الحر أهمية بسبب دورها في الآلية المرضية للكثير من العمليات المرضية وأثرها على معدلات الوفيات والإصابة بالأمراض ،كما ان الجذور الحرة الأكسجين تسبب اكسدة للدهون والمنتج النهائي هو المالونداياالدهيد.

و قد استخدم عقار الديسفرال لكلب الحديد لعدة عقود في المرضى الذين يعانون من مرض الثلاسيميا، نظراً لقدرته على تثبيط نشاط الأكسدة والاختزال من الفلزات الانتقالية من خلال عملية إزالة الحديد.

الهابتوجلوبين (موثق الهيموجلوبين) هو بروتين مرحلة حادة يزيل الهيموجلوبين في حالة تكسير الدم داخل الأوعية أوخارجها ، ويقلل من إمكانية الأكسدة للهيموجلوبين الحر ٠فى الانسان تم تحديد ثلاثة انماط وراثية اساسية للهابتوجلوبين وهى HP1-1,HP2-2,HP2-1 الذى يحدده الاليلات HP1,HP2.

ان الوظائف الفريدة والمميزة لكل نمط وراثي جعلتنا ندقق النظر فى اهمية تعدد النمط الجينى للهابتوجلوبين حيث انه في الآونة الأخيرة، أثبتت العديد من الاختلافات الوظيفية بين انماط الهابتوجلوبين أن لها عواقب بيولوجية وسريرية مهمة. ويرتبط تعدد الأشكال للهابتوجلوبين مع انتشار وتطور للعديد من الأمراض الالتهابية، بما في ذلك العدوى، وتصلب الشرايين، واضطرابات المناعة الذاتية.و يتم تفسير هذه الآثار بانها تعتمد على النمط الظاهري لتعديل الجهد التاكسدى وتصنيع البروستاجلاندين. وتم تأكيد دور الهابتوجلوبين المضاد للأكسدة ، واعتماده على النمط الظاهري لمنع الضرر التأكسدي المحتمل الناجم عن الهيموجلوبين المتحرر و تحرير الحديد خلال الايض.

وقد اجريت هذه الدراسة لمعرفة تاثير تاثير التنوع الجينى للهابتوجلوبين على المظهر الاكلينيكي المتباين فى مرضى انيميا البحر المتوسط - بيتا و علاقته بزيادة الحديد و الجهد التاكسدى.

اجريت الدراسة على خمسين مريض بيتا ثلاسيميا قد سبق تشخيصهم و تتراوح اعمارهم بين ١٢ و٢٠ عاما و يتلقون نقل دم بصورة منتظمة و عقار الديسفيرال لعلاج ارتفاع نسبة الحديد بالدم٠

و قد تم استبعاد المرضي الذين ظهر عليهم اعراض عدوى نشطة.

و شملت الدراسة ايضا مجموعة ضابطة للبيانات من خمسة و عشرين شخص اصحاء المظهر تتراوح اعمارهم بين ١٣ و٢٠ عاما.

المرضى و طرق البحث:

تم عمل الاتى للحالات

تسجيل البيانات التاريخية للمرضي بما في ذلك التاريخ الشخصي والحالى والماضي والعائلى والدوائى.

الفحص الاكلينيكى الباطنى

اختبارات معملية و تشمل

صورة دم كاملة مع الفحص المجهرى-و عد الخلايا الشبكية قبل نقل الدم

اختبار مستوى بروتين سى التفاعلي (للكشف عن اى عدوى نشطة)(

اختبار كومبس (المناعة الذاتية كسبب لانحلال الدم)(

قياس مستوى الحديد و نسبة الفريتين(مخزون الحديد) و تشبع الترانسفرين في مصل الدم

قياس مستوى الهابتوجلوبين (موثق الهيموجلوبين) فى مصل الدم

قياس مستوى المالون دايالدهيد في مصل الدم

ابحاث وراثية (تفاعل البوليمراز المتكرر) لتحديد الطرز الجينية المتعددة للهابتوجلوبين و ذلك عن طريق استخلاص الحامض النووى من كرات الدم البيضاء

النتائج:

وتؤكد البيانات من دراستنا أن انخفاض مستوى الهيموجلوبين في مجموعة الثلاسيما مصحوبا بانخفاض في عدد الكريات الحمراء وتناقص قيم مؤشراتها المحددة (MCH,MCV,PCV)

واظهرت النتائج ارتفاع مستوى الحديد و الفريتين في مصل الدم لمرضى الثلاسيميا مقارنة بالمجموعة الضابطة و كانت هذه الزيادة ذات دلالة احصائية

وكشفت الدراسة أن ٣٢% من مرضى الثلاسيميا ايجابيون لفيروس التهاب الكبدى ج و هى نسبة اعلى من نسبة انتشار المرض المبلغ عنها في مصر(١٥—٢٠%)

اظهرت الدراسة ان ١٦٪ من المرضى كان اختبار كومبس لديهم ايجابى ويعزى ذلك جزئيا إلى عدم وجود فحص روتينى للانماط الشكلية طفيفة الحدوث فى بنوك الدم لدينا

فى هذه الدراسة تم العثور على ارتباط ذو دلالة احصائية بين اختبار كومبس الايجابى و انخفاض مستوى الهابتوجلوبين فى المصل لديهم(٠,٠١٧) الذى يتم استهلاكه لتوثيق الهيموجلوبين المتحرر

من ناحية أخرى، يمكن أن يعزى انخفاض مستويات الهابتوجلوبين فى المصل في مرضى الثالاسيميا إلى وجود أشكال متعددة من الجينات للهابتوجلوبين. ونتيجة لذلك، النمط الوراثي HP1-1 ينتج عنه مستوى أعلى من هابتوجلوبين في المصل بينما الانماط الوراثية HP2-1 وHP2-2 ترتبط مع انخفاض مصل هابتوجلوبين لمستويات تقترب من الدلالة الإحصائية.

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في هذه الدراسة، كان ٥٦% المرضى لديهم النمط الجيني الوراثى HP2-2 يليه Hp2-1

بينما في المجموعة الحاكمة كان الطرز الجينى Hp2-1 مهيمن .

كما كشفت الدراسة أن مرضى الثلاسيميا مع النمط الجينى HP2 2 وجد لديهم اعلى مستوى من الفيريتين فى المصل

في هذه الدراسة، تم العثور على مستوى أعلى بكثير من المالونداياالدهيد فى المرضى عن المجموعة الضابطة، و هو علامة على زيادة الجهد التاكسدى في المرضى مقابل عنصر التحكم ($P < 0.001$), كما وجد ان مجموعة المرضى لديهم النمط الجينيHP2-2 ($P = 0.056$) مقارنة بالاشكال الأخرى.

الاستنتاج:

من هذه الدراسة نستنتج ان
مرضى الثلاسيميا يعانون من خطر زيادة الجهد التاكسدى.

اختلاف النمط الجينى والتعددية الشكلية للهابتوجلوبين له اثر كبير على الجهد التاكسدى فى مرضى الثلاسيميا

مرضى الثلاسيميا ذوى النمط الجينى HP2-2 هم اكثر عرضة للجهد التاكسدى الناتج عن زيادة الحديد عن الانماط الوراثية الاخرى .

الهابتوجلوبين يعمل كمضاد للاكسدة بحكم قدرته على توثيق الهيموجلوبين و منع الاكسدة و تلف الانسجة.

تواتر عمليات نقل الدم التي تعطي شهريا على ما يبدو غير كافية حيث ان ٩٨٪ من المرضى لديهم تدنى فى نسبة الهيموجلوبين قبل نقل الدم.

من حيث مستوي الفريتين فى مصل الدم حوالى ٩٤٪ من المرضى بحاجة الى اعادة النظر فى نظامهم العلاجى لكلب الحديد من الجسم.

التوصيات:

ضرورة تحديد فصائل الدم باستفاضة لمرضى الثلاسيميا.

توصى الدراسة بضرورة تعيين النمط الجينى للهابتوجلوبين فى مرضى الثلاسيميا للعناية أكثر بحاملى النمط HP2-2 من حيث ادخال عدة كلابات للحديد و اضافة مضادات الأكسدة لهم لتقليل الجهد التأكسدى و بالتالى المضاعفات الناتجة عنه.

عمل تحليل المالونداياالهيد علي الاقل مرة كل عام.

النظر في ارتفاع معدل انتشار فيروس التهاب الكبد ج بين المرضى و تحسين الية فحص اكياس الدم خاصة لفيروس ج.

تاثير التعدد الجينى للهابتوجلوبين على المظهر الاكلينيكي المتباين فى مرضى انيميا البحر المتوسط - بيتا وعلاقته بزيادة الحديد والجهد التاكسدى

رسالة علمية

مقدمة الى معهد البحوث الطبية-جامعة الاسكندرية

استيفاء للدراسات المقررة للحصول على درجة

الدكتوراة فى طب وباثولوجيا امراض الدم

مقدمة من

خالد سعد احمد سالم

معهد البحوث الطبية

جامعة الآسكندرية

٢٠١٤

تأثير التعدد الجينى للهابتوجلوبين على المظهر الاكلينيكي المتباين فى مرضى انيميا البحر المتوسط - بيتا وعلاقته بزيادة الحديد والجهد التاكسدى

مقدمة من

خالد سعد احمد سالم

للحصول على درجة

الدكتوراه فى طب وباثولوجيا امراض الدم

لجنة المناقشة والحكم على الرسالة	موافقون
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المشرفون	موافقون
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