BETA THALASSAEMIA AS A CORRELATE OF METABOLIC SYNDROME, TYPE 2 DIABETES MELLITUS AND SOME ENDOCRINE DYSFUNCTIONS

Investigators

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What is Beta Thalassaemia?

- Beta thalassaemia (BT) is an abnormality of haemoglobin synthesis
- There is reduced production of the beta globin chain and excess production of alpha chain
- The excess chain precipitates in the erythroid cells resulting in haemolysis and then anaemia
- The hyperhaemolysis results in iron accumulation in the body and its deposition in organs

ENDOCRINE COMPLICATIONS IN BETA THALASSAEMIA TRAIT

- Iron overload is central to most complications seen in BTT
- Iron is deposited in major organs of the body like the liver, heart, thyroid, pancreas, parathyroid
- This may result in endocrine dysfunctions like DM, hypothyroidism, hypoparathyroidism, stunted growth, delayed puberty and sexual dysfunction

Metabolic Syndrome (MS)

- This is a cluster of metabolic abnormalities which include the following
  - Insulin resistance
  - Central obesity
  - Dysglycaemia
  - Atherogenic lipidaemia
  - Systemic hypertension
  - Oxidative stress
  - Proinflammatory and prothrombotic states

Diabetes Mellitus

- This is an endocrine dysfunction in which there is a failure of glucose metabolism
- Type 2 Diabetes Mellitus results mostly from insulin resistance (Alberti et al, 1990)
- Type 2 DM accounts for over 90% of all cases of DM (Alberti et al, 1990)
- About 46% of individuals with type 2 diabetes mellitus have metabolic syndrome (Ashraf-Sohail et al, 2006).

The Overlap between BTT, MS and DM

- Insulin resistance is the underlying key factor in the pathogenesis of the three disorders
- Systemic hypertension is clearly associated with these disorders
- The observation of atherogenic dyslipidaemia in all three conditions is unlikely to be due to a chance finding
- Proinflammatory, prothrombotic and oxidative stress are equally common findings in the disorders

Epidemiology of the Three Disorders

- It is recently that Beta thalassaemia trait was found to be as high as 26% in the Nigerian population (Kotila et al,2009)
- Recent studies have also shown the presence of MS in 33% of Nigerians (Charles-Davies et al, 2013)
- Though DM affects 18.9% of Nigerians, MS is seen in 46% of patients with DM (Ashraf-Sohail et al, 2006; Nwafor &Owhoji, 2001)

AIM and Objectives of this study

AIM

- The main objective is to investigate the possible association between BTT, MS and some endocrine dysfunctions

Specific Objectives

- Find the prevalence of BTT in individuals with and without DM
- Identify MS in individuals with and without DM
- To evaluate measures of iron stores, insulin resistance & dyslipidaemia, adiposity and other components of the MS in individuals with and without DM
- To determine indices of inflammation, oxidative stress and some endocrine dysfunctions in the individuals with and without type 2 DM.

Methods

PARTICIPANTS

- 100 patients with type 2 DM who are age and sex matched with 100 individuals without DM
- BTT will be diagnosed in all the study population using elevated HbA2, HbF and low red cell indices (MCH, MCV)
- MS will be identified in the study population using the IDF criteria
- Presence of endocrine complications like hypothyroidism, hypoparathyroidism and sexual dysfunction will be documented in both patient and control groups

Methods II

- Ethical approval has been obtained from the UI/UCH Ethical Review Board
- Anthropometric measurements and demographic, family, health, lifestyle, nutritional data will be obtained through a questionnaire.
- Ten millilitres of fasting blood will be collected and distributed into EDTA, fluoride oxalate and plain bottles
- This will be spun at 500g to extract serum/plasma which will be stored frozen (-20^{o}C) in small aliquots till analysis
- DNA samples will obtained from the white blood cells and stored at 4^{o}C for future genetic analysis

Methods III

- HbA2, HbF and red cell indices will be analysed using HPLC
- Hormonal assays, inflammatory markers and antioxidant status will be determined by ELISA.
- Other biochemical parameters will be analysed by standard methods

Statistical Methods

- Numerical data will be presented as mean ± SD.
- Analysis of variance, Post Hoc and Student's t-test will also be used for the comparison of variables.
- Chi-squared test will be used for non-parametric tests.
- Pearson's correlation coefficient and Spearman's correlation coefficient will be employed to explore relationships between quantitative and non-quantitative variables respectively.
- Two-tailed independent t-test of significance, at 95% confidence limit, p-value less than 0.05 ($p<0.05$) will be considered significant for the variables.

Budget

	Year 1	Year 2	Year 3
HbF, HbA2,red cell indices	160,000	800,000	-
Diagnosis of DM	106,500	200,000	-
Lipid Profile	100,000	482,300	120,000
Hormonal assays	215,050	1,300,000	1,000,000
Markers of inflammation, oxidative stress	323,350	80,000	1,000,000
Iron Homeostasis	200,000	600,000	52,700
Equipment	2,412,000	-	-
Personnel, stationeries &Others	220,000	253,550	160,000
TOTAL	3,736,900	3,715,850	2,332,700
Grand Total			9,785,450

Budget Justification

- The major equipment to be purchased is an ELISA reader with automatic washer
- Though an HPLC for Haemoglobin analysis is desired but the cost will be prohibitive
- Samples will be analysed and paid for at the IAMRAT
- The two graduate assistants will receive stipends from the grant

Project Implementation plan

PROJECT DURATION: 3 YEARS

Year 1

1-3months All Investigators

- Collation of study materials, purchase of consumables (for sample collection) and kits for initial laboratory analyses.
- Training of the graduate assistants and other supportive staff on the procedures of the project

4-12 months All Investigators

- Recruitment of participants, collection and storage of appropriate samples, and analyses of initial laboratory tests.
- Purchase of equipment, data entry, statistical analyses of initial data and preparation of first year report

Project Implementation plan (2)

Year 2

1-6months All Investigators

- Recruitment of study participants, purchase of reagents and analyses of other biochemical analyses and data entry

7-12 months All Investigators

- Completion of recruitment of study participants, biochemical analyses, continuation of data entry.
- Preparation of second year report and presentation and publication of findings.

Project Implementation plan (3)

Year 3

1-6months All Investigators

- Completion of biochemical analysis, data analyses and interpretation of results

7-12 months All Investigators

- Writing of final report and presentation/publication of major findings.

Possible Impact of The Study

- Create awareness of the high prevalence of BTT
- Early detection of associated endocrine complications of BTT
- May help to predict people who are likely to develop DM
- May help to predict those likely to be resistant to treatment of type 2 DM
- Results emerging will help in the development of strategies to prevent MS and its progression to DM
- The study will involve the training and mentoring of junior Faculty and Postgraduate students in line with the Mission of the University of Ibadan, Nigeria

References

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2. Charles-Davies M.A., Fasanmade A.A., Olaniyi J.A., Oyewole O.E., Owolabi M.O., Adebusuyi J.R., Hassan O., Ajobo M.T., Ebesunun M.O., Adigun K., Akinlade K.S., Fabian U.A., Popoola O.O., Rahamon S.K., Okunbolade W., Ogunlakin M.A., Arinola O.G. , Agbedana E.O. (2013) Metabolic Alterations in Different Stages of Hypertension in an Apparently Healthy Nigerian Population. International Journal of Hypertension. In press (manuscript no. 351357 accepted on 22/10/2013).
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UI/UCH EC Registration Number: **NIHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: β-Thalassaemia and its Biochemical Indices in Individuals with Metabolic Syndrome and Type 2 Diabetes Mellitus

UI/UCH Ethics Committee assigned number: UI/EC/11/0046

Name of Principal Investigator: **Matthew A. Ogunlakin**

Address of Principal Investigator: Department of Chemical Pathology,
College of Medicine,
University of Ibadan, Ibadan

Date of receipt of valid application: 09/03/2011

Date of meeting when final determination on ethical approval was made: **16/06/2011**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from 16/06/2011 to 15/06/2012. If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC early in order to obtain renewal of your approval to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

UI/UCH EC
16 JUN 2011
APPROVED
COLLEGE OF MEDICINE

Dr. J. A. Otegbayo
Chairman, Medical Advisory Committee,
University College Hospital, Ibadan, Nigeria
Vice- Chairman, UI/UCH Ethics Committee
E-mail:uiuchirc@yahoo.com

Sample Size Calculation

- According to Akinkugbe (1997) prevalence of DM is 2.7%
- Sample size $S = Pq(Z\alpha)^2/e^2$
- P=prevalence=2.7/100=0.027
- Q=1-p=1-0.027=0.973
- Zα=standard deviation=1.96
- E=Margin of error of 5%=5/100=0.05
- $S=0.027x0.973x(1.96)^2/(0.05)^2$
- 40.36=40

BTT

- HbA2 ≥ 3.5%
- Hbf ≥ 1.0%
- MCH > 27pg
- MCV > 80fl

IDF CRITERIA

- Abdominal Obesity; WC≥94cm male; WC ≥ 80cm PLUS any two of the following

1. Hypertriglyceridemia: (plasma Tg > 150mg/dl)
2. Reduced Plasma HDLC-C<40mg/dl male; HDLC-C< 50mg/dl for female.
3. Blood pressure ≥ 130/85mmHg and high fasting glucose ≥ 100mg/dl

TYPE 2 DM

WHO recommendation 2011

1. HbA1C 48mmol/mol (6.5%) is recommended as the cutoff DM.

   Polyuria

   Polydipsia and unexplained weight loss

   +

   A random of blood glucose concentration of ≥ 11.1mmol/l

   or

   A fasting plasma glucose concentration of ≥ 7.0mmol/l or

   2hrs plasma glucose concentration of > 11.1mmol/l after 75grammes of anhydrous glucose in an oral glucose tolerance test


University of Ibadan
Our vision

Our mission

1. To expand the frontiers of knowledge through provision of excellent conditions for learning and research.
2. To produce graduates who are worthy in character and world judgement.
3. To contribute to the transformation of society through creativity and innovation.
4. To serve as a dynamic custodian of society's military values and thus sustain its identity.

UNIVERSITY OF IBADAN

VISION

To be a world-class institution for academic excellence geared towards meeting societal needs.

MISSION

- To expand the frontiers of knowledge through provision of excellent conditions for learning and research
- To produce graduates who are worthy in character and sound judgement.
- To contribute to the transformation of society through creativity and innovation.
- To serve as a dynamic custodian of society's salutary values and thus sustain its integrity.

THANK YOU