

## DECLARATION

I hereby declare that this dissertation represents my own work. It has not previously been submitted for a degree, diploma or any other qualification at this or any other University.

Signed.....

Candidate: Grant Nombwende

Signed .....

Supervisor: Dr Lydia Korolova

Signed.....

Co – supervisor: Dr Trevor Kaile.

## ABSTRACT

**Background:** Diabetic nephropathy (DN) is a kidney disease that is a complication of diabetes. Pathogenesis of DN involves damage to the tiniest blood vessels which is followed by increased concentration of blood urea / creatinine and urine albumin excretion. DN is the leading cause of morbidity and mortality in patients with diabetes mellitus. Multiple factors and mechanisms such as interaction between hyperglycemia-induced metabolic and hemodynamic changes and genetic predisposition have been attributed to the development and outcomes of diabetic nephropathy. Diagnosis of DN in Zambia has been limited to the detection of elevated levels of creatinine, urea and urine albumin in the blood and urine respectively. We set out a comparative study to assess red blood cell distribution width (RDW) as a diagnostic marker of diabetic nephropathy in type 2 diabetes mellitus patients.

**Methods:** A Structured questionnaire was used to capture age, sex, history of blood transfusion and cancer status of the participants. Urea, creatinine and urine albumin concentrations were measured and RDW determined in 122 type 2 diabetes mellitus patients and 61 non diabetic participants. Renal profile tests (creatinine, urea and urine albumin) were used as a proxy marker for diabetic nephropathy in type 2 diabetes mellitus patients. Patients with high renal profile tests (urea > 8.3 mmol/l, creatinine > 120 $\mu$ mol/l, urine albumin > 30mg/l) were considered to have diabetic nephropathy. This study was approved by the University of Zambia Biomedical Research Ethics Committee (Assurance No.FWA00000338, IRB00001131 of IOR0000774) and the ministry of health in Zambia.

**Results:** The results revealed that mean creatinine concentration for type 2 diabetes mellitus patients ( $750 \pm 4.0 \mu\text{mol/l}$ ) was significantly higher than control participants ( $250 \pm 2.1 \mu\text{mol/l}$ ) t – value 5.00; P – value = 0.003. The mean urea concentration for type 2 diabetes mellitus patients ( $4.2 \pm 2.4 \text{ mmol/l}$ ) was significantly higher than control participants ( $2.2 \pm 1.5 \text{ mmol/l}$ ). t – Value = 8.26; p – value 0.002.

The mean urine albumin concentration for type 2 diabetes mellitus patients ( $12.4 \pm 3.3 \text{ mg/l}$ ) was higher than the control participants ( $12.2 \pm 2.9 \text{ mg/l}$ ) but the difference was not significant t – value 5.41; p – value 0.168. The mean RDW for type 2 diabetes mellitus patients ( $32.2 \pm 4.2 \%$ ) was significantly higher than the control participants ( $14.7 \pm 3.8 \%$ ). t – value 7.58; p – value 0.001. The diagnostic performance of RDW and renal profile tests (urea, creatinine and urine albumin; standard proxy) were compared based on sensitivity, specificity, positive predictive value (PPV), negative predictive (NPV) and test efficiency. RDW was found to have sensitivity of 93%, specificity of 96%, PPV 97%, NPV 91% and efficiency of 94% which were very significant parameters to warrant the inclusion of RDW as one of the diagnostic markers of diabetic nephropathy.

**Conclusion:** Using diagnostic sensitivity, specificity, PPV, NPV and efficiency, it was found that RDW was a reliable and suitable biomarker for detecting diabetic nephropathy in type 2 diabetes mellitus patients.

## **DEDICATION**

To my wife Ireen M. Nombwende and my daughters Leah and Dilnoza Nombwende, I owe it all to you.

## **ACKNOWLEDGEMENTS**

It is my sincere gratitude to acknowledge the invaluable contribution and support of my supervisors, Dr L. Korolova and Dr T. Kaile. Their guidance, advice, repeated corrections and revisions helped shape this project the way it is now. I also appreciate the contribution from the laboratory department at Kabwe General Hospital (KGH) and the University of Zambia post graduate forums. Further thanks go to Mrs. Bwalya for helping in translating the information sheet into Bemba Language. I also wish to thank Dr Ahmed, Dr Halwindi and Dr Nzala for their dedication and timeless help, finally thanks to many colleagues, Nurses, Doctors and laboratory staff that helped me to recruit patients and collect data.

To Mrs Elina Malama for her invaluable help and guidance in compiling and typing this dissertation.

To all my patients who kindly accepted to participate in this research.

## TABLE OF CONTENTS

CONTENT	PAGE
0.0 Declaration .....	i
0.1 Abstract .....	ii
0.2 Dedication .....	iii
0.3 Acknowledgements.....	iv
0.4 Table of contents .....	v
0.5 List of figures .....	x
0.6 List of tables.....	xi
0.7 List of abbreviations .....	xii
0.8 List of acronyms /definitions .....	xiii
0.9 Copyright.....	xiv
0.10 Supervisor's certificate.....	xv
0.11 Certificate of approval .....	xvi
<b>CHAPTER ONE</b>	
1.0. Background.....	1
1.1. Statement of the problem.....	2
1.2. Study justification.....	3
1.3. Research question.....	4
1.4. Null hypothesis .....	4
1.5 .General objective.....	4
1.6 .Specific objective.....	4
<b>CHAPTER TWO</b>	
2.0 . Literature review .....	5
2.1 Risk factors and mechanisms for diabetic nephropathy.....	6
2.2 Epidemiology.....	6
2.3 Pathogenesis .....	7

2.3.1 Glycosylation.....	7
2.3.2 Nitric Oxide changes in diabetic nephropathy .....	8
2.3.3 Cytokines.....	8
2.3.4 Oxidative stress.....	9
2.4 Routine blood test of kidney function.....	11
2.5 Pathology .....	11
2.6 Diagnoses .....	12
 <b>CHAPTER THREE</b>	
3.0. Research design and methodology.....	14
3.1 Study design .....	14
3.1.0 Selection of participants .....	14
3.2.0 Case definition .....	14
3.2.1 Inclusion criteria .....	14
3.2.2 Exclusion criteria .....	15
3.3.0 Research site .....	15
3.3.1 Study population .....	15
3.3.2 Target population.....	15
3.4 Ethical considerations.....	15
3.5 Study procedures.....	16
3.5.0 Sample size Calculation.....	16
3.5.1 Variables and indicators of measurements.....	17
3.6 Data Collection.....	19
3.6.1 Questionnaire and Laboratory request forms.....	19
3.6.2 Sample collection .....	19
3.6.3 Laboratory Tests.....	20
3.6.4 Hematology laboratory Tests.....	20

3.6.5 Red Blood cell Distribution Width Estimation.....	20
3.6.6 Principle of the Test .....	21
3.6.7 Procedure.....	21
3.6.8 Chemistry Laboratory Tests.....	21
3.6.9 Blood Glucose Estimation.....	21
3.6.10 Principle of the Test .....	22
3.6.11 procedure.....	22
3.6.12 Blood Urea Estimation.....	22
3.6.13 Test principle.....	22
3.6.14 Test procedure.....	22
3.6.15 Creatinine estimation.....	22
3.6.16 Procedure .....	23
3.6.17 Calculation.....	23
3.6.18 Urine Albumin Estimation .....	23
3.6.19 Principle of the test .....	23
3.6.20 Interpretation .....	24
3.7 Sex and age distribution of participants .....	24
3.7.1 Determination of the association between red blood cell distribution width and diabetic nephropathy .....	24
3.7.2 RDW levels in male and female patients with DN and the control participants .....	24
3.7.3 Determining the association between age and raised RDW in patients with DN .....	24
3.7.4 Assessment of RDW as a biomarker of DN in type2 diabetes mellitus.....	25
3.7.5 Suitability of RDW as a marker of DN .....	25
3.8 Data management and Statistics.....	25
<b>CHAPTER FOUR</b>	
4.0 Results .....	27
4.1 Presentation of Data and Data analysis .....	27

4.2 Assessment of RDW as a biomarker of DN in patients with type2 diabetes mellitus ...	32
4.3: Suitability of RDW as a marker for diabetic nephropathy in diabetes mellitus patients using renal profiles (Creatinine, Urea and Urine albumin) as a standard .....	33

## CHAPTER FIVE

5.0 Discussion of findings .....	35
5.1 Assessment of RDW as a biomarker of DN in patients with type2 diabetes mellitus...	35
5.2 Age and sex distribution of participants. ....	35
5.3 Association between RDW and DN in patients with type2 diabetic mellitus .....	35
5.4 Comparison of mean high RDW between male and female participants .....	36
5.5 Comparison of mean renal profiles in patients with diabetic nephropathy and control Subjects. ....	36
5.6 Assessment of RDW as a biomarker of DN in type 2 diabetes mellitus patients .....	36
5.7 Suitability of RDW as a marker for diabetic nephropathy in type 2 diabetes mellitus patients .....	38

## CHAPTER SIX

6.0 Conclusion.....	40
6.1 Study limitations.....	41
6.2 Recommendations.....	42
References .....	43
Appendix 1: Information sheet (English) .....	52
Appendix 2: Information sheet (Bemba translation).....	55
Appendix 3: Consent form .....	57
Appendix 4: Consent form (Bemba translation) .....	58



Appendix 5: Questionnaire .....	60
Appendix 6: Laboratory data collection forms .....	63
Appendix 7: Permission letters.....	65
Appendix 8: Supervisors Curriculum Vitae .....	69
Appendix 9: Glucose estimation .....	72
Appendix 10: Creatinine estimation .....	75
Appendix 11: Urea estimation.....	79
Appendix 12: Red blood cell distribution width (RDW) .....	83

## LIST OF FIGURES

Figure.2.1 Schematic representation of Nitric Oxide changes .....	8
Figure.2.2 scheme of pathogenesis of diabetic nephropathy.....	10
Figure. 3.4 determination of albumin in urine .....	23
Figure.4.1 Presentation of Data and Data analysis.....	27
Figure.4.2 Age distribution of respondents .....	28
Figure. 4.3 association between RDW and DN .....	29
Figure.4.4 Comparison of mean raised RDW between male and female patients with diabetic nephropathy.....	30
Figure.4.5 Association between age and raised RDW in patients with DN .....	31

## LIST OF TABLES

Table.3. 1: Study Variables and their cut off points.....	18
Table 4.1 renal profiles in patients with diabetic nephropathy and control subjects Independent T – test parameters.....	32
Table. 4.2 Comparison of renal profile results and RDW test results in patients with diabetic nephropathy.....	33
Table.4.3 Reliability of RDW as a biomarker of Diabetic nephropathy .....	34

## LIST OF ABBREVIATIONS:

ACE - Angiotensin converting enzyme  
ADP - Adenosine diphosphate  
AGE - advanced glycosylation end product  
ATP - Adenosine triphosphate  
BMI - body mass index  
DN - diabetic nephropathy  
EDTA - ethylene diamine tetra acetic acid  
ESRD - End stage renal disease  
FBC - full blood count  
FBS - fasting blood sugar  
KGH - Kabwe General Hospital  
MAPK - mitogen activated protein kinases  
MCH - mean cell haemoglobin  
MCHC - mean cell haemoglobin concentration  
NO - Nitric oxide  
OPD - Outpatient department  
PKC - Protein Kinase C  
RBC - red blood cell  
RDW - red blood cell distribution width  
ROS - Reactive oxygen species  
TGF - Tumor growth factor  
UAE - Urine albumin excretion  
USA - United States of America  
VEGF - Vascular epidermal growth factor

## LIST OF ACRONYMS / DEFINITIONS

1. **Acute:** is a disease with either or both of:
  - (a) A rapid onset, as in acute infection
  - (b) A short course as opposed to a chronic course
2. **Anemia:** is a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood.
3. **Epidemiology:** is the study (or the science of the study) of the patterns, causes, and effects of health and disease conditions in defined populations.
4. **Haemoglobin:** a conjugated protein, consisting of haem and the protein globin, that gives red blood cells their characteristic colour. It combines reversibly with oxygen and is thus very important in the transportation of oxygen to tissues
5. **Hemolysis:** (or **haemolysis**)—from the Greek αἷμα (aima, haema, hemo-) meaning "blood" and λύσις (lusis, lysis, -lysis) meaning a "loosing", "setting free" or "releasing" is the rupturing of erythrocytes (red blood cells) and the release of their contents (hemoglobin) into surrounding fluid (e.g., blood plasma).
6. **Nephropathy:** disease of the kidneys.
7. **Diabetic nephropathy:** the nephropathy seen in later stages of diabetes mellitus, with first hyperfiltration, renal hypertrophy, microalbuminuria, and hypertension, and later proteinuria and end-stage renal disease.
8. **Pathophysiology:** is the study of the changes of normal mechanical, physiological, and biochemical functions, either caused by a disease, or resulting from an abnormal syndrome
9. **Prognosis:** is a prediction of the chance of recovery or survival from a disease.
10. **Microvascular complications:** diseases affecting tiny blood vessels.
11. **Proteinuria:** an excess of serum proteins in the urine, as in renal disease or after strenuous exercise.

## **COPYRIGHT**

All rights reserved. No part of this dissertation may be reproduced, stored in any means, electronic, mechanical, photocopying, recorded or otherwise, without prior written permission from the author or the University of Zambia.

## SUPERVISOR'S CERTIFICATE

The dissertation of Grant Nombwende is ready for examination.

Supervisor: .....

Signature

Dr Lydia Korolova

Department of pathology and microbiology

School of Medicine

University of Zambia

Co – supervisor: .....

Signature

Dr Trevor Kaile

Head of Department - Department of pathology and microbiology

School of Medicine

University of Zambia

**CERTIFICATE OF APPROVAL.**

This study dissertation entitled **ASSESSMENT OF RED BLOOD CELL DISTRIBUTION WIDTH AS A BIOMARKER OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETES MELLITUS PATIENTS REVIEWED AT KABWE GENERAL HOSPITAL** by Grant Nombwende has been approved as partial fulfillment of the requirements for the award of the degree of Master of Science in Pathology (Hematology) by the University of Zambia.

Head of department: Name .....

Date: .....

Signature .....

Examiner 1: Name: .....

Date: .....

Signature .....

Examiner 2: Name: .....

Date: .....

Signature .....