UNIVERSITI PUTRA MALAYSIA

RELATIONSHIP BETWEEN ERYTHROPOIETIN AND HEPCIDIN AMONG TRANSFUSED MALAYSIAN ADULT THALASSEMIC PATIENTS

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FPSK(m) 2022 1

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

January 2022

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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January 2022

Chairman :Bahariah Binti Khalid, PhD
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Thalassemia is a genetic blood disorder characterized by insufficient hemoglobin level or due to mutation in the globin chains causing an anemic condition with iron overload despite no history of transfusion; thus, insufficient hemoglobin will cause an imbalance of erythropoietin (an endogenously produced hormone used to maintain the oxygen levels in the blood carried out by hemoglobin molecule) and hepcidin (a peptide hormone responsible for body iron regulation). Besides, many thalassemic patients require a recurrent blood transfusion to survive alongside the different chelation therapy that both remarkably affect these biomarkers. The study was embarked to identify the relationship of serum erythropoietin and human hepcidin in differently transfused thalassemia patients. A cross-sectional study designed for 150 respondents matched by gender; 75 thalassemic patients recruited from Thalassemia Clinic of Ampang Hospital plus 75 healthy participants from FPSK, UPM. The required data which included variables such as body mass index (BMI); hemoglobin (Hb); serum ferritin (SF); liver iron concentration (LIC) and cardiac T2*, gathered using proforma and medical records, except for serum erythropoietin (EPO) and hepcidin (HEPC) assessed using enzyme-linked immunosorbent assay (ELISA kit) from Aug 2020-Oct 2020. HEPC levels correlated inversely with EPO in both MTG (r = -0.618, $P < 0.00001$) and NMTG (r = -0.8243, P=0.000048). In contrast, HEPC correlated positively with SF (MTG: r = 0.7833, $P < 0.00001$ and NMTG: r =0.8587, P=0.000011) and LIC (MTG: r = 0.4348, P= 0.000648 and NMTG: r =0.8817, $P < 0.00001$). Similarly, there was a positive relationship between BMI and Hb (MTG: r = 0.2604, P= 0.0487* and NMTG: r =0.4898, P=0.0463). Most of the thalassemia patients experienced iron overload, had higher levels of EPO, HEPC, SF and LIC when compared to patients with non-iron overload with a p-value of (0.027458, 0.000339, 0.026304 and 0.005644), respectively. The current study showed a significant inverse relationship between serum erythropoietin and human hepcidin in thalassemia patients compared to the healthy population. Thalassemia patients with low Hb seemed to have underweight BMI classification due to the positive relationship between BMI and Hb. The main variables (Hb, EPO, HEPC, SF, LIC and

Cardiac T2*) were more likely to be associated with the phenotypes of thalassemia rather than multi-genes (α and β-globin chains); also, the multiple blood transfusion and the different chelation therapies efficiently affected the values and association of those variables, HEPC specifically. Besides the iron overload that almost all thalassemia patients had, a specific group of those iron overload patients developed pertaining complications to iron overload.

Keywords: Serum erythropoietin, Hepcidin, Thalassemia, Monthly Transfused Group, Non-Monthly Transfused Group, ELISA, Hospital Ampang.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

HUBUNG KAIT ANTARA ERYTHROPOIETIN AND HEPICIDIN DALAM KALANGAN PESAKIT THALASEMIA YANG MEMERLUKAN TRANSFUSI DARAH DI MALAYSIA

Oleh

SHAHAD MOHAMMED IBRAHEEM

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Talasemia ialah gangguan darah genetik yang dicirikan oleh tahap hemoglobin yang tidak mencukupi atau disebabkan oleh mutasi dalam rantai globin yang menyebabkan keadaan anemia dengan kelebihan zat besi walaupun tiada sejarah transfusi; Oleh itu, hemoglobin yang tidak mencukupi akan menyebabkan ketidakseimbangan erythropoietin (hormon yang dihasilkan secara endogen yang digunakan untuk mengekalkan tahap oksigen dalam darah yang dijalankan oleh molekul hemoglobin) dan hepcidin (hormon peptida yang bertanggungjawab untuk pengawalan besi badan). Selain itu, ramai pesakit talasemia memerlukan pemindahan darah berulang untuk terus hidup bersama terapi khelasi yang berbeza yang kedua-duanya sangat mempengaruhi biomarker ini. Kajian ini dimulakan untuk mengenal pasti hubungan serum erythropoietin dan hepcidin manusia dalam pesakit talasemia transfusi yang berbeza. Kajian keratan rentas yang direka untuk 150 responden dipadankan mengikut jantina; 75 pesakit talasemia diambil dari Klinik Talasemia Hospital Ampang serta 75 peserta sihat dari FPSK, UPM. Data yang diperlukan termasuk pembolehubah seperti indeks jisim badan (BMI); hemoglobin (Hb); feritin serum (SF); kepekatan besi hati (LIC) dan T2* jantung, dikumpulkan menggunakan proforma dan rekod perubatan, kecuali untuk serum erythropoietin (EPO) dan hepcidin (HEPC) yang dinilai menggunakan ujian imunosorben berkaitan enzim (kit ELISA) dari Ogos 2020-Okt 2020. Tahap HEPC berkorelasi secara songsang dengan EPO dalam kedua-dua MTG ($r = -0.618$, $P < 0.00001$) dan NMTG ($r = -0.8243$, $P=0.000048$). Sebaliknya, HEPC berkorelasi positif dengan SF (MTG: $r = 0.7833$, $P < 0.00001$ dan NMTG: $r = 0.8587$, $P=0.000011$) dan LIC (MTG: $r = 0.4348$, $P= 0.000648$ dan NMTG8 = 0.000648 dan NMTG8 = 1. < 0.00001). Begitu juga, terdapat hubungan positif antara BMI dan Hb (MTG: $r = 0.2604$, $P= 0.0487$* dan NMTG: $r = 0.4898$, $P=0.0463$). Kebanyakan pesakit talasemia mengalami lebihan zat besi, mempunyai tahap EPO, HEPC, SF dan LIC yang lebih tinggi jika dibandingkan dengan pesakit bukan beban besi dengan nilai p (0.027458, 0.000339, 0.026304 dan 0.005644), masing-masing. Kajian semasa menunjukkan hubungan

songsang yang signifikan antara serum erythropoietin dan hepcidin manusia dalam pesakit talasemia berbanding populasi yang sihat. Pesakit talasemia dengan Hb rendah nampaknya mempunyai klasifikasi BMI yang kurang berat kerana hubungan positif antara BMI dan Hb. Pembolehubah utama (Hb, EPO, HEPC, SF, LIC dan Cardiac T2*) lebih berkemungkinan dikaitkan dengan fenotip talasemia berbanding pelbagai gen (rantai α dan β-globin); juga, pemindahan darah berganda dan terapi khelasi yang berbeza berkesan mempengaruhi nilai dan perkaitan pembolehubah tersebut, khususnya HEPC. Selain kelebihan zat besi yang dialami oleh hampir semua pesakit talasemia, sekumpulan tertentu pesakit kelebihan zat besi tersebut mengalami komplikasi yang berkaitan dengan beban zat besi.

Kata kunci: Erythropoietin serum, Hepcidin, Talasemia, Kumpulan Transfusi Bulanan, Kumpulan Transfusi Bukan Bulanan, ELISA, Hospital Ampang.

ACKNOWLEDGEMENT

Special appreciation to my husband" Bassim Al-Tameemi" and my exceptional cousin" Enas Mohammed" for their continuous support, and encouragement.

I express my sincere appreciation to the Assoc. Prof. Dr Bahariah Khalid for being the mentor for this research and the chairman of the supervisory committee.

Many thanks to Assoc. Prof. Dr Zainina Seman and Prof. Dr Jameela Sather for participating in this study. I would like to express my gratitude for all UPM staff, especially the staff of haematology lab for their unlimited help in this research.

I thank the nursing staff of the haematology and thalassemia clinics in Ampang Hospital for assisting in the recruitment of the patients. Special thanks with great respect must go to all thalassemia patients who were readily participated.

Eventually, I would like to thank God Allah for letting me through all the difficulties.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Alpha
B	Beta
Δ	Delta
Γ	Gamma
EPO	Erythropoietin
HEPC	Hepcidin
EndEPO	Endogenous Erythropoietin
BM	Bone Marrow
Hb	Hemoglobin
LIC	Liver Iron Concentration
MCV	Mean Corpuscular Volume
SCT	Stem Cell Transplantation
HSCT	Haemopoietic Stem Cell Transplantation
HSC	Haemopoietic Stem Cell
RBC	Red Blood Cell
CKD	Chronic Kidney Disease
MDS	Myelodysplastic Syndrome
rhEPO	Recombinant Human Erythropoietin
ELISA	Enzyme-Linked Immunosorbent Assay
TM	Thalassemia Major
TI	Thalassemia Intermedia
BMI	Body Mass Index
MTG	Monthly Transfused Group
NMTG	Non-Monthly Transfused Group
MRI	Magnetic Resonance Imaging
SF	Serum Ferritin

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

An autosomal recessive disorder that can happen due to insufficient amount of hemoglobin production or due to mutation (deletion, absence or reduction) in one or more of the globin chains; two modelled protein molecules in which α-globin chain combines equally to the β-globin chain to form the hemoglobin (Hb) (Agarwal, 2009).

Hemoglobin molecule comprises the heme group and the globin proteins which is functionally important to transport oxygen to lungs and other body tissues (Harewood & Azevedo, 2017). Generally, there are two common types of thalassemia, namely α-thalassemia and beta thalassemia, where each of them is affected by the type and number of transmitted genes. The imbalance of the globin chains causes an anemic condition that varies from an intermediate to a severe manner (clinically, thalassemia intermedia has lesser severity levels compared to thalassemia major).

However, α-thalassemia takes place due to deletion related to α-globin chain, while an absence or reduction of synthesis in the β-globin chain causes β-thalassemia. Moreover, each α-thalassemia or β-thalassemia has numerous forms/subtypes (Refer to Table 1.1) (Origa, 2019) (Akers et al., 2017).

Table 1.1: The genes review of α-thalassemia and β-thalassemia

Alpha Thalassemia (αα/αα)		**Beta Thalassemia (β/β)**	
Genotype	Phenotype	Genotype	Phenotype
- α/ αα	Silent Carrier	β/β+ or β/β0	Thalassemia trait
αα/− − or −α/−α	Thalassemia Minor or Thalassemia trait	β+/β0 or β+/β+	Thalassemia Intermedia, TI
−-/−α	HBH disease	β0/β0, β0/β+ or β+/β+	Thalassemia Major or Cooley's anemia
--/--	HB Bart's hydrops fetalis		

1.2 Problem Statement

Thalassemia is a genetic blood disorder characterized by a deficiency in the hemoglobin level or mutation of the genes (deletion). The prevalence of this disease has escalated on a count of the relative marriage in particular states of Malaysia. A recent study revealed that the state of Sabah in Peninsular Malaysia reported the highest rate of this disease which was prevalent among the Malay race group (Shafie et al., 2020).

The scope of this study was to determine the relationship of serum erythropoietin and serum hepcidin among differently transfused thalassemic patients where the transfusion believingly considered as an important factor in improving ineffective erythropoiesis, the consequent anemia and iron overload (main factor for morbidity and organ mortality in thalassemia patients that generates further complications, for instance, cardiac; endocrinal; gastroenterological and infectious diseases are the most common).

Seemingly, transfusion frequency would have an efficient role as a modulator in this relation/mechanism. However, so far there is an absence of studies reporting the correlation of these two variables (EPO and HEPC) in relation to blood transfusion frequency in differently phenotyped and genotyped thalassemia patients. Despite that, an innovative study on β-thalassemia had proven that hepcidin levels increase directly with the development (severity) of the disease (Eshagh Hossaini & Haeri, 2019).

1.3 Research Questions

1. What would the values of endogenous serum erythropoietin in thalassemia patients in case of genotypes and phenotypes be?
2. Is there any significant relationship between serum hepcidin levels among the Malaysian thalassemia patients relating to the genotypes and phenotypes?
3. What is the association of serum erythropoietin and human hepcidin in comparison with BMI, iron overload and anemia levels?
4. Is there any association between anthropometric status and AS awareness?
5. What are the patterns of iron overload that thalassemia patients experience comparatively to the transfusion frequency?

1.4 Research Objectives

1.4.1 General Objectives

Determination of serum endogenous erythropoietin (EndEPO) and hepcidin levels (HEPC) in Malaysian adult transfused thalassemic patients compared to normal population.

1.4.2 Specific Objectives

I. To determine the serum endogenous erythropoietin (EndEPO) levels in thalassemia patients in relation to the genotype and phenotypes.
II. To find the relationship between HEPC levels in thalassemia patients in relation to the genotype and phenotypes.
III. To determine the relationship of serum erythropoietin (EPO) and hepcidin (HEPC) to BMI, iron overload and the levels of anemia.
IV. To define the pattern of iron overload in relation to the frequency of transfusion.

1.5 Research Hypothesis

1.5.1 Hypothesis

There is an inverse (negative) relationship between erythropoietin and hepcidin in Malaysian adult transfused thalassemic patients.

1.5.2 Null Hypothesis

There is a direct (positive) relationship between erythropoietin and hepcidin in Malaysian adult transfused thalassemic patients.

1.6 Research Significance

Existing studies have not reported the association of serum erythropoietin and human hepcidin in adult patients with different transfused thalassemic while compared to healthy normal individuals in Malaysia. The only innovative study focused on ineffective erythropoiesis and iron among Chinese was reported in China. However, thalassemia disorder is considered one of the public health concerns due to the high prevalence rate particularly in the states of Sabah, Sarawak and Selangor where patients are at risk of developing additional complications related to iron overload which may be fatal to many infants with α-thalassemia.

For this reason, the requisite of this research was to create a new database for thalassemia patients to determine the causative factors as well as to promote discussion of its complications that patients experienced in Malaysian thalassaemic patients and healthy individuals. Secondly, to determine the relationship between serum erythropoietin and hepcidin in transfused thalassemia patients among the Malaysian groups.

1.7 The groups categorization according to blood transfusion frequency

Thalassemia patients are categorized into two groups according to transfusion frequencies. The first group was the monthly-transfused group (MTG) involving patients below the ages of 2 years when began blood transfusion and became blood dependent (once every month). The second group called the non-monthly transfused group (NMTG) comprised of patients who are occasionally blood dependent (once every 5 to 6 months) while commenced the blood transfusion after the age of 2-year-old, and blood independent.

1.8 Prevalence of thalassemia among Malaysian populations

This disease is considered one of the public health problems especially in the South Asia region and has seen to escalate over time (Hossain et al., 2017). In Malaysia, it represents a prevalent inherited disease where about 4.5% of the Malays and Chinese are carriers of the thalassemia trait (Tan et al., 2010).

According to the Malaysian Thalassemia Registry (2109) , β-thalassemia major and HbE/β thalassemia were the most common subgroups, made up of 33.52% and 34.37% of total participants, respectively, followed by patients inherited hemoglobin H (18.26%), while, β-thalassemia intermedia was less common (9.37%) adding to (4.48%) for other rare subtypes such as α-thalassaemia, HbH Constant Spring, Hb Adana, delta-beta thalassaemia, HbAE Bart's Constant Spring (Mohd Ibrahim, 2019).

1.9 The general definition of body mass index (BMI)

BMI is a well-known scale used to measure body fat or obesity according to versatile reference standards. It can also be utilized as an indicator for the prevalence of certain syndromes along with the development of many medical problems (Nuttall, 2015).

1.10 The importance of endogenous erythropoietin role among patients affected of thalassemia

It is a well-known fact that EPO has a prominent role in erythropoiesis to maintain the oxygen levels in the blood. In specific situation particularly in hypoxia manner (low levels of blood oxygen), the kidney will raise the production of the hormone to compensate the oxygen deficiency; thalassemia also augments the release of hormone intentionally to generate more mature RBCs in response to anemia condition which in turn depends on the severity of clinical symptoms of the same disease (Amer et al., 2010).

The majority of thalassemic patients experienced higher levels of erythropoietin regardless of either being blood transfusion-dependent or blood transfusion-independent. However, increased EPO concentration correlated with the severity of the illness in thalassemia major patients who exhibited elevated levels compared to thalassemia minors (Chaisiripoomkere et al., 1999).

1.11 The general conception of Hepcidin "iron master regulator"

To prevent the potential toxicity of iron that manifested by the iron homeostasis where there is no intelligible approach for excretion outside the human body, hepcidin with ferroportin at this stage are committed to regulating the quantity of iron entering the cell to the blood circulation (Wallace, 2016).

On top of that, there are multi-factors such as low oxygen levels in the blood (hypoxia); levels of iron stores; ineffective erythropoiesis and in response to inflammation, influence efficiently on serum/plasma hepcidin levels (Rishi et al., 2015).

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