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EVALUATION OF C3 COMPLEMENT AND INFLAMMATORY MARKER (C-REACTIVE PROTEIN) IN TYPE 2 DIABETES SUBJECTS WITH INSULIN RESISTANCE

Iruegbukpe F.*[1], Davis T. & Ibegbulem E. O.

Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

***Corresponding Author: Iruegbukpe F.**
Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.

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ABSTRACT
Type 2 Diabetes can be caused by Insulin Resistance, a condition in which cells fail to respond to insulin properly and can progressively leads to lack of insulin. Insulin Resistance is directly interlinked with various inflammatory responses. The significance of this study is to evaluate C3 Complement and Inflammatory marker (CRP) in Type 2 Diabetes mellitus with insulin resistance in Yenagoa, Bayelsa state. 220 subjects were recruited into the study work, 100 constitute T2Dm with insulin resistance, 60 T2Dm with no insulin resistance while the remaining 60 were normoglycemic with no insulin resistance. Insulin Resistance was assessed for using anthropometric measurements (hypertensive, hyperglycemia, hypertriglyceridemia, hypercholesterolemia and TG/HDL ratio>2). Result of the analysis showed a significant difference ($p<0.05$) in C3 Complement and CRP values obtained in the studied population. The mean±SD of complement C3 in T2DM with insulin resistance was (235.01 ± 52.67) mg/dl and was (214.82 ± 51.68) mg/dlin T2DM with no IR, while the control gave (160.98 ± 47.89) mg/dl respectively. When compared between groups, there was no significant difference observed. Hs-CRP in T2DM with IR gave a mean ± SD of (5.97 ± 2.89) mg/l, T2DM with no IR gave (2.57 ± 1.77^{a}) mg/l while the control gave a mean ± SD of (4.59 ± 2.70^{ab}) mg/l respectively. When compared between groups, there was a significant difference observed. Sex and age showed no significant difference in the studied biochemical parameters. Correlation between C3 Complement and Hs-CRP showed a significant difference. Conclusively, this study contributes to knowledge and evidences that Hs-CRP and C3 can be assayed as routine test in individuals suspected of having Insulin Resistance because they tend to have a strong association with T2Dm.

KEY WORDS: c3 complement, inflammatory marker, C - reactive protein, type 2 diabetes, insulin resistance.

INTRODUCTION
Diabetes mellitus (DM) is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period (WHO, 2014). Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications (WHO, 2013). Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death (Kitabchi *et al*., 2009). Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, and damage to the eyes.

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced (Shoback *et al*., 2011). As of 2015, an estimated 415 million people had diabetes worldwide (IDF, 2014), with type 2 DM making up about 90% of the cases (Shi *et al*., 2014). This represents 8.3% of the adult population (Shi *et al*., 2014), with equal rates in both women and men (Vos *et al*., 2012). As of 2014, trends suggested the rate would continue to rise (International Diabetics Association, 2014). Diabetes at least doubles a person's risk of early death (DFS). From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes. The global economic cost of diabetes in 2014 was estimated to be US$612 billion (IDF, 2014). In the United States, diabetes cost $245 billion in 2012 (ADA, 2013).

Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight, and avoiding use of tobacco. Control of blood pressure and maintaining proper foot care are important for people with the disease. Type 1 DM must be managed with insulin injections (DFS). Type 2 DM may be treated with medications with or without insulin (WHO, 2013). Insulin and some oral medications can cause low blood sugar (Rippe *et al*., 2010). Weight loss in those with obesity is sometimes an effective measure in those with

type 2 DM (Picot *et al*., 2009). Gestational diabetes usually resolves after the birth of the baby (Cash, 2014).

Insulin is a hormone made in the pancreas, an organ located behind the stomach. The pancreas contains clusters of cells called islets. Beta cells within the islets make insulin and release it into the blood. Insulin plays a major role in metabolism—helps muscle, fat, and liver cells absorb glucose from the bloodstream, lowering blood glucose levels. It also stimulates the liver and muscle tissue to store excess glucose. Insulin resistance (IR) is one of the major hallmarks for pathogenesis and etiology of type 2 diabetes mellitus (T2DM). IR is directly interlinked with various inflammatory responses which play crucial role in the development of IR. Inflammatory responses play a crucial role in the pathogenesis and development of IR which is one of the main causative factors for the etiology of T2DM.

In insulin resistance, muscle, fat, and liver cells do not respond properly to insulin and thus cannot easily absorb glucose from the bloodstream. As a result, the body needs higher levels of insulin to help glucose enter cells. The beta cells in the pancreas try to keep up with this increased demand for insulin by producing more. As long as the beta cells are able to produce enough insulin to overcome the insulin resistance, blood glucose levels stay in the healthy range.Over time, insulin resistance can lead to type 2 diabetes and prediabetes because the beta cells fail to keep up with the body's increased need for insulin. Without enough insulin, excess glucose builds up in the bloodstream, leading to diabetes, prediabetes, and other serious health disorders. The pancreas contains clusters of cells called islets. Beta cells within the islets make insulin and release it into the blood. Although the exact causes of insulin resistance are not completely understood, scientists think the major contributors to insulin resistance are excess weight and physical inactivity. Other causes of insulin resistance may include ethnicity, certain diseases, hormones, steroid use, some medications, older age, sleep problems, especially sleep apnea, and cigarette smoking.

Complement C3 is produced mainly by the liver (Alper *et al*., 1969), but other production sites, such as adipose tissue, may also contribute to systemic C3 levels (Choy *et al*., 1992). C3 is the central component of the complement system, and activation via any of the three major complement pathways results in cleavage of C3 into C3a and C3b and subsequent activation of the terminal complement pathway with concurrent formation of C5a and C5b-C9 (also known as the membrane attack complex) (Walport, 2001). Both the anaphylatoxins C3a and C5a, by acting on their respective receptors, and the (sublytic) membrane attack complex have been shown to induce inflammatory responses (Asgari *et al*., 2013, Laudisi *et al*., 2013).

Impairment of immune and inflammatory homeostasis is thought to cause type 2 diabetes mellitus (T2DM) through affecting insulin resistance (IR) and β-cell function (Shu *et al*., 2012, Sell *et al*., 2012). Complement, C3 is a β-2-protein that constitutes the central component of the complement's classical and alternative pathway. It also acts as an acute phase protein, thus increased levels in serum are related with acute inflammatory diseases. Low levels in serum are found in autoimmune diseases, glomerulonephritis, genetic deficiencies, etc.C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex (Thompson *et al*., 1999). CRP binds to phosphocholine on micro-organisms. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin-mediated phagocytosis), which express a receptor for CRP. It plays a role in innate immunity as an early defense system against infections.

Recent research suggests that patients with elevated basal levels of CRP are at an increased risk of diabetes, (Pradhan *et al*., 2001, Dehghan *et al*., 2007) hypertension and cardiovascular disease. CRP has been considered as one of the most important human acute phase protein that correlates with development of IR (Shi *et al*., 2014). CRP is a systemic inflammatory biomarker and has been considered as one of the major causative factor for the development of T2DM (Rippe *et al*., 2010). It has been evidenced that elevated levels of CRP not only reflect the induction of local inflammation, but also predict the pathogenesis of tissue-specific IR (Picot *et al*., 2014). Several studies have found that strong relationship exists between levels of CRP and development of IR (Vos *et al*., 2012) which indicates that besides other pro-inflammatory mediators, CRP also actively plays its pivotal role for the pathogenesis of IR by inducing local and/or systemic inflammation. The aim of this study is therefore to evaluate C3 and CRP in type2 Diabetes subjects with insulin resistance.

MATERIALS AND METHODS

Study Area

The study willbe conducted at the Federal Medical Centre Yenagoa, Bayelsa State. The hospital is the foremost tertiary health institution as well as a referral centre. Bayelsa state is located within Latitude 4^0 15^1 North and Latitude 5^0 and 23^1 South (Alagoa, 2009). It is also within longitude 5^0 22^1 West and 6^0 45^1 East. It is bounded by Delta State on the North, Rivers State on the East and the Atlantic Ocean on the Western and Southern parts. Bayelsa State has the largest wet land in West Africa and the first State crude oil was struck in commercial quantity. According to the 2006 census

figures, Bayelsa has a population of about 1.7 million people (Alagoa, 2009).

Study Population

The sample size for the study was determined using the formula of Araoye, 2003. A total number of 220 subjects were recruited into the study. 100 constituted type 2 diabetes group with TG/HDL ratio greater than 2, representing insulin resistance, 60 subjects constituted type 2 diabetes with TG/HDL ratio less than 2, which represented no insulin resistance (positive control), while 60 constituted the control group (normal subjects with no diabetes and no insulin resistance). Insulin resistance was extrapolated from Lipid profile, specifically Triglyceride (TG) and High Density Lipoprotein (HDL) ratio. TG/HDL ratio ≥ 2 depicted T2DM with IR while TG/HDL ‹ 2 depicted T2DM without IR (ADA, 2013).

Ethical Approval

The ethical clearance for the study was sort and obtained from the Ethical Committee of the Federal Medical Centre, Yenagoa by obtaining an application form which was duly filled by me and submitted with a copy of my study prop osal. Informed consent of individual participants was sort verbally and the importance of the work was pointed out to them and also how it would benefit the general populace.

Selection Criteria

a) Inclusion

- Subjects with established case of diabetes mellitus (as defined by World Health Organization, that is, fasting plasma glucose ≥ 7mmol/l).
- Subjects with TG/HDL stratification greater than 2 were recruited. That is, they have insulin resistance.
- The control group comprised of subjects with no diabetes and insulin resistance, while positive control were those with Type 2 Diabetes with TG/HDL stratification less than 2, that is, no insulin resistance.

b) Exclusion

- Subjects with history of surgeries and those that have received radiotherapy.
- HIV- positive individual.
- Patients under long term local and systemic drug therapy except(oral hypoglycemics and insulin).

Sample Collection

Purposive and Randomized sampling method was used in this study. Under aseptic conditions 10mls of patient's intravenous blood wasobtained from median cubital vein of the forearm into a plain, K3-EDTA and fluoride oxalate containers respectively. Blood inK3-EDTA container was used for HbA1c analysis as confirmatory test for T2DmSerum resulting from the plain container was used for analysis of Complement (C3), C- reactive protein (CRP), and Lipid profile assay while fasting blood glucose was collected into the fluoride oxalate container. Serum of both the control and experimental groups was utilized for the study.

The sampleswill be analyzed for complement (C3) and Hs-CRP.

Laboratory Procedures

All reagents were commercially purchased and manufacturers Standard Operating Procedures was strictly adhered to. Good laboratory practice ensuring the use of appropriate specimen, storage temperature and appropriate sample identification was also taken into strong consideration.

Determinations Complement (C3)

The C3 assay is a turbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to C3 is added to the sample. Sample containing C3 is incubated with a buffer and a sample blank determination is performed prior to the addition of C3 antibody. In the presence of an appropriate antibody in excess, the C3 concentration is measured as a function of turbidity.

Reagents and procedure for other parameters can be gotten from.

Determination of Hs-CRP

The hs-CRP ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the CRP molecule. This mouse monoclonal anti-CRP antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-CRP antibody is in the antibody enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the CRP molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 minutes incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A tetramethylbenzidine (TMB) reagent is added and incubated for 20minutes, resulting in the development of blue colour. The colour development is stopped with the addition of 1N HCl changing the colour to yellow. The concentration of CRP is directly proportional to the colour intensity of the test sample. Absorbance is measured spectrophotometrically at 450nm.

The procedure and other details for CRP estimation can be found in Appendix 2 along with lipid profile assay.

Statistical Analysis

The data generated from the research work wasanalyzed with SPSS program (SPSS Inc., Chicago, IL, USA; Version 15) and expressed as mean ±SD. ANOVA, Student t-test, correlation and regression analysis was used for comparing values of the measured parameters between experimental and control groups.

Calibration and Quality Control

The reagents and equipment were calibrated appropriately. Standards were provided in the kit.

Randox Level 2 multisera control (Lot No: 461SN) was used with each batch and the values obtained was within the recommended range.

Protein control serum level I (Cod. 31211) and II (Cod. 31212) were used as controls for C3 determinations. Values gotten were within range.

RESULTS

The information about the subjects investigated was shown in Tables 4.1, 4.2, 4.3&4.4. Among the 220 subjects investigated, 100 were hyperglycaemic and have their TG/HDL ratio greater than 2 which represents T2DM with insulin resistance, 60 were hyperglycaemic but their TG/HDL ratio was less than 2 which represents T2DM with no insulin resistance (this serves as positive control) while the remaining 60 serve as negative control with normoglycemic and normal lipid profile results. The mean±SD of complement C3 in T2DM with insulin resistance was (235.01 ± 52.67)mg/dl and was (214.82 ± 51.68) mg/dl in T2DM with no IR, while the mean ±SD of the control was (160.98 ± 47.89) mg/dl respectively. When compared between groups, there was no significant difference observed between T2DM with IR and T2DM with no IR.

Hs-CRP in T2DM with IR gave a mean ±SD of (5.97 ± 2.89)mg/l , T2DM with no IR gave (2.57 ± 1.77[a])mg/l while the control gave a mean ±SD of (4.59 ± 2.70[ab])mg/l respectively. When compared between groups, there was a significant difference between T2DM with IR, T2DM with no IR and control.

Table 4.2 compared the Male and Female subjects of the studied group, there was no significant difference for Complement C3 subjects but a significant difference was observed for CRP in T2DM with IR compared to those with no IR. The mean ± SD of males for C3 was (238.79 ± 48.29) mg/dl for T2DM with IR, and (182.90 ± 44.03)mg/dl for T2DM with no IR while CRP had mean ± SD of (5.79 ± 2.63) and (2.87 ± 1.88) for T2DM with IR and T2DM with no IR respectively. The mean ± SD of females for C3 was (237.39 ± 55.53), (189.80 ± 47.46) and CRP was (6.10 ± 3.08), (2.30 ± 1.66) for T2DM with IR and T2DM with no IR respectively.

Table 4.3 shows the comparison of studied biochemical parameters according to age ranging from between 30 years to 89 years. There was no significant difference observed showing that age is not a determining factor for IR but physical inactivity and obesity is.

Table 4.4 shows the correlation between the studied biochemical parameters (C3 and Hs-CRP) in the studied population. There was a strong positive correlation in subjects that have IR based on Triglyceride and HDL stratification ($p<0.05$) but no correlation in those that have their TG/HDL ratio less than 2 while the control subjects showed a negative correlation.

Table 4.1: Comparison of the studied Biochemical Parameters with respect to TG stratification using simple indices.

	Hs- CRP (mg/l)	C3 (mg/dl)
T2DM with IR	5.97 ± 2.89	235.01 ± 52.67
T2DM with no IR	2.57 ± 1.77[a]	214.82 ± 51.68
Control	4.59 ± 2.70[ab]	160.98 ± 47.89
F – test	32.778	278.549
P – value	0.000	0.005

Legend:

Group A- TG/HDL>2=T2DM with IR

Group B- TG/HDL < 2=T2DM with no IR

Group C- Control

Significant @ p < 0.05 using post hoc LSD; Group A vs Group B or C- a; Group B vs C- b

Table 4.2: Comparison of studied biochemical parameters with respect to sex of the studied population.

	C3 (mg/dl)` TG/HDL>2	C3 (mg/dl) TG/HDL<2	Hs-CRP(mg/l) TG/HDL>2	Hs-CRP(mg/l) TG/HDL<2
Male	238.79±48.29	182.90±44.03	5.79±2.63	2.87±1.88
Female	237.39±55.53	189.80±47.46	6.10±3.08	2.30±1.66
P – value	0.242	0.608	0.029	0.950
F – test	1.384	0.266	4.919	0.004

Significant difference in mean compared with control at p<0.05

Mean±2SD represent the ±values

Table 4.3: Comparison of the studied biochemical parameters according to age.

	C3 (mg/dl) TG>2	C3 (mg/dl) TG<2	Hs-CRP (mg/l) TG>2	Hs-CRP(mg/l) TG<2
30-39	236.22±51.46	182.76±55.25	6.30±2.92	2.85±1.47
40-49	234.00±56.62	187.05±40.89	6.58±2.68	2.11±1.40
50-59	252.62±49.47	187.10±44.54	5.92±2.91	3.04±2.74
60-69	216.45±48.30	177.33±24.40	5.63±3.08	2.98±2.42
70-79	246.45±58.86	207.50±45.27	4.50±3.05	2.36±1.35
80-89	263.75±31.60	112.00±0.00	7.17±1.33	1.40±0.00
P – value	0.246	0.460	0.430	0.694
F – test	1.362	0.944	0.986	0.608

Significant difference in mean compared with control at p<0.05.
Mean±2SD represent the ±values

Table 4.4: Correlation of the studied biochemical parameters.

	r	P – values	interpretation
TG>2 (n=100)	0.543	0.000	S
TG<2 (n=60)	0.072	0.587	NS
Control (n=60)	-0.101	0.444	NS

Significant difference in mean compared with control at p<0.05

DISCUSSION

The results of this study showed a significant difference in the studied biochemical parameters (C3 and Hs-CRP) in those with TG/HDL-C ratio greater than two which represents type 2 diabetes with insulin resistance as against those with TG/HDL-C ratio less than 2 which represents type 2 diabetes with no insulin resistance along with the control group (normoglycemic with normal lipid profile results) that doesn't show any significant difference. Hs-CRP has its p value as P<0.000 while Complement C3 has its p value as P<0.005 in subjects that have their TG/HDL-C ratio greater than two, this shows that lipid profile assay- which is an inexpensive routine test, easy to carry out and offers good precision, can actually be used as a predictor or marker of insulin resistance thereby substituting for HOMA-IR and Euglycemic clamp of insulin assay which is very expensive and time consuming.

This is in line with the work of McLaughlin and colleagues from Stanford University School of Medicine, California in 2003 where they discovered that triglyceride–high-density lipoprotein (HDL-C) ratio are good surrogate markers for identifying insulin resistance when they were trying to find out an easier and simpler way to identify individuals who are overweight and have developed insulin resistance during a recent epidemic of obesity. They also note that lifestyle changes like weight loss and exercise can go a long way to reduce insulin resistance in the supposed patients. In another research conducted by MacLaughlin *et al* and Li *et al*, in 2003 and 2008 respectively, they reported that (TG/HDL-C) ratio has been documented to be closely related to insulin resistance in adult population though the relationship is described more in white adults compared to the black adults.

When mean±SD values were compared between groups, there was a significant difference between Hs-CRP in TG/HDL-C ratio >2 and TG/HDL-C<2 but no significant difference was observed when C3 was compared between group. This shows that Hs-CRP is a better marker for insulin resistance and this is in agreement with the work done by Ridker and his colleagues where they observed that Hs-CRP is a better marker to assay for inflammation and Insulin resistance which have always been related to the development of heart disease (Ridker *et al*., 2003).

In a recent study carried out by Borch-Johnssen, 2007 and WHO in 2008, they both discovered that there is a strong relationship between insulin resistance and chronic inflammatory response which is characterized by abnormal production of cytokine. Ridker, 2003 also observed that Myocardial infarction, ischemic stroke, type 2 diabetes, and hypertension – have a strong and independent prediction by high sensitive CRP assay in the blood which is a systemic inflammatory biomarker . CRP and Cardiovascular disease have been shown to have a strong relationship in a research carried out by Jeppesen and his group and this is in line with the observation made in my present study while on the other hand they also agree that sometimes the relationship can be independent of some risk factors like cholesterol, blood pressure, alcohol consumption, and smoking habit (Jeppesen *et al*., 2008).

Several data gotten either through epidemiology report or experimental design have been shown to have a link with impaired glucose metabolism. Ridker, Nakanishi and his groups also said that future CVD risk including IR can also be predicted by assaying for Hs-CRP aside from it reflecting local inflammation at atherosclerotic lesions (Ridker, 2003).In a research to evaluate the relationship between IR and serum CRP among Peruvian adults after adjusting for BMI, age, and current smoking status, the subjects in the upper tertile (CRP concentration>2.53 mg/l) had twice increased risk of IR more than those in

lowest tertile (CRP concentration <0.81 mg/l) in both men and women. Their observations here are in agreement with other reports from cross sectional and prospective studies ofpopulations that showed associations between IR and CRP and it is also very strongly in agreement with the result of this research work carried out by me.

Different studies have also shown that low-grade inflammation is a novel risk factor in all stages of atherosclerosis and acute coronary syndrome (Hanyu *et al*., 2009) as it is generally believed to be coming from the adipose tissue while visceral adipose tissue particularly, plays a major role in regulating inflammation, therefore elevated CRP concentrations in individuals with IR has been documented by an evolving body and they propose a correlation between the two (Pradhan *et al*., 2001) just as i observed in my research study.

Gabay & Kushner in 1999 also noted that since CRP is primarily synthesized in the liver and regulated by the pro-inflammatory cytokine IL-6 and tumor necrosis factor-alpha (TNF-α) in adiposities, that the relationship of CRP concentrations with fasting insulin, fasting glucose, fasting lipid profile and HOMA-IR could be as a result of a chronic systemic sub-clinical inflammation (Gabay & Kushner, 1999).

Pearson and his colleagues showed that CRP have been endorsed by the Centers for Disease Control and Prevention and the American Heart Association as they publish guidelines that endorses it as the only inflammatory biomarker currently available with adequate standardization and predictive value suitable as an adjunct to traditional risk factor screening (Pearson *et al*., 2003) and makes elevated CRP level a representative of systemic inflammation with etiologic importance in insulin resistance and diabetes.

In a study carried out at the University of Bologna in Italy, Department of Internal Medicine, Cardioangiology, and Hepatology, C3 complement, CRP, ESR, and leukocyte count were compared as a determining factor of the HOMA index in a wide elderly population (Muscari*et al*., 2000) and they found out that serum C3 was more associated with HOMA index thus making it the second strongest covariate of HOMA-IR index after obesity (when represented by waist circumference), but this is in contrast to the study carried out in this research work where CRP appears to be strongest marker for IR.

Weyer also gave another report of a very strong relationship of C3 with IR and fasting insulin in Young adult Pima Indians and in men aged 55-64 years (Weyer *et al*., 2000) which appears to be contrary to the result of this research study work. Engstro¨m and his colleagues have recently showed that C3 can be a strong risk factor for developing diabetes in men between the ages of 38–50 years (Engstro¨m *et al*., 2005), despite adjusting for fibrinogen, haptoglobin, orosomucoid, _1-antitripsin, ceruloplasmin, and C4. They noted that this study did not compare between inflammatory markers (CRP, ESR amd leucocyte count) but C3 was compared with variables like inflammatory indicators of IR because it can also be produced by adipocytes (Choy *et al*., 1992) and activated macrophages, it thus behaves like an inflammatory cytokine and an adipokine (Zimmer *et al*., 1982) aside from being synthesized in the liver like other acute phase proteins (Alper *et al*., 1969). This opinion did not also agree with my findings from this research work and I went further to compare between groups but no significant difference was observed unlike Hs-CRP which gave a significant result even when compared between groups. Yeo et al in 2010 also noted that CRP levels in diabetic subjects are twice that of normal persons therefore showing a positive correlation with insulin resistance. This finding by Yeo and his colleagues is in agreement with my findings CRP shows a strong significance compared to C3 complement and they concluded that a precise predictor of insulin resistance in diabetic patients is CRP.

In table 4.2, a significant difference was observed in Hs-CRP with IR but no significant difference in C3. No significant difference in sex of the study population in C3 Complement which shows that IR has equal chances of affecting both sexes but Hs-CRP has an exception. It can be affected by age once TG/HDL ratio is greater than 2. This is in agreement with Centers for Disease Control and Prevention and the American Heart Association as they publish guidelines that endorses CRP as the only inflammatory biomarker currently available with adequate standardization and predictive value suitable as an adjunct to traditional risk factor screening (Pearson *et al*., 2003). Pearson and his group concluded that elevated CRP level is a distinct representative of systemic inflammation which has etiologic importance in insulin resistance and diabetes.

Table 4.3 shows that age has no contribution to T2DM, as there is no significant difference amongst the different age groups studied. This shows that T2DM can occur at any age due to physical inactivity and obesity.

Table 4.4 shows a strong correlation between the studied biochemical parameters implying that Hs-CRP and C3 can be a marker for insulin resistance T2DM but Hs-CRP has a stronger relationship.

CONCLUSION

In conclusion, this study contributes to knowledge and evidences that Hs-CRP and C3 can be assayed as routine test in individuals suspected of having Insulin Resistance because they tend to have a strong association with T2Dm. for prompt intervention of their health status. However Hs-CRP shows a stronger relationship with IR.

REFERENCES

1. World Health Organization (2014). Noncommunicable diseases and mental health.
2. World Health Organization (*2013). Diabetes Fact Sheet.*
3. Kitabchi, A. E., Umpierrez, G. E., Miles, J.M. & Fisher, J. N. (2009). Hyperglycemic crises in adult patients with diabetes. *Diabetes Care.* 32(7): 1335–1343.
4. Shoback, H. David, G.& Gardner, D. (2011). Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. ISBN 0-07-162243-8.
5. IDF Diabetes Atlas (2014).(6th ed.). International Diabetes Federation.Pp 7.ISBN 2930229853.
6. Shi, Y., Hu, Z. & Frank, B. (2014). The global implications of diabetes and cancer".*The Lancet*, 383 (9933), 1947–1948.
7. Vos, T., Flaxman, A. D., Naghavi, M., Lozano, R., Michaud, C., Ezzati, M., Shibuya, K., Salomon, J. A., Abdalla, S. & Aboyans, V. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380(9859): 2163–2196.
8. American Diabetes, Association (2013). Economic costs of diabetes in the U.S. in 2012. *Diabetes Care*, 36(4): 1033–46.
9. Rippe, U., Richard, S., Irwin, M. & James, M. (2010). Manual of intensive care medicine (5th ed.). Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. Pp. 549.
10. Picot, J., Jones, J., Colquitt, J. L., Gospodarevskaya, E., Loveman, E., Baxter, L. & Clegg, A. J. (2009). "The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation".*Health Technology Assessment*, 13(41): 1–190, 215–357.
11. Cash, J. (2014). Family Practice Guidelines (3rd ed.). Springer. Pp. 396. ISBN 9780826168757.
12. Alper, C. A., Johnson, A. M., Birtch, A. G. & Moore, F. D. (1969). Human C3: evidence for the liver as the primary site of synthesis. *Science*, 163: 286–288.
13. Choy, L.N., Rosen, B. S. & Spiegelman, B. M. (1992).Adipsinand an endogenous pathway ofcomplement from adipose cells. *Journal of Biology and Chemistry*, 267: 12736–12741.
14. Asgari, E., Le Friec, G. & Yamamoto, H. (2013). C3a modulates IL-1β secretion in human monocytes by regulating ATP efflux and subsequent NLRP3 inflammasome activation. *Blood*, 122: 3473–3481.
15. Laudisi, F., Spreafico, R. & Evrard, M. (2013). Cutting edge: the NLRP3 inflammasome links complement-mediated inflammation and IL-1β release. *Journal of Immunology*, 191, 1006–1010.
16. Shu, C. J., Benoist, C. & Mathis, D. (2012). The immune system's involvement in obesity-driven type 2 diabetes. *Seminar on Immunology*, 24: 436–442.
17. Sell, H., Habich, C. &Eckel, J. (2012).Adaptive immunity in obesity and insulin resistance. *National Revi*ew of *Endocrinology*, 8: 709–716.
18. Thompson, D., Pepys, M. B. & Wood, S. P. (1999)."The physiological structure of human C-reactive protein and its complex with phosphocholine". *Structure*, 7(2): 169–177.
19. Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E. & Ridker, P. M. (2001). "C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus". *Journal of American Medical Asociation*, 286(3): 327–334.
20. Dehghan, A., Kardys, I., de Maat, M. P., Uitterlinden, A. G., Sijbrands, E. J., Bootsma, A. H., Stijnen, T., Hofman, A., Schram, M. T. & Witteman, J. C. (2007). "Genetic variation, C-reactive protein levels, and incidence of diabetes". *Diabetes*, 56 (3): 872–878.
21. Alagoa, E. J. (2009). The land and people of Bayelsa State: Central Niger Delta. Onyoma Research Publication.
22. McLaughlin, T., Abbasi, F., Cheal, K., Chu, J., Lamendola, C. & Reaven, G. (2003).Use of metabolic markers to identify overweight individuals who are insulin resistant. *Annals of Internal Medicin*e, 139: 802–809.
23. Li, C., Ford, E. S., Meng, Y. X., Mokdad, A. H. &Reaven,G. M. (2008). Does the association of the triglyceride to high-density lipoprotein cholesterol ratio with fasting serum insulin differ by race/ethnicity? *Cardiovascular Diabetolology*, 7: 4.
24. Ridker, P. M., Buring, J. E., Cook, N. R.& Rifai, N. (2003). C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14,719 initially healthy American women. *Circulation*, 107: 391-397.
25. Borch-Johnsen, K. (2007).The metabolic syndrome in a global perspective.The public healthimpact. *Danish Medical Bulletin*, 54: 157-159.
26. Weyer, C., Tataranni, P. A. & Pratley, R. E. (2002). Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. *Diabetes Care*, 23: 779 –785.
27. Engstro¨m, G., Hedblad, B., Eriksson, K. F., Janzon, L. & Lindgarde, F. (2005). Complement C3 is a risk factor for the development of diabetes: a population-based cohort study. *Diabetes*, 54: 570 –575.
28. Zimmer, B., Hartung, H. P., Scharfenberger, G., Bitter-Suermann, P. & Hadding, U. (1982). Quantitative studies of the secretion of complement component C3 by resident, elicited and activated macrophages: comparison with C2, C4 and lysosomal enzyme release. *European Journal of Immunology*, 12: 426–430.