جامعة عين شمس كلية العلوم قسم الحيوان

اسم الطالبة: هبه عثمان عبد الدايم

الدرجة العلمية: ماجيستير علم الحيوان

القسم التابع له: قسم الحيوان

اسم الكلية: كلية العلوم

الجامعة: جامعة عين شمس

سنة التخرج: ١٩٩٩م.

سنة المنح: ٢٠٠٩م.

موضوع الرسالة: تأثير ارتفاع نسبة الصفراء في الأطفال حديثي الولادة على سريان الدم وعلى غشاء كريات الدم الحمراء و الدهون المكونة له

جامعة عين شمس كلية العلوم قسم الحيوان

رسالة الماجيستير في العلوم

اسم الطالبة: هبه عثمان عبد الدايم عنوان الرسالة: تأثير ارتفاع نسبة الصفراء في الأطفال حديثي الولادة على سريان الدم وعلى غشاء كريات الدم الحمراء و الدهون المكونة له

لجنة الإشراف: ١- أ.د./ نهيسة حسين مكي أستاذ فسيولوجيا الحيوان - كلية العلوم جامعة عين شمس

٧- أ.د./ وائل رفعت مولس

أستاذ البثولوچيا الإكلينيكية- كلية الطب جامعة الأزهر

"-د./ نازك كامل سعفان أخصائية بمستشفى عين شمس الجامعي كلية الطب

لجنة الممتحنين:

تاريخ التسجيل: / / م.

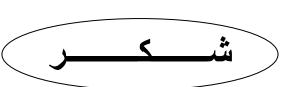
الدراسات العليا:

ختم الإجازة أجيزت الرسالة بتاريخ

مو افقة مجلس الجامعة

بتاريخ

جامعة نمين شمس كلية العلوم قسم الحيوان



أشكر السادة الأساتذة الذين قاموا بالإشراف وهم:

- اً .د./ نفيسة حسين مكي أستاذ فسيولوجيا الحيوان كلية العلوم الديران مكي جامعة عين شمس
- ٢ أ.د./ وائل رفعت حبلص أستاذ الباثولوچيا الإكلينيكية- كلية الطب جامعة الأزهر
- الجامعي عين شمس الجامعي د./ نازك كامل سعفان كلية الطب كلية الطب

وأشكر كل من ساهم في إخراج هذه الرسالة على هذا النحو



Acknowledgment

First of all, I wish to express my sincere thanks, great indebtedness and supreme gratitude to my great and kind **ALLAH....** without his wide mercifulness; I wouldn't be... without his kind guidance; I wouldn't do... without his generous gift; I wouldn't see or hear, know or learn.

I would like to express my deep gratitude and Thanks to **Prof. Dr. Nefissa H. Meky,** Professor of physiology, Zoology Dep. Faculty of science, Ain Shams University, for the great role she played in my life that helped me fulfill my dream (which was the master degree) by teaching me at college, she gaved me the first hope then by accepting me to register with her she makes my dream so close.

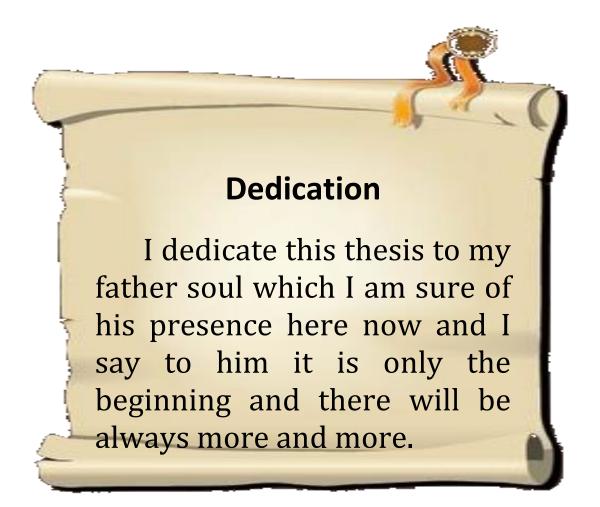
All the thanks and great gratitude to **Prof. Dr. Wael R. Hablas,** Professor of Clinical pathology Dep., Faculty of Medicine, Al- Azhar University, who by being in my practical life helps me to gain so much of knowledge. Also, by all his help and instructions I gained great experience and knew how to be a person with both knowledge and ethics together.

At last and not least to the one who shared by great part also for me being here now **Dr. Nazek Kamel Saafan**, Fellow in Ain Shams university hospitals, Clinical pathology Dep.,Ain shams university, because she with all love and sincierely she treated me as her daughter. I can never find any words to express my thanks to her but I hope I can one day pay it back to her.

Also, I would like to thank **Mrs. Magda Ades** because she was the first one who helped me during the first year of my university studies and I owe her a lot.

Last but not least, I would like to thank my mother, my sister and brothers and my close friends for their encouragement and unlimited support.

Heba Othman



ABSTRACT

Effects of Neonatal Hyperbilirubinaemia on Blood Rheology as well as Erythrytrocyte Membrane and Lipid Constituents

Key words Neonates, hyperbilirubinemia, jaundice, erythrocyte morphology, osmotic fragility-membrane lipids, membrane protein, deformability, glucose-6-phosphate dehydrogenase.

The current study aimed to evaluate the alterations of RBC morphology, osmotic fragility, RBCs deformability as well membrane protein and lipids composition due to unconjugated hyperbilirubinemia in neonates.

The results revealed that, hyperbilirubinemia in neonates produced haematological disorders manifested by a significant decrease in RBCs membrane cholesterol and phospholipids level, increased osmotic fragility, decreased deformability as well as morphological changes. The mechanism by which hyperbilirubinemia induced such disturbances might be due to the deposition of bilirubin in the membrane bilayer and disintegeration of the lipids with the loss of cholesterol and phospholipids for the membrane.

On the other hand, hyperbilirubinemia in neonates showed no significant change in RBCs count, haemoglobin content, hematocrit value, G6-PDH level nor in membrane protein composition.

List of Tables

Table No.	Table Title	Page No.
1	Composition of 15% resolving gel and 3.9% staking gel	80
2	Total plasma bilirubin (mg/dl) in different studied groups	87
3	Direct plasma bilirubin (mg/dl) in different studied groups	90
4	Indirect plasma bilirubin (mg/dl) in different studied groups	93
5	Effect of hyperbilirubinemia on RBCs count (x106/mm3) in different studied groups	96
6	Hemoglobin level (g/dL) in different studied groups	96
7	Hematocrit value (PCV%) in different studied groups	102
8	G6PD level (U/gHb) in different studied groups	105
9	Filteration rate (ml/min) of blood samples obtained from different studied groups through 8 µm pore diameter polycarbonate filters	108
10	Colesterol level of red cell membrane (mg/dL) in different studied groups	111
11	Phospholipids content of red cell membrane (mg/dL) in different studied groups	114

List of Figures

Figure No.	Figure title	Page No.
1	Pathway for the degradation of heme to bilirubin substituuent, M=methyl, P=Proprionic, V=Vinyl	6
2	Hepatic transport and conjugation of bilirubin	10
3	The metabolism of bilirubin	12
4	The fluid mosaic model of membrane structure	44
5	A Schematic Representation of Apparatus	71
6	Exploded view of Pop-Top Holder	72
7	APELEX gel electrophoresis system	81
8	Total plasma bilirubin (mg/dl) in different studied groups	88
9	Direct plasma bilirubin (mg/dl) in different studied groups	91
10	Indirect plasma bilirubin (mg/dl) in different studied groups	94
11	Effect of hyperbilirubinemia on RBCs count (x106/mm3) in different studied groups	97
12	Hemoglobin level (g/dL) in different studied groups	100
13	Hematocrit value (PCV%) in different studied groups	103
14	G6PD level (U/gHb) in different studied groups	106
15	Filteration rate (ml/min) of blood samples obtained from different studied groups through 8 µm pore diameter polycarbonate filters	109
16	Colesterol level of red cell membrane (mg/dL) in different studied groups	112
17	Phospholipids content of red cell membrane	115

Figure No.	Figure title	Page No.
	(mg/dL) in different studied groups	
18	Osmotic fragility of red cell membrane in different studied groups	116
19 A	Scanning electron micrographs of erythrocytes obtained from healthy neonatal group	118
19 B1	Scanning electron micrographs of erythrocytes obtained from jaundiced neonatal group I: with magnification of 4000	118
19BII	Scanning electron micrographs of erythrocytes obtained from jaundiced neonatal group I: with magnification of 6000	119
19BIII	Scanning electron micrographs of erythrocytes obtained from jaundiced neonatal group I: with magnification of 4000	119
20 A	without incubation with bilirubin, showing normal biconcave disc appearance	120
20 BI	After incubation with bilirubin (8 mg/dl) for 3 hours (echinocytosis) with magnification 6000	120
20 BII	After incubation with bilirubin (8 mg/dl) for 3 hours (echinocytosis) with magnification 5000	121
21	The electrophoresis bands show the presence of all membrane proteins	121

List of Abbreviation

ABO...... Blood group ADP Adinosine di phosphate ATP Adinosine triphosphate Bb Bilirubin Co Carbon monoxide G6PD.....Glucose-6-phosphate dehydrogenase Hb...... Hemoglobin HE Hereditary elliptocytsis HOs Heme oxygenase Ht Hematocrit IgG.....Immunogolubin G NADPH Nicotinamide adenine dinucleotide phosphate OFR Oxygen free radicals P Probability PC..... Phosphotidyl choline PE.....Phospatidyl ethanolamine PSA..... Periodic acid shift RBCs Red blood cells Rh.....Rhesus factor S..... Significant

Contents

	Page
Abstract	i
List of Table	ii
List of Figure	iii
List of Abbreviations	v
☐ Introduction	1
Aim of the Work	3
Review of Literature	4
Sources of bilirubin.	6
Bilirubin metabolism and excretion	9
Neonatal jaundice	13
The causes of hyperbilirubinemia	15
Cellular toxic effects of bilirubin	26
Neurotoxic Effecs of Bilirubin	30
Membrane biology of red blood cells	34
• Role of spectrin, Ankyrin in the shape and flexibility of the red blood cell	40
Lipid Organization	42
Red cell deformability	45
Role of cholesterol in deformability	48
Osmotic fragility	49

•	• Effect of bilirubin on membrane lipid	51
•	• Glucose-6-phosphate dehydrogenase	52
	Materials and Methods	60
	Results	85
	Discussion	122
	Summary	131
	References	136
	Arabic Summary	1-5

تأثير ارتفاع نسبة الصفراء في الأطفال حديثي الولادة على سريان الدم وعلى غشاء خلايا الدم الحمراء والدهون المكونة له

رسالــة

توطئة للحصول على درجة الماجستير في العلوم في فسيولوجيا علم الحيوان

> مقدمة من الطالبة / هبه عثمان عبد الدايم

تحت إشراف

الأستاذة الدكتورة / نفيسه حسين مكي أستاذ بقسم علم الحيوان كلية العلوم علم علم الحيوان جامعة عين شمس

الأستاذ الدكتور / وائل رفعت حبلص أستاذ الباثولوجيا الإكلينيكية كلية الطب كلية الطب جامعة الأزهر

الدكتور / نازك كامل سعفان أخصائية بمستشفى عين شمس الجامعي قسم البثولوچيا الإكلينيكية كلية الطب كلينيكية جامعة عين شمس

كلية العلوم جامعة عين شمس