Qualitative and Quantitative C - reactive Protein Concentration in Tissue Injury

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Abstract
C-reactive protein (CRP) is a β1-globulin protein synthesized primarily in the liver and released following soft tissue injury. Venous blood samples were collected from fifty test subjects with tissue injury (25 males and 25 females, age 30 -35 years) and fifty control subjects matched for sex and age. The presence of CRP was investigated by antigen-antibody agglutination test and quantified by serial two-fold dilution of the samples collected. The test subjects showed positive agglutination indicating the presence of CRP while the control subjects yielded negative result. Quantitatively, the mean CRP level in control subjects was 6.6 ± 0.6 mg/l and 60 ± 36mg/l in test subjects. Female test subjects had higher percentage increase (89%) than the male test subjects (81.7%). These increases are statistically significant ($p<0.05$). CRP concentration is elevated in acute tissue injury and aids assessment and treatment.

Keyword: *C - reactive protein, Soft Tissue, Injury, Quantity*

INTRODUCTION

C-reactive protein is an acute phase reactant, a protein made by the liver and released into the blood within a few hours after tissue injury, the start of infection or other inflammations. It is found in trace amounts in healthy people (mold et al., 2010; Jabs et al., 2011). CRP forms an integral component of innate immunity and serves primarily to recognize potential pathogens and damaged cells through opsonisation and activates the complement cascade (Allin and Nordesguard, 2011; Bermer et al., 2012). CRP belongs to a highly conserved phylogentically ancient family called pentraxins which have five identical non-covalently linked subunits (Shrive et al., 2006; Gabbay and Kushner,

2009). C-Reactive proteins are characterized by their homopentameric structure and calcium dependent ligand binding affinity for the phosphocholine moiety. CRP gene is located on the first chromosome. It has 224 amino acids, a monomer of molecular mass 25106 Da with an annular pentameric discoid shape (Pepys, 2010, Pue et al., 2009).

RATIONALE OF STUDY

Soft tissue injuries involve damage to the ligaments, tendons and muscles. Injuries can result from repeated overuse or a sudden single injury, for example, a fall or twisting of the area.

Some tissue injuries arise with trauma. A blow, excessive force extending or flexing beyond the normal range of motion, awkward movement like twisting of a joint and strenuous physical activity that an individual is not occasioned for, can cause soft tissue injuries. With more severe force, soft tissue injuries may occur with a bone fracture or dislocation in the joint. Thus the aim of this study was to determine quantitatively the concentration and relationship between CRP and tissue injury.

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation, percentage statistical analysis was performed using analysis of variance and correlation coefficient between the mean values of patients and the control. Statistical significance was defined as $p<0.05$.

METHODOLOGY

Venous blood samples were collected into plain bottles from fifty patients with tissue injury. This comprised twenty five males and twenty five females, between 30-35 years. Blood samples were also collected from fifty apparently healthy subjects to serve as controls. The blood samples were allowed to clot at room temperature, centrifuged at 3000 revolutions per minute and the supernatant serum transferred to a clean plain bottle. Qualitative and Quantitative analyses of C-reactive protein were carried out using CRP Bio system kit reagents for both the neat and two-fold serial dilutions.

Qualitative Analysis

Fifty microlitres each of the test serum samples and controls were placed on separate circles of the test card and fifty microlitres of the latex reagent added to each circle, mixed and placed on a rotator for one minute. Each reaction mixture was observed for visible agglutination.

Quantitative Analysis

Positive samples were titred by making serial two-fold dilutions of the serum samples in 9g/l sodium chloride solution in test tubes and using the procedure above to observe for agglutination. The serum titre was defined as the highest dilution showing a positive (agglutination) result. The concentration of CRP in the sample was obtained by multiplying the titre by a factor.

RESULT

Tables

Mean value for male test subjects was 36±12 mg/l and 60± 36 mg/l for female test subjects. This result showed higher percentage increase in females (89%) than in males (81.7%).

Table 1: Mean Valves for Control and Test Subjects

	No. of subjects	Mean Mg/L
Controls	50	6.6 ± 0.6 (6.0 – 7.2)
Test Subjects	50	60 ± 36 (24 – 96)

Table 2: Mean Valves for Male and Female Test Subjects

	No. of subjects	Mean Mg/L	Percentage Increase
Controls	50	6.6 ± 0.6 (6.0 – 7.2)	
Male	25	36 ± 12 (24 – 48)	81.7%
Female	25	60 + 36 (24 – 96)	89%

DISCUSSION

This study assessed C-reactive protein concentration in tissue injury. CRP binds to the phosphocholine expressed on the surface of dead or dying cells and some bacteria. This activates the complement system, promoting phagocytosis by macrophages which clears necrotic and apoptotic cells and bacteria (Cermak et al., 2010).

Blood serum of test subjects showed marked positive agglutination indicating increased release of CRP after tissue injury. This acute phase response is as a result of a rise in the concentration of interleukin-6, which is produced by macrophages and adipocytes. As part of acute phase response CRP level rises sharply to facilitate non-specific immune functions and assist with repair processes. CRP has the ability to recognize disease causing agents, damaged cells and to mediate their removal by acting as an opsonin (Gershow et al., 2012). Opsonisation involves coating of the agents surface by other cells of the immune system specifically neutrophils and macrophages (Lu et al., 2010). Inflammation is suppressed through the inhibition of interleukin-1b (IL -1b) and interleukin-1ra (1L-1ra). Additional anti-inflammatory properties exhibited by CRP include the ability to decrease the expression of cell adhesion (L-selectin) and reduce neutrophil superoxide production (Gabbay et al., 2009; Zouki et al., 2011).

CRP concentrations correlated significantly (0.92) with surface area and depth of tissue injuries in both male and female subjects. Percentage increase of CRP concentration was higher in female subjects (89%) than in males (81.7%). This difference may be attributed to the differing 'puffing' behaviour in women and the taking of oral contraceptives containing oestrogen which has been found to correlate significantly with elevated C-reactive protein (Nakamura et al., 2011). CRP concentration is determined by the rate of production and hence the severity of the precipitating cause, therefore, CRP is a screen of inflammation. It is recommended that CRP evaluation be part of routine investigations whenever there is tissue damage or injury to minimize the risk of inducing other health conditions associated with elevations of CRP concentration.

REFERENCES

Allin, K.H. and Nordestgarrd, B.G. (2011). Elevated C-reactive protein in the diagnosis, prognosis and cause of cancer. *Critical Reviews in clinical laboratory sciences* 48(4), 155-170.

Berman, S., Gerwuz, H. and Mold, C. (2012). Binding of C-reactive protein to nucleated cells leads to complement activation without cytolysis. *Journal of immunology:* 136: 1384-9

Cermak, J., key, N.S. and Bach, R.R. (2010). C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Journal of medicine* 115:702-5.

Gershow, D., Kim, S. and Brot, N. (2012). C-reactive Protein binds to Apoptotic Cells, Protects the Cells from Assembly of the Terminal Complement Components and Sustains an Inflammatory Innate Immune Response: Implications for Systemic Auto-Immunity. *Journal of Expert Medicine:* 192: 1353-64.

Jabs, W.J., Logering, B.A. and Gerke, P (2011). The Kidneys as a Second Site of Human C- Reactive Protein Formation in vivo. Europe *Journal of Immunology*: 33:152-61.

Gabbay, C. and Kushner, I. (2009). Acute Phase Protein and other Systematic Responses to Inflammation. National England *Journal of Medicine*: 340: 448-54.

LU, H., Ouyang, W. and Huang, C. (2010). Inflammation, a Key Event in Cancer Development. *Molecular Cancer Research* 4 :(4): 221-33.

Mold, C., Gewurz, H. and DU-dos, T.W. (2010). The Regulation of Complement Activation by C-Reactive Protein. *Immunopharmacology*: 42:23-30.

Pepys, M.B. and Hirschfield, G.M.D. (2000). C-reactive Protein: A Critical Update. *Clinical Invest*: 111: 1805 -12.

Pue, C.A, Mortenser, F. and Marsh, C.B. (2009). Acute levels of C-reactive protein. *Journal of Immunology:* 156: 1594

Shrive, A.K., Cheetan, G.M. and Holden D.C. (2008). Three Dimensional Structure of Human C-reactive Protein. *Natural Structural Biology:* 3:346-354.

Nakamura, T., Grimer, R.J. and Gaston, C.I. (2011). The Prognostic Value of the Serum Level of C-reactive Protein for the Survival of Patients with Primary Sarcoma of Bone. *Joint Journal:* 95:411 -418

Zouki, T., Beauchamp, M. and Baron, C. (2011). Prevention of in vitro Neutrophil Adhesion to Endothelial cells through Shedding of L-selectin by C-reactive Protein and Peptides derived from C-reactive Protein. *Journal of Clinical Invest:* 100:522-9

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Epidemiological Transitions Associated with Preterm Deliveries: A Case Study in A Secondary Health Care Centre North-Western Nigeria

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Abstract
Preterm birth is an increasingly common complex condition with multiple risk factors and has substantial medical, psychological, economic and social impacts. Complications of preterm birth are the leading direct cause of neonatal morbidity and mortality, and progress is dependent on achieving high coverage of evidence-based interventions to prevent preterm delivery and to improve survival for preterm newborns. The study investigated the determining and epidemiological transition in 64 preterm deliveries using a retrospective survey. Data was obtained using a standardized data collection form based on CDC/WHO criteria and analyzed using descriptive statistics. The results showed that most subjects were male babies (60.9%) and late preterm were the most common form of preterm deliveries and most subjects were of low birth weight (65%). Findings also showed that the most common medical condition associated with preterm was respiratory disease syndrome (62.5 %) followed by jaundice with (34.4 %.) respectively in a decreasing frequency. It was concluded that, respiratory disease syndrome was the most common medical condition associated with preterm deliveries.
Keywords: *Preterm Birth, Survival Chances, Preterm Conditions*

INTRODUCTION

Preterm birth is a major risk factor for morbidity and mortality among infants worldwide, and imposes considerable burden on health, education and social services, as well as on families and caregivers [1].The morbidity impact of preterm birth is not limited to the neonatal period, but also extends into later periods in life resulting in cognitive

developmental impairments, learning difficulties, social and behavioral problems [2]. Additionally, morbidity and mortality resulting from preterm birth is reported to be highest among early (born at less than 28 weeks gestational age) and moderate (born between 28 and 32 weeks gestational age) preterm infants [3]. Neonates born very early are at risk of dying in their first few weeks of life, or if they survive, such children may have damage to their developing brain, manifesting as cerebral palsy, blindness, deafness or cognitive dysfunction [4]. Mortality rates increase with decreasing gestational age, and neonates who are both preterm and small for gestational age are at even higher risk[5]. According to Schendel & Bhasin (2008) neonates born too soon are between 6 and 26 times more likely to die during the first four weeks of their lives than neonates born at term[5]. However, presently preterm birth is the single most important cause of neonatal death [5], and the second most common cause of under-5 deaths after pneumonia [6].
According to the WHO [7], preterm is defined as neonates born alive before 37 weeks of pregnancy or 259 days of gestation are completed. The American Academy of Pediatrics advocates the use of the term "preterm" infant to mean any infant of less than 37 weeks' gestation [8].The preterm neonates are classified into three (3) main categories according to gestational age at birth with those born from 32 to 36 weeks classified as mild preterm, 28 to 31 weeks very preterm and less than 28 weeks classified as extremely preterm for birth [8].

However, Infants born preterm compared to term infants experience more difficulty with feeding, blood glucose control, jaundice, temperature instability, apnea, respiratory distress and sepsis either singly or in combination [9].Approximately 45% to 50% of the causes of preterm births are idiopathic, 30% are related to preterm rupture of membranes and another 15% to 20% result from medically indicated or elective preterm deliveries [10].

The treatment modalities of preterm born demand maintenance of adequate oxygenation, continuous electronic cardiac and respiratory monitoring, frequent monitoring of vital signs, thermoregulation, infection control, hydration, provision of adequate nutrition and sensory stimulation, and emotional support for the parents [10]. In addition,

highly skilled neonatal nurses, feeding support, use of intravenous fluids, and safe oxygen use with careful tracking of oxygen saturation as well as follow-up services are highly essential [11].

Globally, about 11% of all births, translating to an estimated 15 million births per year, are estimated to be preterm [1] [9][12].Additionally, preterm deaths constitute 28% of the estimated 4 million annual neonatal deaths with 99% of these deaths occurring in developing countries [13].Since preterm birth is the leading cause of neonatal deaths globally [13], and the second leading cause of mortality among under five, improving child survival requires achieving higher coverage of evidence-based interventions to prevent preterm birth and to improve survival for preterm newborns[14].

The vast majority (85%) of global preterm births occur in Africa and Asia [1] where health systems are weak and access to and utilization of health services are limited, contributing to the higher risks of death and disabilities in preterm infants [14]. More so, over 60% of global preterm births occurred in these regions [12].

In countries like Nigeria preterm birth is a public health problem, where one in every three newborn deaths is attributed to preterm birth complications [8].In Nigeria, prematurity is reported to be the leading cause of neonatal death with an estimated 85,700 newborn deaths due to preterm birth complications every year [8].A study conducted by Mukhtar & Iliyasu established that preterm neonates accounted for 32.1% of prenatal mortality [15].

Complications of preterm birth are the leading direct cause of neonatal mortality, accounting for an estimated 27% of the almost four million neonatal deaths every year, and act as a risk factor for many neonatal deaths due to other causes, particularly infections [16]. Neonatal deaths account for more than 42% of under-five deaths [17]. However, while under-5 mortality rates are improving in many countries worldwide, neonatal mortality rates have shown much less progress [18].

METHODOLOGY

Study Setting

The study was conducted at Special Care Baby Unit (SCBU) of Murtala Muhammad Specialist Hospital (MMSH). A secondary referral hospital located at the centre of Kano, north-western Nigeria.

Target Population

The study targets population consisted of all live births during the study period January 2013 to January 2015.

Sample Size

The census method was used for the study, to obtain a total sample size of 64 subjects.

Sampling Technique

Using a retrospective cross-sectional study design, purposive sampling was used to recruit the subjects until the desired sample size was reached.

Research Instrument

A standardized data collection instruments was employed based on the CDC/WHO criteria. Information obtained included, gender, birth weight, date of admission and discharge, clinical manifestations, diagnoses and treatment modalities.

DATA ANALYSIS

The data were analyzed descriptively using statistical package for the social sciences (SPSS) version 16.0.

Ethical Consideration

Ethical clearance was sought and granted from the research ethical committee of the study hospital.

RESULTS

Table 4.1 Socio-Demographic Data of the Subjects

Variable No=64	Frequency	Percent (%)
Gender		
Male	39	60.9
Female	25	39.1
Total	**64**	**100.0**
Age (in days)	**Frequency**	**Percent (%)**
1-5	2	3.1
6-10	3	4.7
11-15	10	15.6
16-20	17	26.6
21-25	21	32.8
26-30	11	17.2
Total	**64**	**100.0**

Table 4.1 above shows that most subjects (60.9 %) were males while only (39.1 %) were females. With regard to the age most of the subjects (32.8 %) were between 21-25 days while only (3.1 %) were between 1-5 days.

Table 4.2 Categories of Preterm Babies

Variable No=64	Frequency	Percent (%)
Based on gestational age		
Extremely preterm (<28 weeks)	6	9.4
Very preterm (28-<32 weeks)	39	4.7
Moderate preterm (32-<34 weeks)	16	25
Late preterm (34-36 weeks)	3	60.9
Total	**64**	**100.0**
Based on birth weight	**Frequency**	**Percent (%)**
Extremely low birth weight (<1000g)	6	9.4
Very low birth weight (1000-<1500g)	42	65.6
Low birth weight (1500-<2500g)	16	25
Total	**64**	**100.0**

Table 4.2 above shows that most subjects (69.9 %) were late preterm while only (4.7 %) were very preterm. With regard to birth weight most subjects (65 %) were low birth weight while only (9.4 %) were extremely low birth weight.

Figure 4.4 Medical Conditions Associated with the Subjects.

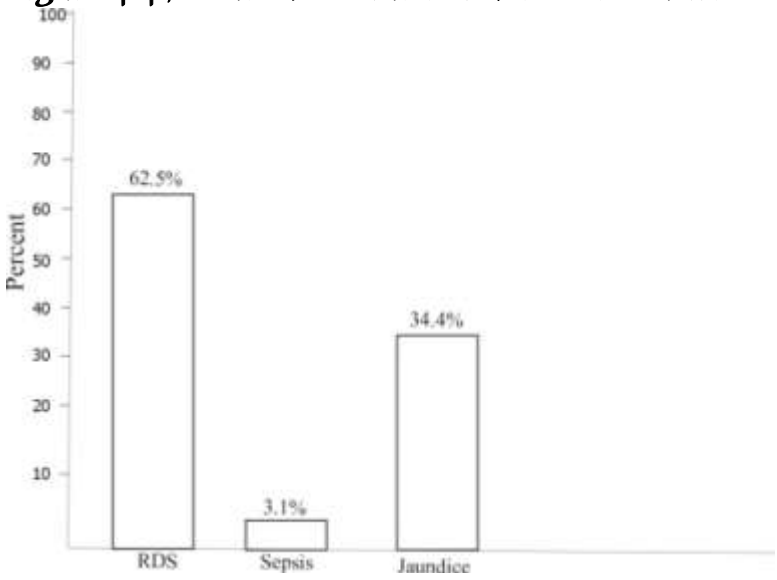

Figure 4.4 Most of the subjects (62.5 %) have respiratory distress syndrome (RDS) while only (3.1 %) have sepsis.

Table 4.5 Subject Treatment Modalities

Variable No=64	Frequency	Percent (%)
Thermal care		
Incubator	22	34.4
Kangaroo	42	65.6
Total	**64**	**100.0**
Feeding support	**Frequency**	**Percent (%)**
Breastfeeding	62	96.9
Others	2	3.1
Total	**64**	**100.0**
Care of jaundice	**Frequency**	**Percent (%)**
Phototherapy	20	90.9
Exchange blood transfusion and phototherapy	2	9.1
Total	**22**	**100.0**
Care of RDS	**Frequency**	**Percent (%)**
Oxygenation and CPAP	38	97.4

Assisted ventilation	1	2.6
Total	**39**	**100.0**

Table 4.5 Most of the subjects (65.6%) were managed with kangaroo mother while only (34.4%) were managed with incubator. With regard to feeding support, it was revealed that most subjects (96.9%) were managed with adequate breastfeeding while only (3.1%) were managed with other modalities. Over 90% of the subjects had phototherapy and few had EBT. It was also revealed that more than 97% of the subjects with (RDS) were treated with oxygenation and CPAP, while very few were treated with assisted ventilation.

DISCUSSION

The study showed that, majority (60.9%) of preterm were males. Similar demographic observation was obtained by another study conducted by [12] reported that irrespective of gestational age, preterm baby boys are 14% more likely to be born than female preterm, but have lower chance for survival. The reason could have been linked to the theoretical assertion that male babies carried higher risks of adverse neonatal outcomes, including preterm birth[19].Speculation exists regarding the male fetal hormonal input into the onset of labour, or the genetic disadvantage of the male fetus, evidenced by the excessive male sex percentage in adverse pregnancy outcomes [20]. However, despite numerous studies on the mechanisms of preterm labour influenced by fetal gender, an explanation for the chances for survival remains uncertain. The study found that, the most common medical condition associated with preterm was respiratory distress syndrome (RDS), followed by jaundice. The reason for this could have been link to the developmental immaturity of the lungs, leading to inadequate pulmonary surfactant production or delay in embryological development of the lungs tissue in utero.

CONCLUSION AND RECOMMENDATIONS

It was concluded that, respiratory distress syndrome was the most common medical condition associated with preterm among the studied group and therefore recommended that the government should provide more facilities and equipment for proper management of preterm neonate most especially incubators particularly at the study setting

and more health personnel should be trained by government and non-governmental organizations through organizing workshops and seminars on the management of preterm babies.

REFERENCES

Beck, S., Wojdyla, D., Say, L., Betran, A.P., Merialdi, M., Requejo, J.H., Rubens, C., Menon, R. and Van, L. (2010) PFA: The Worldwide incidence of Preterm Birth: a Systematic Review of Maternal Mortality and Morbidity. *Bull World Health Organ, 88: 31 – 38*

Van Baar, A.L., Vermaas, J., Knots, E., de Kleine, M.J., and Soons, P. (2009) Functioning at School Age of Moderately Preterm Children Born at 32 to 36 Weeks' Gestational Age. *Pediatrics, 124: 251 –255*

Goldenberg, R.L., Culhane, J.F., Iams, J.D., and Romero, R. (2008). "Epidemiology and Causes of Preterm Birth". The Lancet371, 9606: 75–84.

Saigal, S. and Doyle, L. W. (2008). An Overview of Mortality and Sequelae of Preterm Birth from Infancy to Adulthood. The Lancet371 9608: 261–269.

Schendel, D., and Bhasin, T.K. (2008). Birth Weight and Gestational Age Characteristics of Children with Autism, Including a Comparison with Other Developmental Disabilities. *Pediatrics, vol. 121(6): 1155 – 1164.*

Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J.E., Cousens, S., Mathers, C., and Black, R.E. (2014). Global, Regional, and National Causes of Child Mortality in 2000—13, With Projections to Inform Post - 2015 Priorities: An Updated Systematic Analysis. Supplementary Appendix. *The Lancet, (Early Online Publication).*

WHO. (2007) Pocket Book of Hospital Care for Children: Guidelines of the Management of Common Illnesses with Limited Resources. Geneva: World Health Organization.

Lockwood, C.J., Ramin, S.M., and Barss, V.A. (2011). Overview of Preterm Labour and Delivery. *Up To Date, Vol. 19: No. 1.*

Engle, W.A., Tomashek, K.M., Wallman, C. and the Committee of Fetus and Newborn. (2007). "Late-Preterm" Infants: a Population at Risk. A Clinical Report. *Pediatrics, 120: 1390-1401. (http://dx.doi.org/10.1542/peds.2007-2952)*

Pennell, C.E., Jacobsson, B., Williams, S.M., Buus, R.M., Muglia, L.J. and Dolan, S.M. (2007). Genetic Epidemiologic Studies of Preterm Birth: Guidelines for Research. *American Journal of Obstetrics & Gynecology,196:107-118.*

Sola, A., Saldeno, Y.P. and Favareto, V. (2008). Clinical Practices in Neonatal Oxygenation: Where Have We Failed? What Can We Do? *Journal of Perinatology: California Perinatal Association,* 28 Suppl 1:28-34.

Blencowe, H., Cousens, S., Oestergaard, M., Chou, D. and Moller, A.B. (2012). National, Regional and Worldwide Estimates of Preterm Birth Rates in the Year 2010 with Time Trends for Selected Countries since 1990: A Systematic Analysis. *The Lencet, in Press.*

Federal Ministry of Health, (2011). Saving Newborn Lives in Nigeria. Newborn Health in the Context of the Integrated Maternal, Newborn and Child Health Strategy. Revised 2nd Edition, 2011.

Lawn, J.E., Gravett, M.G., Nunes, T.M., Rubens, C.E. and Stanton, C. (2010). Global Report on Preterm Birth and Stillbirth (1 of 7): Definitions, Description of the Burden and Opportunities to Improve Data. BMC Pregnancy and Child Birth, 10(Supply 1).

Mukhtar-Yola, M. and Iliyasu, Z. (2007). A Review of Neonatal Morbidity and Mortality in Aminu Kano Teaching Hospital, Northern Nigeria. *Trop Doct: 37:130-2.*

Bhutta, Z.A., Ahmed, T., Black, R.E., Cousens, S., Dewey, K. (2008). What Works? Interventions for Maternal and Child under Nutrition and Survival. *The Lancet, 371(9610): 417-440.*

Lawn, J.E., Gravett, M.G., Nunes, T.M., Rubens, C.E., and Stanton, C. (2010). Global Report on Preterm Birth and Stillbirth (1 of 7): Definitions, Description of The Burden and Opportunities to Improve Data. *BMC Pregnancy and Child Birth, 10(Suppl 1), S 1.*

Sheiner, E., Levy, A., Katz, M., Hershkovitz, R., Leron, E. and Mazor, M. (2004). Gender Does Matter in Perinatal Medicine. *Fetal Diagn Ther: 19(4):366-9.*

Astolfi, P., and Zonta, L. A. (2009). Risks of Preterm Delivery and Association with Maternal Age, Birth Order and Fetal Gender. *Human Reproduction. Vol. 14, No. 11, (Nov 2009): 2891- 2894.*

Raju, T.N., Higgins, R.D., Stark, A.R. and Leveno, K.. (2006). Optimizing Care and Outcome for Late-preterm (Near-Term) Infants: A Summary of The Workshop Sponsored by The National Institute of Child Health and Human Development. *Pediatrics, 118:1207–1214*

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Knowledge, Attitude and Practice towards Voluntary Counseling and Testing (VCT) for Human Immunodeficiency Virus (HIV) Among Students of Schools of Nursing and Midwifery in Kaduna State, Nigeria

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Abstract

The uptake for voluntary counseling and testing is low especially in Nigeria which rank among the top countries in the world with death from human immune deficiency virus. This study was therefore carried out to determine the knowledge, attitude and practice towards Voluntary Counseling and Testing (VCT) for HIV among students of schools of Nursing and Midwifery in Kaduna State. Survey method was adopted for the study. Two schools of Nursing and Midwifery were randomly selected from the four schools in Kaduna state and used for the study. Data were collected by the use of questionnaire containing fifty questions which comprised of both open and closed ended questions. Multistage sampling technique was adopted and 178 students were selected by simple random sampling method. The data collected were analysed by the use of Statistical Package for Social Sciences Version 21 and presented in frequency tables, pie charts and chi square further used to test the significant difference between variables. The findings showed that, all the respondents (100%) had heard about HIV/AIDS, were all aware of its cause as being viral and were aware of unprotected sexual intercourse as a route of HIV transmission (82%). Majority (95.5%) of the respondents strongly believed that HIV infection can be prevented by abstinence from sexual intercourse. Majority (89.9%) of the respondents were aware of Voluntary Counseling and Testing (VCT) and 67.4% indicated that VCT was available in Hospitals while all the respondents indicated that VCT was not available in their schools. Most (91%) of the respondents were of the view that they can benefit from VCT. Half (51.7%) of the

respondents considered fear of being positive as the most important factor discouraging utilization of VCT. More than half (59.5%) of the respondents had been tested before voluntarily. Result of the cross tabulation showed that, there was a significant statistical relationship between gender and perception of necessity of VCT (X_2 = 1.08, df = 1, P < 0.05, P value = 3.841). There was also a significant statistical relationship between level of study and knowledge that an infected person can appear infected (X^2 = 11.77 df = 4 P< 0.05, P value = 19.675). In view of these findings, there is need for schools to provide proper enlightenment/orientation programmes about VCT so that students can go for test at will and understand the benefit of screening.

Keywords: *Knowledge, Attitude, Practice, Voluntary, Counseling, Testing, Human Immunodeficiency Virus*

INTRODUCTION

Infection with the human immunodeficiency virus (HIV) is a global pandemic. Sub-Saharan Africa, although home to only 10% of the world's population, has approximately 70% of all the persons living with HIV infection/acquired immunodeficiency syndrome (AIDS)[1]. Sub-Saharan Africa remains the most serious affected region with AIDS, still the leading cause of death, with increasing number of orphans and widows. Nigeria has the third highest population of people living with HIV after South Africa and India[2]. The adult prevalence rose from 1.8% in 1991 to 5.4% in 2003 and estimates indicates that more than 3.5 million Nigerians are infected. However, in 2005, epidemic rates had dropped to 4.4%[3]. In Nigeria and most of sub-Saharan Africa, its consequences are already threatening the gains made by the health care delivery and economic development with the already existing high prevalence of communicable disease and deprivation disease. African governments are progressively becoming unable to meet their obligations of providing quality health and social services[4]. HIV/AIDS is transmitted through three main routes:

These are; penetrative sexual intercourse (unprotected) as the main route of transmission, parenteral transmission is the second most common source (20 – 30%) in Nigeria and is the most efficient source of them all[5] blood transfusion is responsible for about 10% of HIV in

Nigeria[6] and finally, vertical transmission from infected mother to her child before, during and shortly after birth is the third main route of transmission of HIV. According to UNAIDS[7], mother to child transmission accounts for more than 90% of global infections in infants and children. Mother to child transmission (MTCT) during pregnancy, delivery or through breastfeeding is the second most common mode of spread of HIV – 1 in the developing world. It is observed that rates of MTCT of HIV-1 have been consistently higher in Africa than in Europe or North America (30 – 40%) as compared with 15 – 20% respectively.

According to FMOH[8], Voluntary Counseling and testing for HIV/AIDS therefore is a process through which an individual or couples undergo counseling to enable him/her /them reach or make an informed decision about being tested for HIV. This involves three (3) major components: Pre-test counseling, HIV testing and Post-test counseling. Thus voluntary counseling and testing (VCT) is a cornerstone for early access to prevention as well as care and support services. Its need is increasingly compelling as HIV infection rates continue to rise. In spite of its huge potential, the success of Voluntary Counseling and Testing (VCT) in tackling the HIV problem in Nigeria has been limited. Various preventive strategies have been employed to curb the spread of this infection as there is presently no cure. Abstinence, avoidance of multiple sexual partners, condom use, voluntary counseling and testing (VCT) and treatment of HIV-infected individuals form the cornerstone of HIV prevention. VCT has been introduced in many low-resource settings as it helps to create awareness of an individual's HIV status and offers the opportunity for counseling on risk behavior modification. It also lessens stigma and has become a first step to accessing care[9].

Research conducted by the Ministry of Health to assess the availability of VCT services of Nigeria showed that 54% males and 43% females had knowledge of where to get an HIV test, about 89% of respondents from South East had the highest knowledge of where to get the HIV test, 41% of respondents from rural areas reported less knowledge as to more than 63% from urban areas. However, about 81% respondents had

higher knowledge compared to 26% who had not been to school and 36% who had Qur'anic education respectively. In terms of age, HIV/AIDS knowledge was lowest among 42% respondents aged 50 – 64 years and 54% stood at the peak between the age bracket 25 and 29 years. In the same study respondents were asked reasons for desiring an HIV test. 79% of them were willing to take the test to know their HIV status, 18% to allay fear and anxiety, 2% indicated that they want to satisfy mandatory marriage requirement and only 1% for employment purpose[3]. In another study carried out by Illiyasu[10] among 210 pregnant women attending antenatal clinics in Aminu Kano Teaching Hospital, Kano, Nigeria on the awareness and attitude towards HIV/VCT, most respondent were aware of it, 87% were quite knowledgeable about VCT and its purpose. The remaining 13% neither knew what it was nor could state the usefulness of the test. 81% approved VCT, 13% disapproved of it and were not willing to undergo the test while the remaining 6% were undecided of these approval, the main reasons were "I am afraid of stigma if the result comes out positive", I am afraid of isolation", "it may affect my marriage", "It is not necessary" and "I just don't want it".

Acceptability of VCT has been studied among various student populations. In Tanzania, for example, 34.6% of students of health care professions and 43.3% of medical students had VCT [11]. Reports from organizations like the World Health Organization (WHO), National Action Committee on AIDS (NACA) have shown the world prevalence with sub-Saharan Africa being particular. Despite this, the knowledge of HIV/AIDS and uptake of voluntary counseling and testing (VCT) is still low even among the most susceptible in Nigeria. In Nigeria, acceptance rate of VCT among students of tertiary institutions range from 8.3% to less than 30%[12, 13]. Students from schools of Nursing, besides being at risk of occupational exposure to HIV, are mostly within the age group at risk of contracting the infection through sexual practices. On the other hand, they also can function as role models among their peers and the general public in the fight against HIV/AIDS, including VCT. Kaduna State has a prevalence rate of 5.6 people infected with AIDS[3]. In Nursing and Midwifery schools in Kaduna State, it has been observed that despite the knowledge of the

benefits of voluntary counseling and testing for HIV in the fight against HIV epidemics, the uptake among the students is still quite low as only few of these students had reported to have visited the voluntary counseling and testing centre for their services. This prompted the researchers to carry out this study.

The objectives of this study were; to determine the knowledge about Voluntary Counseling and Testing for HIV/AIDS among nurses and midwives in Kaduna State; determine the attitude of the students towards voluntary counseling and testing of HIV/AIDS; identify the preventive measures among the students, assess the uptake and factors affecting this among the students. The study also determines the relationship between gender and perception of necessity of VCT and also determine the relationship between level of study and the knowledge that an infected person can appear healthy.

MATERIALS AND METHODS

The study was conducted in Kaduna State, which was created in 1987 from the former Northern region by the then regime of General Murtala Mohammed. It is located at the centre of Northern Nigeria. The state shares boundaries with Niger State to the West, Katsina, Zamfara, and Kano to the north, Bauchi and Plateau to the East, FCT Abuja, and Nasarawa State to the South. The State has a population of over four million people with the Northern part of the state mainly habited by the Hausa/Fulani tribe who are mainly Muslems, while the Southern part is mainly habited by tribes such as Bajju, Atyap, Gwong and Ham. These tribes are mainly Christians by religion. The major towns in Kaduna State are Zaria, Kafachan, Kaduna North and Kaduna South. The settings used for this study are the Schools of Nursing and Midwifery Kafachan, which is located in the East of Kaduna State and Ahmadu Bello University Teaching Hospital, Zaria. The population of study comprises of all the students (491) of both schools of Nursing and Midwifery.

RESEARCH DESIGN

A descriptive cross sectional design was adopted for this study.

Data collection: This was by the use of structured administered questionnaire which were distributed to 197 students by simple random selection of students from the two Schools in the State.

SAMPLE SIZE AND SAMPLING PROCEDURE

Forty percent (40%) of the total students in both schools were used as the sample size for the study i.e a total of 197 students. This proportion was adopted from Nwana[14] who stated that, if the population of study is a few hundred, 40% is sufficient for the study. Therefore, the sample size determination for the study in ABUTH was 40/100 x 130 = 52 students and for Kafachan was 40/100 x 361 = 145 students. Thus, making a total of 197 students as sample size for the study.

SAMPLING TECHNIQUES

A multistage sampling technique was adopted. Firstly, simple random sampling technique was adopted to select 2 schools of Nursing and Midwifery out of four (4) schools of Nursing and Midwifery namely; Schools of Nursing and Midwifery ABUTH, St. Lukes Wusasa, Kafachan and St Louis Zonkwa. Secondly, a cluster sampling method was adopted to divide the schools into 4 clusters (2 schools offering Basic Nursing progammes and 2 schools offering Post Basic Midwifery programmes), thereby making the 2 schools a primary sampling unit. The total number of students in each of the clusters of the schools was gotten from their administrative departments. Thirdly, a quota sampling method was adopted in the allocation of questionnaires according to the population of students in each level of study. 52 students were selected out of 130 students from school of Nursing and Midwifery ABUTH according to their levels of study and from school of Nursing and Midwifery Kafachan 150 students were selected to fill the questionnaires using the paper basket method. Identification numbers of each student were written on a piece of paper and each folded, mixed thoroughly and each randomly selected numbers was used as sample to fill the questionnaires.

INSTRUMENT FOR DATA COLLECTION

A structured self-administered questionnaire containing 50 questions was designed in such a way that it explored the respondents knowledge

on causes of HIV/AIDS, mode of transmission, awareness of prevention, awareness of VCT, attitude towards it and practice of uptake among the respondents consisting of both closed and open ended questions. 178 questionnaires were adequately filled, returned within four days of administration and used for analysis.

METHOD OF DATA ANALYSIS

The data collected were coded and analysed using the Statistical Package for Social Sciences (SPSS) Version 21 using descriptive and inferential statistical measures and presented in pie charts, tables with percentages and chi squares.

RESULTS

A total of 178 respondents participated in the study. Their sociodemographic characteristics are presented in Table 1.

Table 1: Socio-Demographic Characteristics of Respondents

Variables	Male		Female		Total	
	Freq	Percent	Freq	Percent	Freq	Percent
Age Distribution						
15-19	6	3.4	15	8.4	21	11.8
20-24	12	6.7	60	33.7	72	40.4
25-29	31	17.4	30	16.8	61	34.2
30-34	4	2.3	20	11.3	24	13.6
TOTAL	**53**	**29.8**	**125**	**70.2**	**178**	**100**
Marital status						
Married	8	4.5	11	6.2	19	10.8
Single	45	25.3	114	64.0	159	89.2
TOTAL	**53**	**29.8**	**125**	**70.2**	**178**	**100**
Religion of Respondents						
Islam	20	11.3	42	23.6	62	34.9
Christianity	33	18.5	83	46.6	116	65.1
TOTAL	**53**	**29.8**	**125**	**70.2**	**178**	**100**
Ethnic Group						
Hausa	18	10.1	33	18.5	51	28.6
Igbo	3	1.7	13	7.3	16	9
Yoruba	5	15.2	65	36.5	19	10.7
Others	27	15.2	65	36.5	92	51.7
TOTAL	**53**	**29.8**	**125**	**70.2**	**178**	**100**
Level of Study						
100	25	14.0	68	38.2	93	52.3
200	18	10.1	44	24.7	62	34.8
300	10	5.6	15	7.3	23	12.9
TOTAL	**53**	**29.8**	**125**	**70.2**	**178**	**100**
Schools of Respondents						
Nursing	35	19.7	74	41.6	109	61.3
Midwifery	18	10.1	51	28.6	69	38.7
TOTAL	**53**	**29.8**	**125**	**70.2**	**178**	**100**

Table 1 revealed that the respondents were within the age range of 15 – 34 years, with a mean age of 22 years ±2. Most of the respondents fell within the age group of 20 – 24 years (40.4%). Students were drawn from

all levels of study (100 – 300level). Most respondents were from year 1 (52.3%), year 2 (34.8%) and the least were from year 3 (12.9%). Majority of the respondents were females (70.2%). This can be related to the fact that the profession initially was dominated by females. Of these, 28.67% were Hausa, 10.7% Yorubas, 9% Igbo and others 51.7% which include (Edo, Idoma, Igala, Ikulu, Bajju, Kagoro, Binawa, Atijap, Adara etc). Majority of the respondents were Christians (65.1%) and others were Muslims (34.9%). Most of the respondents were single (89.2%) with only 10.8% of them married.

Table 2: Knowledge on Human Immunodeficiency Virus and AIDS

a). Source of Knowledge and Means of Transmission		
Source of Knowledge of AIDS	FREQUENCY	PERCENT (%)
Mass Media	26	14.5
Health Workers	132	74.2
Family Members	10	5.6
Friends	3	1.7
Religious Institutions	7	4
Others	-	-
TOTAL	178	100
b). Means of Transmission of HIV/AIDS		
Route of Transmission	Frequency	Percentage of cases (%)
Unprotected Sex	155	87.1
Unscreened Blood Transfusion	146	82.0
Sharing Sharp Object	98	55.1
Barbing Instrument	52	29.2
Pedicure And Manicure	49	27.5
Unprotected Sex	155	87.1
Unscreened Blood Transfusion	146	82.0

Responses Not Mutually Exclusive

All the respondents (100%) had heard about HIV/AIDS and they were all aware of its cause as being Human Immunodeficiency Virus. This is

higher than 87% reported in NARHS (2003) but similar to those carried out on HIV/AIDS in Nigeria; for example in a study among women attending antenatal clinic in Lagos, Nigeria by Aghoghobia (2002) and that at Kano[10] which showed 100% awareness among respondents. The knowledge on cause of HIV/AIDS was 100%. This was also expected based on the type of respondents and level of study. A study carried out by Abiniku (2003) in Jos Plateau State revealed that among women attending ANC in the State Specialist Hospital, 51.2% attributed cause to virus and 12.9% to diabetes. Table 2 (a) showed that most of the respondents got the information from Health workers (74.2%). Table 2(b) showed that, most of the respondents were aware of unprotected sexual intercourse as a route of HIV transmission (82%). All the respondents had heard about HIV/AIDS at one time or the other in their life. This is expected as the study population is that of a highly educated group of people on the subject matter.

The major source of information about HIV/AIDS was health workers (74.2%). In this study, knowledge about symptoms of HIV/AIDS was also very high, weight loss (92.7%), persistent cough (69.1%), persistent fever (58.4%). Almost all respondents (91.0%) agreed that one could not tell if a person is infected with HIV by his/her appearance. This could be attributed to be as a result of the popular campaign slogan "AIDS no dey show for face" generally used by behavioural change communication (BCC) materials such as posters, bill boards and radio etc. The knowledge about the prevention was also high, common with abstinence, screening blood before transfusion; avoid infecting with unsterilized needles and being faithful to one partner. However, despite the attention focused on its use by programmes such as behavioural change communication (BCC), only 64.4% of respondents thought that the use of condom could prevent transmission of HIV infection.

Majority (91%) of the respondents were aware that an infected person can appear healthy. This is represented in the pie chart (figure 1).

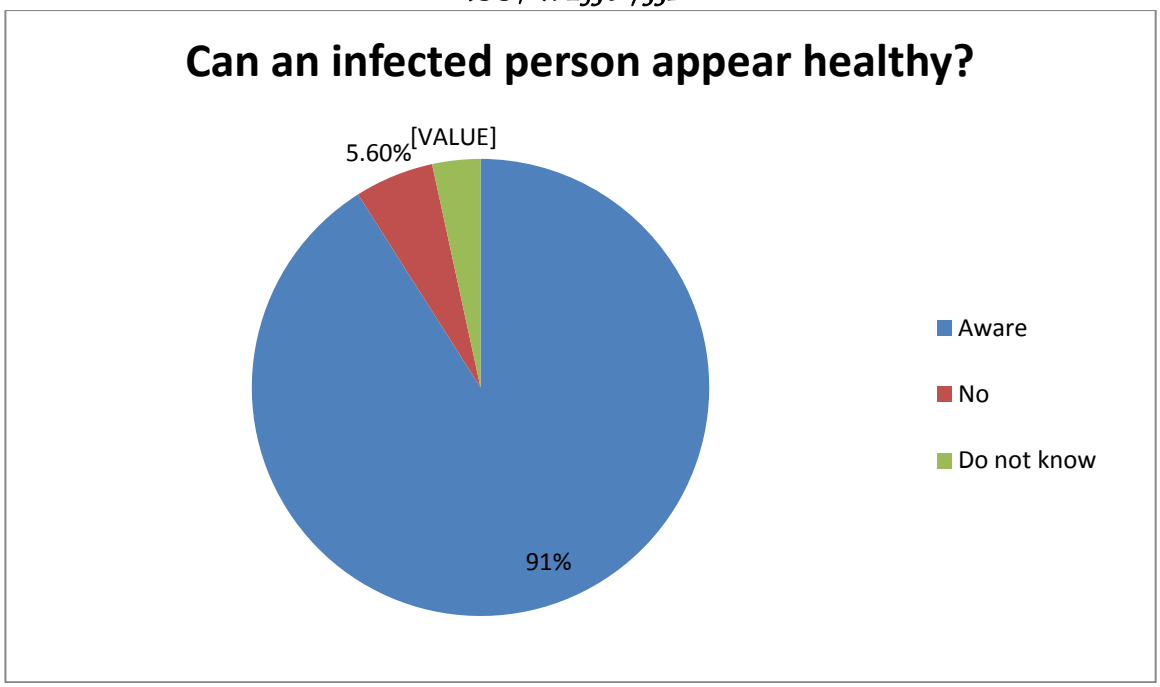


Figure 1: Pie Chart showing response to question "Can an infected person appear Healthy?

Figure 2 showed that, 96.6% of the respondents were aware that HIV/AIDS can be prevented.

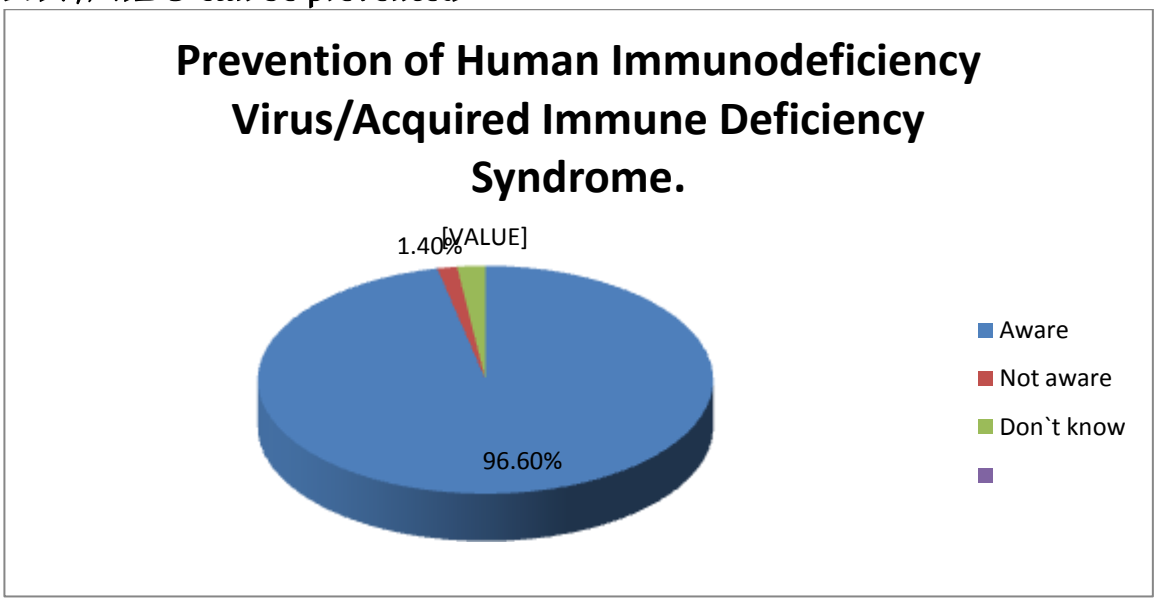
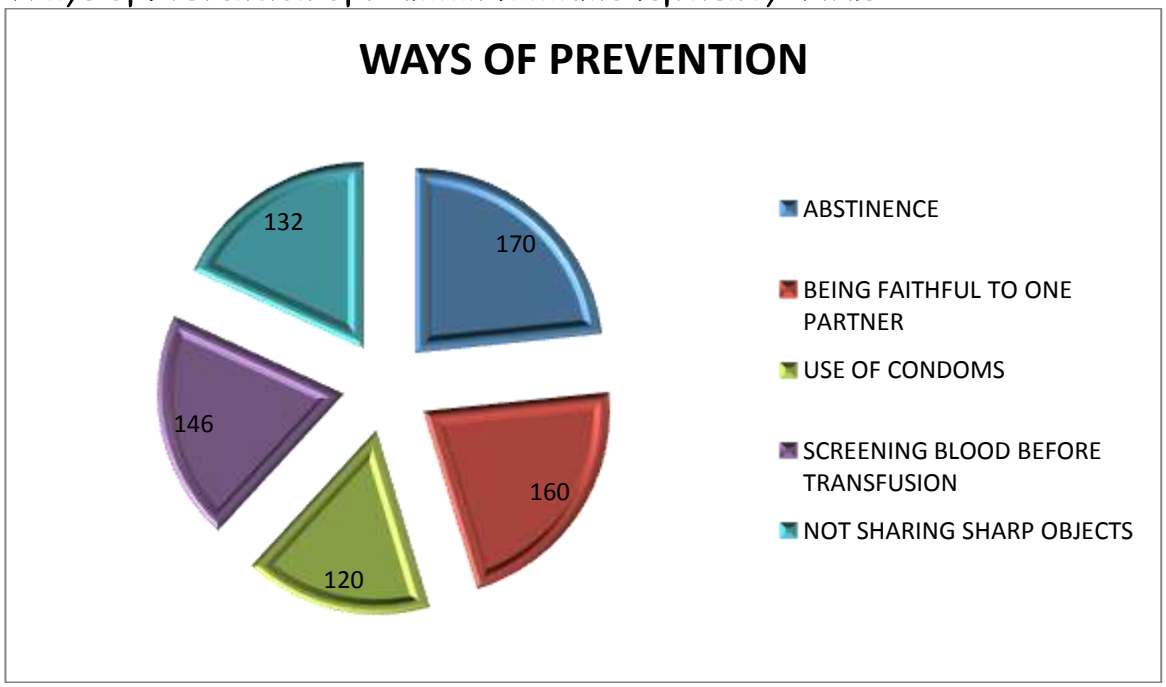


Figure 2: Knowledge on the Prevention of Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome.

Ways of Prevention of Human Immunodeficiency Virus



Responses not Mutually Exclusive

Figure 3: Pie Chart showing response to Ways of Prevention of Human Immunodeficiency Virus

Figure 3 shows that, 95.5% of respondents strongly believed that HIV infection can be prevented by abstinence from sexual intercourse, while 89.8 suggested being faithful to partner.

Table 3: Awareness, Understanding, Source and Purpose of Voluntary Counseling and Testing (VCT).

Awareness of VCT		
Status	Frequency	Percent (%)
Aware	160	89.9
Not Aware	18	10.1
TOTAL	**178**	**100**
Understanding of VCT		
Class of Understanding		
Self Interest HIV Testing	149	83.7

Mandatory Testing	17	9.6
Testing Without One's Knowledge	12	6.7
TOTAL	**178**	**100**
Awareness of Sources of VCT		
Source		
Hospitals	120	67.4
Mass media	30	16.8
Family members	18	10.1
Religious institution	10	5.6
TOTAL	**178**	**100**
Awareness of the Test Counseling		
STATUS		
Aware	154	86.5
Not Aware	24	13.5
TOTAL	**178**	**100**
Purpose of VCT Interpretation		
To Understand And Prepare For HIV Status	92	
To Measure To Protect Oneself To Provide	61	51.7
Entry Point For Prevention, Early Treatment And Care For Infected	25	14.1
TOTAL	**178**	14.1

Table 3(a) showed that, 89.9% of the respondents were aware of VCT. This was higher than that reported in the study in Kano[10], which revealed awareness rate of 71.6% among a formally educated population. Table 3(b) shows that, 83.7% of the respondents understood VCT as self-interest HIV testing. Table 3(c) showed that, 67.4% of the respondents indicated that VCT is available in Hospitals. The study in Kano[10] revealed that only 26% of respondents knew where they could get

the service while none of the respondents understood what it really meant and the steps involved. All respondents indicated that VCT is not available in their schools. Like the information about HIV/AIDS, here, the source of VCT for most respondents was health workers in hospitals (67.4%). Table 3(d) showed that, 86.5% of the respondents were aware of the practice of the pre-test counseling. Table 3(e) revealed that, 51.7% of the respondents considered VCT as necessary to understand ones HIV status.

Result on the attitude towards voluntary counseling and testing showed that all respondents indicated that VCT is necessary.

Table 4: Attitude and Perception towards Voluntary Counseling and Testing

Do you Think you can Benefit from VCT?		
Benefit from VCT	**Frequency**	**Percentage**
Yes	162	91
No	16	9.0
TOTAL	**178**	**100**
Perception of benefits of VCT		
Benefit of VCT		
Opportunity For Early Access to Treatment	36	20.2
Increased Perception About HIV	128	70.8
Living Positive Life Irrespective of Outcome	12	6.7
Allay Anxiety	4	2.3
TOTAL	**178**	**100**
Can Voluntary Counseling and Testing Reduce HIV Transmission?		
Whether VCT can reduce HIV transmission		
Yes	162	91
No	16	9.0

I don't know	10	5.6
TOTAL	178	100
d) Method of Encouraging Students to Utilize Voluntary Counseling and Testing		
Method		
Availability And Accessibility to Centres	45	25.2
Improved Quality Of Services	40	22.5
Free Services	38	21.3
Easy Access To Treatment And Prophylactics	35	19.5
Improve Staff Attitude	20	11.3
TOTAL	178	100
Perception of Factors Discouraging Utilization of VCT		
Factor		
Fear Of Being Positive	92	51.7
Stigmatization	60	33.7
Rejection	26	14.6
TOTAL	178	100
Should VCT be made Mandatory for Students?		
Response		
Yes	100	56.2
No	53	29.8
I don't know	25	14
TOTAL	178	100
Unwillingness to Undergo VCT		
Response		
Willing	91	51.1
Unwilling	87	48.9
TOTAL	178	100

Table 4(a) indicated that, 91% of the respondents were of the view that they can benefit from VCT. Table 4(b) showed that, majority (70.8%) of the respondents considered it a means of measuring perception about HIV. Table 4(c) showed that, 79.8% of the respondents indicated that it can reduce HIV transmission. Table 4(d) showed that, 25.2% of the respondents considered availability and accessibility to centers for screening as an important means of encouraging students to utilize VCT. Table 4(e) showed that, half (51.7%) of the respondents consider fear of being positive as the most important factor discouraging utilization of VCT. Half (51.1%) of the respondents were of the opinion that VCT should be made mandatory for all students. Reason is that it encourages positive sexual behavior and Table 4(f) indicated that, 51.1% of the respondents were willing to undergo testing in order to know their status. Attitude towards voluntary counseling and testing among the respondents was not far from expected. However, the rate of willingness of respondents to take VCT to the rate of unwillingness was marginally higher with only 51.1% willing and 48.9% not willing. Study on knowledge, attitude and practice towards voluntary counseling and testing among university students in North West Ethiopia[19] showed that 73.3% had positive attitude towards VCT for HIV.

Table 5: Uptake of Voluntary Counseling and Testing (VCT).

Prior HIV Testing among Respondents.		
Response	**FREQUENCY**	**PERCENT (%)**
Have been tested before	106	59.6
Have never been tested	72	40.4
TOTAL	**178**	**100**
Reason for Prior Testing.		
Reason		
During illness	31	29.2
Marriage	12	11.3
Voluntarily	63	59.5
TOTAL	**106**	**100**

Where the Test was Carried Out?		
Response		
Hospital	41	38.7
Other places	65	61.3
TOTAL	**106**	**100**
d) Whether Counseled When Receiving Voluntary Counseling and Testing		
Response		
Yes	37	39.1
No	69	60.9
TOTAL	**106**	**100**
e) Results of Prior Testing of Respondents		
Reason		
Positive	3	2.8
Negative	76	71.7
no response	27	25.5
TOTAL	**106**	

Table 5(a) indicated that, 59.6% of the respondent had been tested before. This was not in conformity with the study at Kano[10] where 98.6% of respondents never had VCT previously. This was also not in conformity with the findings obtained in NDHS[15] where more than 80% of Nigerians had never been tested. But it was similar to a study conducted in Zaria, Kaduna Sate[15] where just a few women (14.5%) had carried out the test. A study on HIV voluntary counseling and testing practices among 350 military and civilian cantonment residents in Southeastern Nigeria[16] found that one hundred and forty-five (41.4%) respondents had ever been tested for HIV. Another study at Ethiopia[17] on factors affecting voluntary HIV counseling and testing among men found that 21.9% of urban men and 2.6% of rural men had ever tested for HIV through VCT. Fear of positive results, stigma and discrimination following the positive results were reported as main barriers for VCT uptake. This is similar to the results from these studies which revealed that reasons for not taking VCT was basically fear of being positive, stigmatization, rejection and divorce. Table 5(b) showed that, 59.5% of the respondents were tested voluntarily and not as part of marriage

counseling or investigation for ill health. Table 5(c) indicated that, only 38.7% of the respondents were tested in the hospital, while others tested in other places like chemists, laboratories and pharmacies. Only 39.1% of the respondents were counseled when they received VCT (Table 5d). Majority (71.7%) were tested and found negative, 2.8% were found positive while 25.5% did not respond to the question (Table 5e).

Table 6: Opinion on How Voluntary Counseling and Testing can be Improved

Factors	Frequency	Percent (%)
Proper Enlightenment Of HIV/AIDS	60	33.7
Stressing The Importance Of Knowing One's Status And Opting For VCT	20	11.2
Testing Should Be Mandatory For Students	17	9.6
VCT Should Be Made Free For All Students	43	24.1
VCT Should Be Taken By Every Student Individually	38	21.4
TOTAL	**178**	**100**

Table 6 showed that, 33.7% of the respondents considered proper enlightenment on HIV important in improving uptake of VCT.

Table 7: Knowledge of Voluntary Counseling and Testing among Respondents

Knowledge Of VCT	Frequency	Percentage (%)
Poor	10	5.6
Fair	60	33.7
Good	108	60.7
TOTAL	**178**	**100**

Only 0.7% of the respondents had good knowledge of VCT (Table 7).

Table 8: Attitude towards Voluntary Counseling and Testing among Respondents.

Attitude	Frequency	Percentage (%)
Positive	130	73.0
Negative	48	27.0
TOTAL	**178**	**100**

Majority (73%) of the respondents had a positive attitude towards VCT (Table 8).

Table 9: Cross Tabulation between Gender and Perception of Necessity for Voluntary Counseling and Testing.

	YES	**%**	**NO**	**%**	**TOTAL**
Male	51	96.2	2	3.8	53
Female	115	92	10	8	125
TOTAL	**166**		**12**		**178**

$X2 = 1.08$, df = 1, $P < 0.05$, P value = 3.841

The cross tabulation shows that, there is a significant statistical difference between gender and perception of necessity of VCT ($X2 = 1.08$, df = 1, $P < 0.05$, P value = 3.841). Majority (96.2%) of the male respondents and females (92%) were of the view that VCT is necessary (Table 9). An Ethiopian study[19] found that sex showed a statistically significant association with Voluntary Counseling and Testing.

Table 10: Cross Tabulation of Level of Study by the Knowledge that an Infected Person can Appear Healthy.

Year of study	**Yes**	**No**	**I don't know**
100	86	6	1
200	58	3	1
300	18	2	3
TOTAL	**162**	**11**	**5**

$X^2 = 11.77$ df = 4 $P < 0.05$, P value = 19.675

Table 10 showed that, there is a significant statistical relationship between level of study and knowledge that an infected person can appear infected ($X^2 = 11.77$, df = 4 $P < 0.05$, P value = 19.675).

CONCLUSION

The study has demonstrated that majority of the students of Schools of Nursing and Midwifery, Kaduna State are knowledgeable about HIV/AIDS and VCT. They showed a positive attitude towards VCT and their views on how to improve its coverage. These groups of respondents are in contact with the populace and will therefore help create awareness especially on stigmatization which is a major barrier preventing uptake of VCT. There is need for the Government to provide more VCT centres and improve access to services through innovative, efficient and practical modes of delivery.

REFERENCES

USAID.http://data.unaids.org/pub/EPISlides/2007/2007_epiupdate_en.pdf(accesse 19th September 20011).

1. National Action Committee of AIDS: Nigeria HIV/AIDS National Strategic Framework, Federal Republic of Nigeria, 2006.
2. National Action Committee of AIDS: Nigeria HIV/AIDS National Strategic framework, Federal Republic of Nigeria, 2005.
3. United Nations, (United Nation Joint Programme on AID/WHO AIDS Epidemic Update). WHO Journal. 2003; 39(3) 4 – 34.
4. William D.C.: HIV Infection among Men with STDs. New England Journal of Medicine, 1998; 319: 274 – 278.
5. Akinsete I., (Safe blood transfusion in Nigeria). A Necessity. A paper Presented at the First Nigerian National Conference on HIV/AIDS in Abuja, Nigeria, 2001.
6. USAID: Nigeria Country Profile (HIV/AIDS). www.usaid.gov/location/Sub-saharanafrica/countries/nigeria. visited on August 15, 2007.
7. Federal Ministry of Health, Nigeria: National Guideline on Prevention of Mother to Child Transmission of HIV/AIDS (PMTCT), 2005.

8. Sweat M., Gregorich S., Sangiwa G., Furlonge C., Balmer D., Kamenga C., Grinstead O., Coates T., (Cost-effectiveness of Voluntary HIV-1 Counseling and Testing in Reducing Sexual Transmission of HIV-1 in Kenya and Tanzania). Lancet 2000;356: 113-121.

9. Illiyasu Z., Isa S., Mohammed K., (Knowledge of HIV/AIDS and Attitude Towards Voluntary Counseling and Testing among Adults), 2006.

10. Charles M.P., Kweka E.J., Mahande A.M., Barongo L.R., Shekalaghe S., Nkya H.M., Lowassa A., Mahande M.J., (Evaluation of uptake and attitude to voluntary counseling and testing among health care professional students in Kilimanjaro region, Tanzania). BMC Public Health 2009; **9**:128 doi:10.1186/1471-2458-9-128. 9.

11. Ikechebelu I.J., Udigwe G.O., Ikechebelu N., Imoh L.C., (2006). (The knowledge, attitude and practice of voluntary counseling and testing (VCT) for HIV/AIDS among undergraduates in a polytechnic in Southeast, Nigeria). Niger J Med 15: 245-249.

12. Ike S.O., Aniebue P.N., (HIV/AIDS perception and sexual behavior among Nigerian University students), Niger J Clin Pract 2007; 10: 105-110.

13. National Population Commission (2008): "Nigerian Demographic and Health Survey

14. Adelaiye R.S., (Factors Influencing the Uptake of Voluntary Counseling and Testing (VCT) of HIV in Antenatal Clinic in Ahmadu Bello University Teaching Hospital, Zaria), A Thesis Submitted to the Post Graduate School, Ahmadu Bello University, Zaria in Partial Fulfillment of the Requirements for the Award of Master in Public Health Degree, 2005.

15. Azuogu B.N., Ogbonnaya L.U., Alo C.N., (2011), (HIV voluntary counseling and testing practices among military personnel and civilian residents in a Military cantonment in Southeastern Nigeria). National Library of Medicine, National

Institute of Health. HIV/AIDS Research and Palliative Care. 2011; 3: 107–116, Published online 2011 Oct 13. doi: 10.2147/HIV.S23774.

16. Tesfaye, Ingvild, Sandøy and Knut (Factors affecting voluntary HIV counseling and testing among men in Ethiopia: a cross-sectional survey). BMC Public Health 2012; **12**:438, **DOI:** 10.1186/1471-2458-12-438© Leta et al.; licensee Bio Med Central Ltd. 2012

17. Zelalem A., Aregawi Y., Yitayal S., Abebe A., Wubet B., Biniam M., Belayenesh T., Knowledge, Attitude and Practice towards Voluntary Counseling and Testing among University Students in North West Ethiopia: a cross sectional study. BMC Public Health, 2013: **13**:714. **DOI:** 10.1186/1471-2458-13-714

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Control of Post-Harvest Fungal Rot Agents of Tomato (*Lycopersicon esculentum* Mill) with *Vernonia amygdalina* (Del.) and *Moringa oleifera* (Lam.) in Yola, Adamawa State

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ABSTRACT

Post harvest spoilage caused by fungal pathogens has been reported to reduce the quality of tomato fruits. Identifying these problems to prolong shelf life will make tomato available in Yola. Based on the foregoing, experiments were conducted in the Department of Plant Sciences of Modibbo Adama University of Technology, Yola. A two (2) factorial fitted into completely randomized design was used with 3 replications, the results were analyzed with Statistic for Applied Sciences package and Least Significant Difference was used to separate means that were significant. The result obtained showed that five species of fungi identified as *Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Fusarim solani* and *Geotrichum candidum* were associated with the spoilage of tomato fruits. All the fungi were found to be pathogenic on tomato in Yola. The biocidal activities of two plant material namely *Moringa oleifera* and *Vernonia amygdalina* were investigated on the fungal isolates. Varying concentrations (40%, 60% and 80%) of aqueous leaves extract were applied in-vivo on the fungal isolates and the mycelia growth inhibition was monitored. The aqueous leaf extracts were found to be effective in inhibiting the mycelial growth of the pathogens and higher concentrations of the plant extracts exhibited more inhibition. The findings from this study are significant as it contributes information on the post harvest pathology and management of tomato in Yola using plant extract.

Keywords: Control, pathogens, post-harvest, rot, tomato

INTRODUCTION

Tomato is perhaps the most important popular vegetable crop grown all over the country, both the wet and dry season cropping systems contribute immensely to the national requirement, but the bulk production is from the dry season cropping system grown yearly under irrigation in southern states (Ojo *et al.*, 2009). Tomatoes are considered to be one of the most economically important crops of all those that exist in the world (Rahman *et al.*, (2003). Tomatoes are also one of the main ingredients in hundreds of dishes and products that are sold in supermarkets throughout the developed world, this means that the demand of tomatoes from countries will be extremely high (Mourvaki *et al.*, 2005).

Tomato commercialization is limited by rotting caused by *Alternaria alternata* (Fr.:Fr.) Keissl. Or by *Botrytis cinerea* Pers.:Fr. (Jones et al., 1991). In developing countries, losses of fruits and vegetables during post- harvest fluctuate between 20 and 50% (Kader, 2002). It has been estimated that 20 - 50% of tomato fruits harvested for human consumption are lost through microbial spoilage. Other losses result from damage by dynamic stresses during transit, and through rough handling during loading and unloading (Aworth, 1985).

A number of economically important tomato diseases caused by fungi are transmitted by seeds or transplants (Ivanović and Mijatović, 2003). Tomatoes are parasitized by a number of pathogens, including *Fusarium oxysporum* Schlecht, the causal agent of fusarium wilt of tomato (Aleksić *et al.*, 1990; Ivanović and Mijatović, 2003), which is one of the most important species as tomato pathogen (Jones *et al.*, 1982; Smith *et al.*, 1988; Agrios, 2005). In an indoor environment due to high temperature and humidity, *F. oxysporum* can cause significant damage (Mijatović *et al.*, 2007).

Due to increased awareness about the risks involved in the use of synthetic pesticides, much attention is being focused on alternative method of pathogen control. The spiraling up cost of chemical fungicides particularly in those countries where pesticides are important, pollution to soil, water and air by the accumulation of obnoxious chemical

residues due to continuous use of fungicides and development of resistant races to these chemicals are there (Momein and Abeer, 2012). This research is intended to provide alternative methods of controlling post-harvest rot of tomato which are ecologically friendly, cheap, safe and specific to fungal pathogens.

MATERIALS AND METHODS

Source of Samples

Tomato fruits with symptoms of spoilage were randomly collected from the market stalls at Yola Main market and Jimeta Modern market on the 16 July, 2013. Fresh tomato fruits were also collected and packed into a sterilized polythene bag and taken to the laboratory for pathogenicity test on the 14 August, 2013.

Medium for Isolation and Identification

The medium that was used for this study was Potato dextrose Agar (PDA) (Smith and Onion, 1983) .

Preparation of Potato Dextrose Agar (PDA)

The sterilized potato dextrose agar was prepared from fresh healthy Irish potato tubers (*Solanum tuberosum* Lin.). Tubers were washed with tap water and peeled with a knife. Two hundred grams (200g) of the peeled and sliced pieces of the Irish potato tubers were gently boiled in 600ml of distilled water in a liter Erlenmeyer conical flask over a PHYWE hot plate for one hour. After cooling the supernatant was decanted into a liter Griffin Ambergrade measuring cylinder to obtain one liter of the supernatant.

Twenty grams of each of plain agar and glucose were dissolved into 1 liter of the supernatant and homogenized in an Arnold and sons (Basildon) Ltd. autoclave. This was then dispensed into bottles and autoclaved at 121°C 1.1kg cm^2 for 15 minutes. After cooling, streptomycin (a broad spectrum antibiotic) was added at 250mgl^{-1} of potato dextrose agar (Smith and Onion, 1983).

The resulting medium was then dispensed into 9 cm^3 sterile Petri-dishes (about 20ml per Petri-dish) under aseptic conditions with the cover of

the Petri-dishes only slightly opened. The plates were left on clean and alcohol swapped table in the inoculation chamber under ultra-violet light to solidify prior to inoculation.

Isolation of the pathogens

The method of Thomas (1979) was used; under aseptic conditions, the diseased tissues (DT) from the periphery of the rotted tomato fruits were sectioned into 5mm square pieces with a heat sterilized scalpel. Pieces were picked with flamed, then cooled pair of forceps. These portions were transferred into 0.1% mercuric chloride solution contained in a sterile 9cm Petri-dish for surface sterilization for 30 seconds.

The sterilized portions were washed in three changes of sterile distilled water and dried between sterile filter papers. With a flamed and cooled pair of forceps a sterilized piece of the tomato was then plated out on sterile solidified potato dextrose agar (PDA) and incubated at temperature of 27 $\pm$ 2°C for seven days before sub-culturing on new set of sterilized PDA plates. Pure isolates of fungal species were obtained from hyphal tips of growing colonies by using a sterilized needle and repeated sub-culturing on solidified sterile dextrose agar slants in Mc Cartney bottles until pure cultures were obtained. These were appropriately labeled according to organisms. The slants were initially corked loosely to enable the content fungus to grow. They were then tightly corked and stored at a temperature range of 0-10°C in a refrigerator to serve as stock cultures.

Identification of isolated fungi

Microscopic examination was made after examining the colony characteristics on media. A sterile needle was used to take a little portion of the hyphae containing spores on to a glass slide which was stained with Lactophenol cotton blue and observed under the microscope for the structures of the fungi (Frazier, 1978). Morphological structures were observed under the microscope and compared with the structures in (Alexopoulus *et al.*, 2002).

Pathogenicity Test

Pathogenicity test was carried out using techniques of Okigbo *et al.* (2009). Fresh tomato fruits were washed with sterile distilled water, wiped dry using Whatman No.1 filter paper and surface sterilized with 0.1% mercury chloride solution to remove surface contaminants and rinsed in three changes of sterile distilled water. A sterile razor blade was used to make a 2mm cut on the fruits and then culture of the isolates were introduced into the open cut surface and replaced with the core and sealed with Vaseline jelly. Fruits were inoculated in three replicates. The fruits were incubated for 5 days. On establishment of disease symptoms, inocula from the infected fruits were taken and cultured until pure cultures were obtained. The morphological and microscopic characteristics of the Isolates were observed and compared with those of Alexopoulos *et al.* (2002).

Collection and Preparation of Leaf Extracts

The method of Ijato *et al.* (2011) was used to prepare aqueous leaf extracts; fresh leaves of *Vernonia amygdalina* and *Moringa oleifera* plants were collected from Sangere village, Girei local Government, Adamawa State on 21 August, 2013. These plants were taken to the Plant Sciences Department of Modibbo Adama University of Technology, Yola.

The collected plants were washed thoroughly under running tap water and were allowed to air dry for 7 days. These were grinded separately. Thirty grams of each sample was added to 15ml of distilled water in separate conical flasks. This was vigorously shaken and left to stand for 24 hours. The samples were filtered with 3 layers cheese cloth and filtrate extract preparation of 80, 60 and 40% concentrations were used as the aqueous extract.

Effect of Plant Extracts on Fungal Mycelia Growth

The approach of Ijato (2011) was used to evaluate the effect of the extract on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plate. The point of intersection indicates the centre of the plates. This was done before dispensing PDA into each of the plates. The extracts were poured into

the flask plugged with cotton wool and heated for about 10 minutes to avoid contamination (Madari and Singh, 2005). About 2 ml of the extract of *V. amygdalina* and *M. oleifera* were separately introduced into the Petri-dish containing the media and the pure isolate (poisoned food method), Control experiments were without addition of any plant extract but sterile distilled water. Fungi growth inhibition was determined in terms of percentage spore germination (Nene and Thalpiyal, 2000).

Inhibition percentage (%) = DC- DT / DC X 100

Where: DC - Average Diameter of fungal spore germination in control

DT - Average diameter of fungal spore germination with treatment.

Experimental Design and Data Analysis

The experimental layout was a completely randomized design of two plant extracts. Each plant extract was at the same concentration. The experiment was replicated three times. All the data were analyzed using analysis of variance (ANOVA) according to Gomez and Gomez (1984). Least Significant Different (LSD) according to Scheff (1953) was used to separate the means where there was significant difference. The statistical package used to analyze the results was Statistics for Applied Sciences.

RESULTS

Isolation and Identification of Fungi

The fungal isolates from the tomatoes studied in this research which was found through pathogenicity test to be responsible for the post harvest rot of Tomato fruits in Yola were identified as *Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Fusarim solani* and *Geotrichum candidum.*

Description of isolates

Rhizopus stolonifer (Ehrenb. Ex. Fr) Lind. (Zygomyces)

Colony colour was whitish three days after plating becoming brown 5-6 days due to brownish s*porangiophores* and brown black sporangia, with extensive mycelia growth in culture, Sporangiosphores in groups from almost colourless to dark brown with slightly rough-walled stolons opposite the branched rhizoids which grew and covered the Petri-dish six days after plating, Sporangia is globose which became blackish-brown at maturity. Sporangiospores are irregular in shape.

Fusarium oxysporum Schlecht. f. sp. (Deuteromycetes)

On potato-dextrose agar fungus isolated from diseased tomato fruits formed light pink aerial mycelium and red pigment in the agar. All observed isolates formed macroconidia as eliptical, gradually pointed or curved edges (pointed end). Most often they were short and had three septa. They formed a large number of unicellular, elyptical, oval-shaped or kidney- shaped microconidia clustered into so-called false heads. Microconidia or chlamydospores were not formed. Macroconidia were hyaline.

Geotrichum candidum Link (Deuteromycetes)

On Potato dextrose agar, colonies are fast growing covering the face of the Petri-dish in five days, the colour changes from white to cream, dry and finely suede-like with no reverse pigment. Hyphae are hyaline, septate, branched and break up into chains of hyaline, smooth, one-celled, subglobose to cylindrical arthroconidia. They are released by the separation of a double septum.

Fusarium solani Mart. (Saccharomyces).

On potato dextrose agar medium, *F. solani* produces sparse to abundant, white cream mycelium. Macroconidia have three to four septa on average, which are slightly curved, wide and thick walled and have a slightly blunted apical end. Microconidia are abundant, oval to kidney shaped, and formed in false heads on very long monophialides. Chlamydospores are abundant.

Aspergillus niger (Deuteromyces)

On potato dextrose agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large, globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae.

Pathogenicity Test

The result of the pathogenicity tests showed that all the isolated fungi were pathogenic on tomato fruits, from visual observation the symptoms were similar to those of the rotten tomato fruits from which the organisms were isolated, under the microscope, the fungi re-isolated were the same as those used for the pathogenicity test. Also the wound created by the razor blade initiated spoilage as it formed opening for the easy penetration of the pathogens.

Rhizopus stolonifer completely disintegrated the affected tissue (100% infection) with extensive *mycelia* growth covering the fruit and on PDA within 4-5 days. The rot induced by *Fusarium oxysporum and Fusarium solani* exhibited moderate to severe infection 4-7 days after inoculation. Water soaked lesions that spread rapidly covering the fruit was the usual pattern of pathogenicity. The fruit then became covered by white cottony mycelium topped by pinkish mass of fungal spore that completely disintegrated the tomato 7 days after inoculation. *Geotrichum candidum* infection became manifested 2 days after inoculation and became severe 4-7 days later. This was characterized by whitish cheesy-like lesion on rotted fruits and on agar plate. Rot development on inoculated fruit was gradual. In all cases soft rot was induced that became watery accompanied by offensive odour.

Effect of aqueous plant extracts on isolated organisms

Analysis of Variance (ANOVA) on the effect of aqueous plant extracts on isolated organisms showed that all the means are highly significant ($P < 0.01$). Table 1 showed that the highest inhibition caused by the aqueous extract of *M. oleifera* and *V. amygdalina* was on the

growth of *F. solani* (62.26% and 61.96%) respectively followed by *A. niger* (61.93% and 59.51%) respectively and the least mean percentage inhibition was recorded on the growth of *F. oxysporum* (43.00% and 53.45%) respectively. The mean inhibition of *V. amygdalina* was higher (57.38) than that of *M. oleifera* (54.50).

Table 1: Inhibition Effect of Aqueous Plant Extract on the Mycelial Growth of Fungi Pathogens

Organisms	*Moringa oleifera*	*Vernonia amygdalina*
Geotrichum candidum	50.43	55.48
Aspergillus niger	61.93	59.51
Fusarium oxysporum	43.00	53.45
Fusarim solani	62.26	61.96
Rhizopus stolonifer	54.88	56.55
Mean	**54.50**	**57.38**
LSD	**2.62**	**2.32**
P – Value	**0.001**	**0.001**

Interaction of aqueous plant extracts and concentration on isolated organisms

The interaction effect of aqueous plant extracts and concentration on isolated organisms showed that at 40% concentration both *Moringa oleifera* and *Vernonia amygdalina* had the lowest mean percentage inhibition of 44.18% and 46.10% and the highest mean percentage inhibition was recorded to be 66.14% and 69.15% at 80% concentration respectively, the mean inhibition of *V. amygdalina* was higher (57.38) than that of *M. oleifera* (54.50) at $P < 0.01$ all means are highly significant (Table 2).

Table 2: Interaction of Aqueous Plant Extracts and Concentration on Isolated Organisms

Concentration (%) *Vernonia amygdalina*		*Moringa oleifera*
40	44.18	46.10
60	53.18	56.10
80	66.14	69.14
Mean	**54.50**	**57.38**
LSD	**2.03**	**1.80**
P – Value	**0.001**	**0.001**

DISCUSSION

Isolation and Identification of Rot Agents

Five fungal isolates from the tomatoes studied in this research were responsible for the post harvest rot of Tomato fruits in Yola and these were *Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Fusarim solani* and *Geotrichum candidum*. These pathogens also gained entry through injuries caused by rough handling, poor road and storage facilities (Liu and Ma, 1983).

Ijato *et al.* (2011) reported that *Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporum,* Geotrichum *candidium* were isolated from infected tomato fruit parts in Ado-Ekiti. Uke *et al.* (2012) also reported that *Helminthosporium solani, Aspergillus niger, Penicillium digitatum and Mucor piriformis* were responsible for post harvest fungal rot agents of tomatoes in Nsuka. *Fusarium moniliforme, Rhizopus stolonifer and Geotrichum candidum* were also reported associated with the spoilage of tomato fruits (Chuku *et al.,* 2010).

Effect of Plant Extract on Mycelia Growth of Fungi Pathogens

Aqueous leaf extracts of *M. oleifera* and *V. amygdalina* inhibited the growth of the pathogens. Higher concentrations exhibited more inhibition than lower concentrations. This result is in line with the report of Ijato *et al.* (2011) who reported aqueous extract of *V. amygdalina* (80%) had high inhibitory effect against *Geotrichum*

candidium and *Aspergillus niger* than 60% aqueous concentration of the test plant extracts, it is also in line with the work of Chuku *et al.* (2010) who reported that at higher quantity (3gram) Ginger and Garlic had higher inhibition percentage of fungal mycelia growth than at lower quantity of 1gram.

Ijato *et al.* (2011) reported that the inhibitory effect of the plant extracts could be alluded to the presence of antimycelia substances. Greater inhibition of fungal growth was observed at higher concentrations of the aqueous and extracts. *R. stolonifer, F. oxysporum,* F. solani, *G. candidium* and *A. niger* are common pathogenic fungi which cause tuber rot, fruit and vegetable rot. The results of the present investigation are vivid indications for the potential of plant extracts to control fungal pathogens.

It is also clear from the result that both the test plant extracts significantly reduce the radial growth of isolated fungi. It seems that the antifungal and the antimicrobial effects are the results of many compounds acting synergistically (Bangamboula *et al.* 2004).

CONCLUSION

This work has shown that the pathogens responsible for the post harvest rot of tomato fruits in Yola were *Rhizopus stolonifer, Fusarium oxysporum, Aspergillus niger, Fusarim solani* and *Geotrichum candidum*. Aqueous leaf extracts of *Moringa oleifera* and *Vernonia amygdalina* are found to be effective in inhibiting the mycelial growth of the pathogens and higher concentrations of the plant extracts exhibited more inhibition than lower concentrations. *Fusarium oxysporum* was the least inhibited pathogen and *Fusarium solani* was the most inhibited.

REFERENCES:

Agrios, G.N. (2005). **Plant Pathology**, (5th Edition), Academic Press, New York. p 922.

Aleksić, Ž., Aleksić, D. and Šutić, D. (1990). Bolesti povrća i njihovo suzbijanje. Nolit, Beograd.

Alexopoulos, C.O., Mims, C.W. and Blackwell, M. (2002). **Introductory Mycology.** (3rd Edition), John Wiley and Sons, New York, pp 204-340

Aworth, O.C. (1985). Preservation of Perishable Food Commodities through Processing. *Nigerian Food Journal,* 2:2.

Bangamboula, C.F, Uyttendaele M., Debevere J. (2004). Inhibitory effect of thyme and basic essential oils, carvacrol, thymol, estragol, *Microbial.,* **21**, 33-43

Chuku, E.C, Osakwe, J.A and Daddy-West, C. (2010). Fungal Spoilage of Tomato *(Lycopersicon esculentum mill),* Using Garlic and Ginger. *Journal of Research in Biosciences* **6**(1), 86-90

Frazier W.C. (1978). **Food Microbiology**. Tata Mcgraw hill Co. Inc. USA, pp 20-40

Gomez, K. A. and Gomez A. A. (1984). **Statistical procedures for Agricultural Research** (2nd ed). John Wiley and Sons, p. 680

Ijato J. Y, Otoide J. E, Ijadunola J .A and Aladejimokun A. O. (2011). Efficacy of antimicrobial effect of *Venonia amygdalina* and *Tridax procumbens* in *in vitro* control of tomato (*Lycopersicum esculentum*) post harvest fruit rot. Report and Opinion, 2011; **3**(1), 120- 123, Retrieved 15 march, 2013, from: **http://www.sciencepub.net/reports**

Ivanović, M. and Mijatović, M (2003). Patogene gljive semena povrća. Poljoprivredni fakultet, Novi Sad.

Jones, J.P., Jones, J.B. and Miller, W (1982). Fusarium wilt on tomato. Fla. Dept. Agric. & Consumer Serv., Div. of Plant Industry. *Plant Pathology Circular.* **237**.

Jones, J.B., Jones J. P., Stall R. E., Zitter T. A. [eds.] (1991). *Compendium of Tomato Diseases. The American Phytopathological Society*, St. Paul, Minnesota.

Kader, A.A. (2002). *Post-harvest Technology of Horticultural crops.* University of California, **Agriculture and Natural Resources**, p 535.
Liu, M.S. and Ma, P.C. (1983). *Post-harvest problems of vegetables and fruits in the Tropics and subtropics.* Asian Vegetable Research and Development Center. 10th Anniversary monograph Series. Taiwan, China, p14.

Madari,S and R.P Singh (2005). Management of mushroom pathogens through botanicals. *Ind.Phyto Pathology,* **58**, 189-193.

Mijatović, M., Obradović, A., Ivanović, M. and Zaštita povrća (2007). Comparison of three molecular methods for the characterization of *Fusarium oxysporum* strains. **Phytopathology**, **85**, 579-585.

Momein H. El-Katatny and Abeer S. Emam (2012). Control of Postharvest Tomato Rot by Spore Suspension and Antifungal Metabolites of *Trichoderma Harzianum. Journal of Microbiology, Biotechnology and Food Sciences*. **1(6),** 1505-1528

Mourvaki, E.; Gizzi, S.; Rossi, R.; Rufini, S. (2005). "Passion flower Fruit — A "New" Source of Lycopene". *Journal of Medicinal Food,* **8**(1), 104–106.

Nene, Y and Thapilyal L. (2000). **Poisoned food technique of fungicides in plant disease control** (3rd Ed). Oxford and TBH Publishing Company, New Delhi

Ojo M.A, Ibrahim O.A and Mohammed U. S. (2009). Profitability and production function of small scale irrigated tomato production in Niger state, Nigeria. *Continental Journal of Agricultural Economics* **3**, 16–22.

Okigbo, R.N., Ramesh P. and Achusi, C.T (2009). Post-Harvest Deterioration of Cassava and its Control Using Extracts of *Azadirachta indica* and *Aframomum melegueta. E-Journal of Chemistry* **6**(**4**), 1274-1280.

Rahman, S. A. and Lawal, A. B. (2003). Economic analysis of maize-based cropping systems in Giwa Local Government Area of Kaduna State, Nigeria. *International Journal of Agricultural Sciences, Science, Environment and Technology.* **3**, 139-148

Scheff, H. (1953). A method of judging all contrast in the Analysis of Variance. **Biometrica. 40**, 104-107

Smith, D. and Onion, S. A. (1983). The Preservation and Maintenance of Living Fungi. Commonwealth Mycological Institute Kew, pp 63

Smith, I.M., Dunez, J., Phillips, D.H., Lelliott, R.A, Archer, S. A. (eds.) (1988). **European Handbook of Plant Diseases**. Blackwell Scientific Publications, Oxford, UK, pp 1-58.

Thomas, G. A. (1979). **Medical Microbiology**. Macmillan publishers London, p 403.

Ukeh, J.A and Chiejina, N.V. (2012). Preliminary Investigations of the Cause of Post - harvest Fungal Rot of Tomato. *Journal of Pharmacy and Biological Sciences.* **4**(**5**), pp 36-39.

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Quality Control of Haemoglobin (HB) and Packed cell Volume (PCV) Results in some Haematology Laboratories in Port Harcourt Metropolis

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Abstract
The quality control of haemoglobin (Hb) and packed cell volume (PCV) results in some haematology laboratories in Port Harcourt metropolis has been determined in this study. A total of nine laboratories were selected from Port Harcourt to participate in this study and they were identified with codes. 50.0ml of blood sample was collected from a female of 24 years of age. 10,ml of blood was dispensed into each of 50 plain specimen bottles to make 50 samples. The 50 samples were divided into five groups making a total of 10 samples in each group. Five samples were picked randomly one from each group and dispatched to each of the participating laboratories while the remaining five samples were used by the control research laboratory. Six of the laboratories estimated haemoglobin by cyanmethaemoglobin method while seven of them estimated the packed cell volume by the microhaematocrit method. It was shown from the results that the overall performance of all the participating laboratories were generally of poor accuracy and imprecise. The study also concluded from the findings that the poor performance was due to the non compliance to the standards that ensures good quality of laboratory determinations and therefore recommends for the institution of quality assurance programmes to ensure the highest standard of performance in accuracy and precision that is both practical and useful for diagnostic purposes.
Keywords: Quality, Control, Haemoglobin, Microhaematocrit, Imprecise, and Inaccuracy

INTRODUCTION

A quality control (QC) protocol for hematology, as for other sections of the laboratory, should encompass both internal and external QC programs. The extent to which a hematology laboratory should be

involved depends upon various factors, including availability of facilities, financial resources, range of tests, workload, the number of staff and their levels of training and the overall organization of the laboratory. (Frean *et al;* 12012, Saxena *et al;* 2007 Lawson *et al;* 1995). To ensure quality patien care, the intra laboratory QC program must include a least the minimal measures of monitoring and control at each step from collection of blood specimens, through the actual processing and analysis, to reporting of the results. Quality assurance in laboratory haematology includes – constant checking of test reliability by internal quality control, external quality assessment by an independent agency to check performance of a number of laboratories at intervals in order to obtain a retrospective indication of their ability and proficiency control by supervision of the pre-test and post-test phases of laboratory work, from specimen collection to delivery of the report to the clinician (Who Regional Office of Africa 2012), Elbireer and Amukele 2012).

The procedures which comprise quality control include use of control preparations with control charts, constancy of daily means of the blood count, indices of 'absolute values', duplicate testing, clinical correlation and the important role of the blood film to check the instrument-derived blood count measurements. (Schroeder and Amukele 2012). Continuing education is also an integral part of an effective QC propram. Three very important aspects of QC in hematology are calibration of automated instruments, monitoring of accuracy and precision of instruments and procedures, and verifying the reliability of test results. In the absence of a true primary reference/standard for calibration of instruments for the complete blood count, the most commonly performed hematologic test, the use of commercial calibrators is acceptable.

A combination of commercial controls (three levels) and retained or fresh patient blood specimens is recommended for monitoring of accuracy and precision on a long and short-term basis. Patient red-cell indices moving average data allow continuous monitoring of instrument performance and should be used as an adjunct to other QC approaches to detecting instrument calibration drift. (Maha *et al;* 2014, Aulit *et al;* 2012). Correlation of results of related parameters and careful review of

blood films remain the two most important and widely used approaches to ensure reliability of results obtained from automated hematology instruments. Participation in an external QC program offers the most practical means of monitoring overall work performance in comparison with instrument, method, and/or reagent-based peer group data. (Halbergetal 2013, Naleway *et al;* 2014). A laboratory may choose to participate in one or more national and/or regional QC programs, depending upon the range of tests it performs and the requirements of accreditation and regulatory agencies. Most of the accreditation agencies require participation in programs covering at least all of the routinely or frequently performed tests and, if available, also in those for infrequently performed tests.

Internal quality control is based on monitoring the actual analysis performed in the clinical laboratory. Internal quality control is intended to ensure that there is continual evaluation of the reliability of work of the laboratory and that control is exercised over the release of test results. However, it is primarily a check of precision (ie reproducibility) but not necessarily accuracy (Sah *et al;* 1999, Lewis 1995, Dacie and Lewis 1994, Schroeder and, Amukele 2014, Terranella *et al;* 2013).

The External Quality Assessment (EQA) scheme for haematology in spain started in 1984 with 56 laboratories increasing to 473 by 1994. Participants came from public health services (70%) and from private laboratories (30%). Surveys are performed monthly or quarterly depending on the tests and on each occasion whole blood for cell counts (erythrocytes and leucocytes) was sent to participating laboratories. After preparation, the control material is sent to participants in the scheme where the requested tests are performed and the result reported back to the organizer. Results are evaluated and the mean, deviation index (DI) and coefficient of variation was determined. More than 80% of laboratories responded regularly for blood counts and haemoglobin.

There is an improvement on the individual performances each year when compared to the previous year (Vines-Corrons *et al;*1995, Oparkiattikul and Bejrachandra 2002).

Quality assessment in haematology has been in Germany for many years. Cell count, haemoglobin measurement and differential count on smear with morphology was carried out among others. Though the exercise is not mandatory, efforts are being made to change the circumstance. The role of reference laboratories and reference values as well as difficulties for the adequate reference material was discussed (Heller 1995).

This study is therefore, aimed at evaluating the reliability of haemoglobin and packed cell volume (PCV) results obtained in some haematology laboratories in Port Harcourt metropolis.

MATERIALS AND METHODS

Selection of participating laboratories

A total of nine medical laboratories selected in Port Harcourt participated in this study. They include two public and seven private laboratories. They were identified with codes as AA, AB, AC, AD, AE, AF, AG, AH and AI. Six laboratories estimated haemoglobin by the cyanmethaemoglobin method while seven laboratories estimated packed cell volume (PCV) by the microhaematocrit method.

Collection of blood samples

50.0ml of blood was collected by venepunture from a female of 24 years of age and placed into a glass bottle containing 7ml of citrate phosphate dextrose (CPD) anti coagulant. 1.0ml of blood was dispensed into each of 50 plain specimen bottles with the aid of a Pasteur pipette to make 50 samples. The 50 samples were divided into five groups making a total of 10 samples in each group. Each of the five groups were given five different names as if they were from five different patients whereas all the ten samples in a group were given the same name. Five samples were picked randomly one from each group and labeled with five different names as if they were from five different patients. These were dispatched to each of the participating laboratories while the remaining five samples were used by the control research laboratory

Haemoglobin Determination using Cyanmethaemoglobin Method

20.0μl of blood was added to 4.0ml of the diluents and the tubes containing this solution were stoppered inverted several times. They were allowed to stand at room temperature for three minutes. Absorbance of test was read in a colorimeter at a wavelength of 540nm using the diluents as a blank. Haemoglobin concentration was read off from a calibration curve in g/dl.

Packed Cell Volume (PCV) Determination by the Microhaematocrit method

The blood sample was adequately mixed by slow inversion and allowed to enter the capillary tubes by capillary attraction, leaving about 15mm unfiled. The tubes were sealed at one end with a plastic sealant (cristaseal). The capillary tubes were centrifuged using the microhaematocrit centrifuge at 12000 rpm for 5 minutes. The PCV was measured using the microhaematocrit reader.

STATISTICAL ANALYSIS

Using the variance index (V.I), performance was graded as follows:
V.I of 0 $\leq$ 0.5 = excellent performance
V.I of > 0.5 – 1 = reasonable/satisfactory performance
V.I of > 1-2 = acceptable performance
V.1 of < 0 and > 2 = rejectable performance
For the purpose of this study, determinations whose V.I falls in between the "excellent" and "acceptable" range are considered accurate while those that fall outside this range were considered inaccurate.

Precision was assessed by the application of cut off coefficient of variation (CV) using 2SD of the reference laboratory's values.

$$C.V = \frac{2SD}{x} \times 100\%$$

RESULTS

Haemoglobin

The results of the mean haemoglobin, standard deviation and coefficient of variation obtained by the 6 participating laboratories were shown in Table 3. Two of the laboratories had results with good precision. A total

of 23 out of the 30 Hb results from the 6 participating laboratories were inaccurate with rejectable variance indices (Table 4). Table 5 showed that 3 of the laboratories obtained grossly inaccurate Hb results from all the 5 aliquots processed. Only one laboratory (16.7%) obtained Hb results that were of good accuracy and precise (Table 6).

Packed Cell Volume

Seven laboratories estimated packed cell volume (PCV). All the laboratories had PCV results with good precision (Table 8). Seventeen of the 35 PCV results posted by the laboratories were inaccurate (Table 9). Three of the laboratories had variance indices that were rejectable. Table 10 showed that 2 of the laboratories failed to record any result that was accurate from all the 5 aliquots investigated. Only 3 laboratories returned results that were of both good accuracy and precision (42.9%), Table 11).

The results of Haemoglobin (Hb) and packed cell volume (PCV) obtained from the reference research laboratory and all the participating laboratories were tabulated and presented below. The mean, standard deviation (SD), coefficient of variation for the parameters were determined. Precision and accuracy performance of the participating laboratories for the parameters were also determined.

Results from the Research Laboratory.

Table 1: Table showing Mean, Standard deviation (S.D), 2SD, and Coefficient of variation (CV) for Haemoglobin (Hb) and Packed cell volume (PCV) from the research laboratory.

Parameters	Sample number								
Determined	1	2	3	4	5	Mean	S.D	2SD	CV
Hb (g/dl)	9.9	9.9	10.00	10.1	10.0	10.0	0.08	0.16	0.8
PCV (%)	3.1	3.1		3.2	3.0	31	0.7	1.4	2.3

RESULTS FROM THE PARTICIPATING LABORATORIES

Haemoglobin

Table 2: Table showing Hb results of all the participating laboratories (g/dl)

Participating laboratories	Sample Number				
	1	2	3	4	5
AA	9.6	9.8	10.0	9.8	9.7
AB	9.6	9.6	9.9	9.0	9.9
AC	8.9	10.1	10.1	10.1	9.7
AF	10.5	10.1	10.3	10.7	10.0
AH	9.8	9.9	9.8	10.0	9.7
Al	11.2	10.0	9.6	10.8	10.8

Table 3: Table showing mean, standard deviation (SD), and coefficient of variation (CV) of haemoglobin obtained by each participating laboratory for the estimation on 5 aliquots of the same sample.

Performance Laboratories	Mean	SD	CV(%)
AA	9.78	0.1	1.0
AB	9.6	0.4	4.2
AC	9.78	0.5	5.1
AF	10.32	0.3	2.9
AH	9.84	0.1	1.0
Al	10.48	0.7	6.7

Weighted Mean: (Mean calculated from results of all the participating laboratories) = 9.96

Weighted SD: SD calculated from all results of the participating laboratories that are within $\pm$ 2 SD of the reference laboratory value = 0.1

Reference range = 9.9-10.1

Cut off CV for precision using 2SD

Table 4: Table showing variance index (V.I) for each determination carried out by the participating laboratories.
Hemoglobin

Sample Aliquots	AA	AB	AC	AF	AH	Al
1	3.6	3.6	10.6	-5.4	1.6	-12.6
2	1.6	3.6	-1.4	-1.4	0.6	-0.4
3	-0.4	0.6	-1.4	-3.4	1.6	3.6
4	1.6	9.6	-1.4	-7.4	-0.4	-8.4
5	2.6	0.6	2.6	-0.4	2.6	-8.4

Key:
V.I of O $\leq$ 0.5 = Excellent
V.I of $\geq$ 0.5 – 1 = Satisfactory
V.I of $\geq$ 1.0 – 2.0 = Acceptable
V.I of < 0 and > 2 = Rejectable

Table 5: Table showing Haemoglobin Performance Percentages of each of the Participating Laboratory

Variance Index Ranges	AA	AB	AC	AF	AH	Al
Excellent (0$\geq$0.5)	-	-	-	-	-	-
Satisfactory ($\geq$ 0.5-1.0)	-	2(40)	-	-	1(20)	
Acceptable ($\geq$ 1-2)	2(40)	-	-	-	2(40)	
Rejectable (<0 and >2)	3(60)	3(60)	5(100)	5(100)	2(40)	5(100)

Table 6: Table showing precision and accuracy performances of the participating laboratories for haemoglobin estimation

Performance	AA	AB	AC	AF	AH	AI	% of Lab
Good accuracy	-	-	-	-	+	-	16.7
Good precision	+	-	-	-	+	-	33.4
Good accuracy and precision	-	-	-	-	+	-	16.7
Poor accuracy	+	+	+	+	-	+	83.5
Poor precision	-	+	+	+	-	+	66.8
Poor accuracy and good precision	+	-	-	-	-	-	16.7
Good accuracy and poor precision	-	-	-	-	-	-	
Poor precision and poor accuracy	-	+	+	+	-	+	66.8

Key: + = Yes
- = No

Packed cell volume

Table 7: Table showing Packed Cell Volume (PCV) results of all participating laboratories (%)

Participating laboratories	Sample Number				
	1	2	3	4	5
AA	29	29	30	30	29
AB	30	27	30	29	29
AD	28	27	27	28	27
AE	30	32	31	33	30
AF	30	33	32	32	31
AG	33	34	33	34	32
AH	34	33	34	35	34

Table 8: Table showing mean, standard deviation (SD) of PCV obtained by each participating laboratory for the estimation on 5 aliquots of the same sample.

Participating laboratories	Mean	SD	CV (%)
AA	29.4	0.5	1.7
AB	29.0	1.2	4.1
AD	27.4	0.5	1.8
AE	31.2	1.3	4.2
AF	31.6	1.1	3.5
AG	33.2	0.8	2.4
AH	34.0	0.7	2.0

Weighted mean = 30.8
Weighted SD = 2.4
Reference Range = 30-32
Cut off CV for precision using 2 SD = 4.5%

Table 9: Table showing variance index (V.I) for each determination carried out by each participating laboratory for the PCV estimation

Sample Aliquots	AA	AB	AD	AE	AF	AG	AH
1	0.8	0.3	1.2	0.3	0.3	-0.9	-1.3
2	0.8	1.6	1.6	-0.5	-0.9	-1.3	-0.9
3	0.3	0.3	1.6	-0.1	-0.5	-0.9	-1.3
4	0.3	0.8	1.2	-0.9	-0.5	-1.3	-1.8
5	0.8	0.8	1.6	0.3	-0.1	-0.5	-1.3

Key:
V.I of $0 \leq 0.5$ = Excellent
V.I of $\geq 0.5 - 1$ = Satisfactory
V.I of $\geq 1.0 - 2.0$ = Acceptable
V.I of < 0 and > 2 = Rejectable

Table 10: Table showing PCV performance percentages of each of the participating laboratories

Variance Index Ranges	AA	AB	AD	AE	AF	AG	AH
Excellent (0≥0.5)	2(40)	2(40)	-	2(40)	1(20)	-	-
Satisfactory (≥ 0.5-1.0)	3(60)	2(40)	-	-	-	-	-
Acceptable (≥ 1-2)	-	1(20)	5(100)-	-	-	-	-
Rejectable (<0 and >2)	-	-	-	3(60)	4(80)	5(100)	5(100)

Table 11: Table showing precision and accuracy performance of the participating laboratories for PCV estimation

Performance	AA	AB	AD	AE	AF	AG	AH	% of Lab
Good accuracy	+	+	+	-	-	-	-	42.9
Good precision	+	+	+	+	+	+	+	100
Good accuracy and precision	+	+	+	-	-	-	-	42.9
Poor accuracy	-	-	-	+	+	+	+	57.2
Poor precision	-	-	-	-	-	-	-	
Poor accuracy and good precision	-	-	-	+	+	+	+	57.2
Good accuracy and poor precision	-	-	-	-	-	-	-	
Poor precision and poor accuracy	-	-	-	-	-	-	-	

Key + = Yes
\- = No

DISCUSSION

This study has investigated the quality control of haemoglobin (Hb) and packed cell volume (PCV) results in some haematology laboratories in Port Harcourt metropolis.

The results showed that two of the laboratories had Hb results with good precision. Majority of the participating laboratories had results that were inaccurate with rejectable variance indices (Table 4) whereas only one laboratory obtained HB results that were of good accuracy and precision. (Table 6).

For the PCV results, it was shown that all the participating laboratories had results with good precision (Table 8) whereas about half of the results from the laboratories were inaccurate (Table 9). Three of the laboratories had variance indices that were rejectable and only three laboratories also had results that were of both good accurancy and precision (Table 11).

From the Hb and PCV results obtained by the participating laboratories, it can be seen that only few laboratories obtained results that were of both good accuracy and precision where as many of them obtained results that were grossly inaccurate with rejectable variance indices. The findings in this study is in conformity with the results of other researchers in several centres in Nigeria. In their studies, the participating laboratories had a rather poor performance. It was observed that poor compliance with standards that ensure quality of laboratory determinations is a contributing factor of this low performance (Emeribe and Irene 1994).

Aside from the above factor, other contributing factors to the very poor performance mostly for Hb determination include insufficient mixing of blood, incorrect timing for the conversion of methaemoglobin to cyanmethaemoglobin and instrument.

The performance of PCV seems to be somehow better than that of Hb probably because of the ease in carrying out PCV determination. Though the most common cause of errors in its determination include

improper packing of cells, incorrect use of haematocrit reader and inadequate mixing of blood samples.

This poor performance therefore suggests that there is the need to establish an external haematology laboratory quality control programmes for the purpose of improving on the rather poor inter laboratory quality control habit, since this is one of the ways to enhance the reliability and credibility of laboratory test results.

REFERENCES

Aulit K.A., Heine R.P. and Riley L.E (2012). Pragmatic and research priorities for improving influenza immunization of pregnant women. *American Journal of obstetrics and Gynaecology.* 207 (3 supp.): 75-77.

Dacie J.V. and Lewis S.M. (1994). Quality assurance. In:practical Haematology 8th Ed. Chuchil Livingston,Edinburgh Pp. 35-47.

Elbreer A. and Amukele (2012). A survey of clinical laboratories in Kampala, 2012. Paper presented at 1st international conferences of the African society for laboratory medicine; December 2012, Cape Town, South Africa.

Emeribe A.O. and Irene P.A. (1994). Quality assessment of Haematology results in some Medical laboratories based in Calabar. *Journal of Medical Laboratory Science.* 4:144 -150

Frean J., Perovic O. and Fensham V. (2012). External quality assessment of National public health laboratories in Africa, 2002-2009. *Bull World Health Organ.* 90:191-199.

Halberg S.E., Trogstad L. and Gunness N. (2013). Risk of fetal death after pandemic influenza virus infection or vaccination. *New England Journal of Medicine.* 68(4):333-340

Heller S. (1995). External quality assessment scheme for haematology. *Methods in Haematology* 22:14-19.

Lawson P.L., Lawson N.S and Ross J.W. (1995). The stability of survey-assigned assay values when surveyed control materials are used in a daily inter laboratory quality control program. The College of American Pathologists Chemistry Survey-Quality Assurance Service shared poolexperience. *Arch Pathology Lab Med.* 119:312-333.

Lewis S.M. (1995). Quality assurance programme in the United. *Ann-1st super sanitation.* 31(1): 42-49.

Maha E.R., Higa D. and Naugler C. (2014) Accuracy of the cella vision DM 96 platform for reticulocyte counting *Journal of pathology information* 14:17-24.

Naleway A.L., Irving S.A. and Henninger M.L (2014). Safety of influenza vaccination during pregnancy. A review of subsequent maternal obstetrics events and findings from two recent cohort studies. *Vaccine* 32(26): 3122-3127.

Opartkiattikul N. and Bejrachandra S. (2002). The external quality assessment schemes in Thailand. *Rinsho Byori.* 50:121-125.

Sah S.P. Raj G.A, and Prackash M.B. (1999). Quality assurance programme in the eastern region of Nepal. *Indian Journal of Pathology and microbiology.* 42(2):145 149.

Saxena R., Katoch S.C. and Srinivas U. (2007). Impact of external haematology proficiency testing programme on quality of laboratories. *Indian J. Med. Res.* 126: 428-432

Schroder L. and Amukele (2012). ISO 15189 and CAP accredited laboratories in sub-Saharan Africa. Paper presented at 1st International Conference of the African Society for Laboratory Medicine; December 2012 Cape Town, South Africa.

Schroeder L.F., and Amukele T. (2014) Medical Laboratories in sub saharon Africa that meets international quality standards. *American Journal of Clinical Pathology* 141: 791-795

Terranella A., Asay G.R., Messonier M.L., Clark T.A. and Liand J.L. (2013). Pregnancy dose Tdap and postpartum cocooning to prevent infant pertusis. *A decision analysis.*5:27.

Timan I.S. Aulia D and Santoso W. (2002). External quality assessment scheme and laboratory accreditation in Indonesia. *Rinsho Byori.* 50:126-130.

Vines – Corrons J.L. Gutirrez G. Jou J.M. and Reverler J.C. (1995). Characteristics and performance of the External quality assessment scheme (E.Q.A.S) for haematology in Spain. Ten years of experience. *Ann-1st supper-sanita* 31(1):95-101.

Who regional office for Africa (2012). Strengthening public health laboratories in the WHO African region: a critical need for disease control. Available at: Accessed September 6.

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Anti-Hemorrhoid Evaluation of Selected Medicinal plants used in North-East Nigeria for the Treatment of Hemorrhoids (Pile)

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Abstract

Medicinal plant is any plant in which one or more of it organs contain substance that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. In this research, four(4) selected plants were screened for antihemorrhoid activities in mice. The extracts were obtained from whole plant or part of plants such as root, stem, leaves and seeds and include the following plants: Khaya senegalensis, Euphorbia hirta, Parkia biglobosa , Newbouldia leavis and Prosopis africana. Hemorrhoid (pile) was induced in group of five mice of five animals per group using Jatropha oil(Jatropha curcas, Euphorbiaceae)(I.P) and using Pilex granule as the control drug. Group I received 5mg/kg Pilex granule, and 200, 250, 300, 350 mg/kg b.w of A. leiocarpus, N. leavis, P. africana, and KEP for groups II, III, IV and V respectively. A. leiocarpus and KEP(mixture of K. senegalensis, E. hirta and P. africana) showed the best antihemorrhoidal activities in mice than the other plants and compared with the standard drug Pilex granule. However, all the plants extracts showed significant rectoanal coefficient at potent levels. The study showed that the extracts of the plants investigated possessed antihemorrhoid activities with A. leiocarpus demonstrating the best activity in mice.

Keywords: Anti-hemorrhoid, medicinal plants, mice, pilex granule, Jatropha oil.

INTRODUCTION

Hemorrhoids represent the dilation of varicose of the vessel of the superior of inferior rectal plexuses of veins. They have be noted common human affliction from the down history. The exact incident in population of developing countries has not been determined but in spite of ascertain to the contrary. The condition is frequently encountered in

developing country [1]. Various dilation of the internal hemorrhoids; physiological dilation present already in infancy is presumed to develop into varicosities under the influence of wide range of factors. The predisposing factors include hereditary, age, sex pregnancy are the prepared state and even paramount.

The precipitating factors comprise cathartic abuse, diarrhea, enemata, constipation infection and spasms or a tony of the oral sphincter obesity and arise in extra-abdominal pressure [2]. There are two types of piles, internal piles and external piles. Internal piles expand inside, along the anal. The common symptoms of internal piles are the painless blood loss. The internal piles are the totally prolapsed. External piles extend close to the anus. The colour of external piles is same as the skin. The outside piles forms thrombus. The outside piles are painful. When the external pile ruptures it bleeds. The blood loss is more disturbing and blood loss is typical cause for considering a doctor. Prolapsed is on the other hand, oral dysfunctional special effect, and the other undeniable warning sign soreness, impatient are fewer dependable problem solving criterion [3].

MATERIALS AND METHODS

Plant Collection and Identification

The plants species (*Parkia biglobosa, Prosopis africana, Euphorbia hirta, Khaya senegalensis, Newbouldia leavis* and *Anogeissus leiocarpus*) were collected from Bali town and were identified by Mr. Cletus A. Ukwubile of Science Laboratory Technology Department, Federal Polytechnic Bali, where voucher numbers were deposited for the plants

Attempt has been made to expel scyballous masses from the rectal by traditional medical practitioners using different plant species in either of the following preparation methods: concoction, decoction and maceration. This research aims to identify the plant species used for the treatment of such ailment and also to identify the plant species which has the best anti-hemorrhoids properties, also the ecology, scientific names and method of preparation of the drug and also to make a herbarium press of the plant species.

Preparation and Extraction of Plant Materials

The plants were air-dried for two weeks and then, plants parts were ground into powder and weighed and stored for onward use. One of the plants was extracted with aqueous solution (*Newbouldia leavis*) while others were extracted with absolute ethanol 99.1% (v/v), which was soaked for 24 h using cold maceration technique.

Grouping of Swiss albino rats

The animals were grouped into five (5) groups of 5 animals according to each plant extracts.

Experimental Animals

Inbred male and female Swiss albino mice (18-29g weights) that were housed in standard conditions of temperature (22 ± 3°C), relative humidity (55 ± 5%), and light (12h light-dark cycle) before and during the study were included in the experiment. They were fed with standard pellet diet (obtained from animal house of Department of Pharmacology and Clinical Therapeutics, Ahmadu Bello University Zaria) and water *ad libitum*. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of the Ahmadu Bello University Zaria and Health Research Extension Act of 1985(Public Law November 20, page 99-158) USA. The animals received humane care as per the guidelines prescribed by committees for the purpose of control and supervision of control and supervision of experiments on animals (CPCSEA), the Ministry of Environment and Forests, Nigeria.

Experimental Protocols

Two sets of experiments were carried out. The first set was used to improve an existing experimental model of hemorrhoids mentioned by [3], and to validate the same by using pilex granules (PG), *Newbouldia leavis* extract (NE), and a combination of both extracts. The protocol was designed to quantify the extent of plasma exudation and to determine the levels of inflammatory cytokines such as TNF-α and IL-6 associated with hemorrhoids. In the second set, the effect of PG, AL, and a combination of some plant extracts were further confirmed by

determining the rectoanal coefficient (RAC), severity score, and the histopathological evaluation[4].

Evaluation of Anti-hemorrhoid property against Jatropha oil-induced hemorrhoid in mice

Mice of both sexes (20-29g) were randomized based on their body weights and were divided into 5 groups (G-1-G-5), with each group consisting of 5 animals (n=5). G-5 animals received PG (Pilex granule) (10mg/kg) and served as positive control; G-1 animals received AL (200mg/kg):G-2 and G-3 animals received NL and KEP (200 and 400mg/kg b.w; i.p , respectively). Haemorrhoids were induced to all the groups, except normal control group, by applying croton oil preparation. 24h hours after induction, all the animals were subjected to respective treatment as assigned to the groups once daily for five days. On the fifth day, 1 h after the treatment, all the animals were euthanized by exsanguinations under deep isoflurane anaesthesia and rectoanal tissues (20mm in length) were isolated. They were evaluated for the severity score, weighed, and fixed in 10% formalin solution for histological examination.

The RAC was calculated using the formula

$$\text{Rectoanal coefficient} = \frac{\text{Weight of rectoanal tissue (mg}}{\text{Body weight (g)}}$$

Histological observation of the rectoanal tissue was carried to determine the appearance of inflammatory cells, congestion, haemorrhage, vasodilatation, and medium to high degrees of necrosis [5].

RESULT

Table 1: LD_{50} Determination of Jatropha oil from *Jatropha curcas*

Dosage (mg/kg)	***No of animal died*** / ***no of animal survive***
100	0/5
200	0/5
250*	1/4
300	ND

LD_{50} = 1118 mg/kg b.w (Lorke , 1983), ND (not determine), * toxic dose

Table 2: Effect of Jatropha oil on the body weights of mice before and after induction

Test groups	Weight Before (g)	Weight After (g)
Group I Control	29.6	25.2
100mg/kg	28.6	24.1
Group II 200mg/kg	25.2	20.1
Group III 250mg/kg	28.0	22.2
Group IV 300mg/kg	26.6	20.2
Group V 350 mg/kg		

Table 3: Effect of extracts on rectum after drug administration (i.p)

Extract dose (mg/kg b.w) (n=5)	Rectoanal coefficients (g)	Inference
Group I Control Pilex (5mg)	0.6 ± 0.22	Moderate healing
Group II AL 100	0.1 ± 0.20™	*Strong healing
Group III NL 200	0.4 ± 0.18	Moderate healing
Group IV PA 400	0.3 ± 0.15	Moderate healing
Group V KEP 600	0.2 ± 0.10™	*Strong healing

AL (*Anogeissus leiocarpus*), NL (*Newbouldia leavis*), PA (*Prosopis africana*), KEP (*Khaya senegalensis, Euphorbia hirta, Parkia biglobosa*),

Results are means ± SEM. The lower the values, the more efficacy the drug, ™ More efficacy.

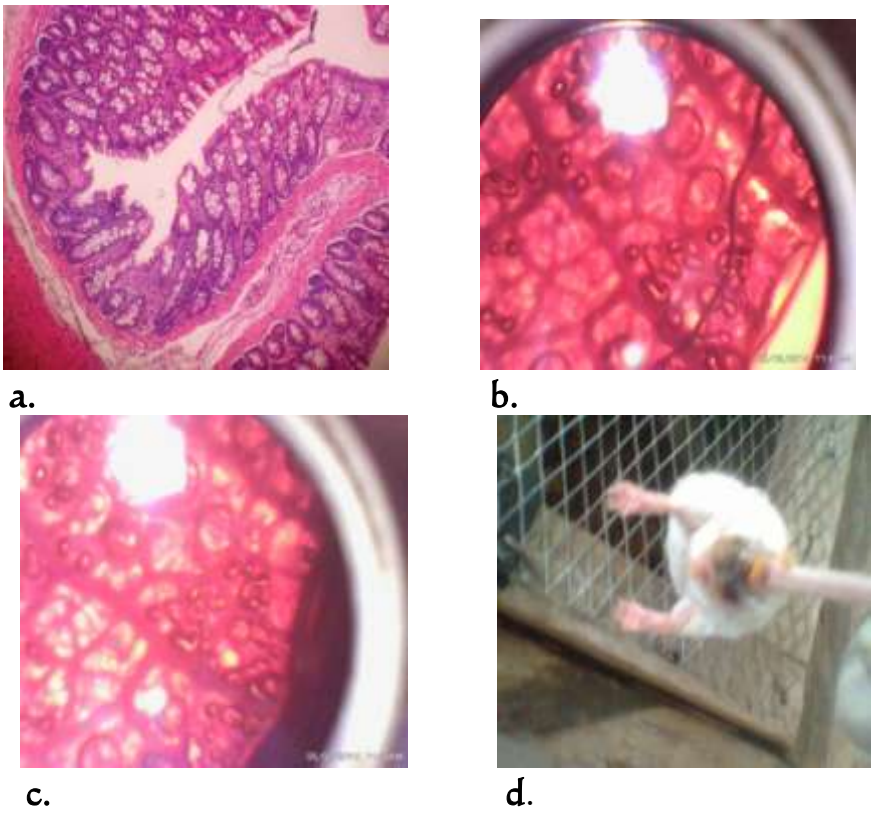

Figure 1: Medicinal plants effects on rectoanal tissue in mice in Jatropha oil-induced hemorrhoids; a; hemorrhoid induced in recto anal tissue, b-c; healing of tissue after drug administration with *Anogeissus leiocarpus*, d; mouse developed pile after five days of induction with Jatropha oil.

DISCUSSION

It is well proved that hemorrhoids are a pathological condition, which is characterized by a severe vasodilation at the rectoanal region, which leads to inflammation of the surrounding tissues, thus further leading to secondary complications such as extravasations of fluid into interstitial space mainly due to increased vascular permeability and migration of large quantity of inflammatory cells (granulocytes and monocytes)[6].

In the present study, Jatropha oil from seeds of Jatropha *curcas* (*Euphorbiaceae*) has been used as inducer/phlogiston agent to induce experimental hemorrhoids. Jatropha oil causes inflammation due to the

release of soluble factors involving inflammatory lipid metabolites [7].These factors, alone and/or in combination, regulate the activation of resident cells (Fibroblasts, endothelial cells, macrophages, and mast cells) and newly recruited inflammatory cells (Monocytes, lymphocytes, neutrophils, and eosinophils) leading to systemic response to inflammation [8-9].

The normal control group showed normal cell architecture of the rectoanal region. The results showed the loss of weights in the animals after induction (Table 2), which is a symptom of the disease. However, intraperitoneal administration of plant extracts of AL, NL, PA, and KEP showed remarkable vasoconstriction of the rectum (Table 3). The greatest healing of the rectum were shown by AL (*Anogeissus leiocarpus*) and KEP (*K. senegalensis, Euphorbia hirta, Parkia biglobosa*), and these signify the constriction of the mucosa linings of the anus in the mice by the plant extracts. These results were comparable with that of the standard control drug (Pilex). All the extracts produced a better rectoanal coefficient values than the first line drug (Table 3).

CONCLUSION

Medicinal plants are a source of many biological ingredient which cannot be ignored. The study therefore showed that extracts of *Anogeissus leiocarpus, Khaya senegalensis, Euphorbia hirta, Parkia biglobosa* and *Newbouldia leavis* posses antihemorrhoid properties in mice, and can be use as medication for the treatment of hemorrhoid (pile). These plants thus, represent sure source towards the development of orthodox medicine for the treatment of piles than surgery, which normal is expensive and risky. However, the precise molecular mechanism behind the antihemorrhoidal activities of these plant extracts need to be explored in future studies.

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REFERENCES

1. Abayomi, S . Medicinal plant and Traditional Medicine in Africa. Spectrum Books, Ibadan.2006: 1-23.

2. Alma-Ata, K.F. Conference on plants in the Development of Modern Medicine. University College London, November 22-25th,2009, UK.

3. Elvin, M. Traditional Medicine in Nigeria. (Ed.). Federal Ministry of Health, Nigeria Bulletin No 120:2014; Vol. 45: 56-59.

4. Evans, W. F and Trease, G.E. Naturally Occurring Phytol Esters. (Ed.) C.R.C Press. London; 2004: 78

5. Jaijoy, K.L. Anti-inflammatory, activity of the water extract of *Terminalia chebula* Retz. in Rats. *Plant Medica*.2012; Vol. 78:112

6. Janju, H.M . African Traditional Medicine, A Case Study of Hausa. In: Medicinal Plant 1964, Ibadan Federal Department of Forestry Research.2008; Vol I and II.

7. Lestrange, R. A History of Herbal Plant (Eds.) Augurs and Robertson, London, 2013:90-98.

8. Lewis, W.H and Evin – Lewis, M.F (. Medical Botany: Plant Affecting Man's Health. John Wiley and Son London 2nd edition; 2008:56-78.

9. Noto, T. Melanogenesis inhibitory, anti-inflammatory, and chemo preventive effects of limonoids from the seeds of *Azadirachta indica* A. Juss. (Neem) .*Journal of Oleo Science*.2009; Vol. 58 (11):581-594.

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The Distribution of ABO and Rhesus D Blood Group Antigens in Nembe Community of Bayelsa State

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Abstract

This study investigated the distribution of ABO and Rhesus D blood group antigens in Nembe community of Bayelsa State in Nigeria. A total of 204 students in a coeducational institution in Nembe community were used in this study. They were made up of 124 males and 80 females. 5.0mls of blood were collected from each of the patient by venepuncture and transferred into a bottle containing EDTA. Fresh red cell suspension and 20% suspension of known A, B and Rhesus D antigens were prepared. Tile agglutiaion technique was used in the determination of ABO and Rh.D grouping system. The resuls showed that for the distribution of ABO Groups in the studied population 66 (32.3%) of the males were group O which was the highest while 6(2.9%) of the males were AB which was the least. For the females 28(13.7%) were group O and the same number of females had group A also which was the highest while the least number of 2(0.9) females were group AB. None of the 123 males, 116(56.8%) were Rh.D positive while 8(3.9%) were Rh.D negative. And out of 90 females 74(36.2) were Rh.D positive while 6(2.9%) were Rh.D negative. The frequency distribution of Rh.D positive and Rh.D negative subjects in ABO blood groups in males, females and in the total subjects studied were assessed, the result showed that blood group O had the highest number and frequency of Rh.D positive and negative in all the above parameters assessed. On the basis of the findings the study therefore concludes that blood group "O" and Rh.D positive are the most common and prevalent blood group antigens in Nembe community of Bayelsa State.

Keywords: Antigens, ABO Rh.D, Distribution, agglutination and Blood.

INTRODUCTION

The human blood can be grouped according to some criteria. The criteria mostly used are according to the presence or absence of certain substances on the blood of the individual. These substances are called blood group antigens. They are known to be present on the surface of the red blood cells forming part of their cell membrane on the leucocytes and in the serum portion of blood (Garrison *et al;* 1976, Dali *et al;* 2011).

Antigens are substances that are capable of stimulating the production of antibodies under favourable condition (Anstee 2010). Blood group antigens therefore are the various antigens found on the blood that are used in blood grouping. Various blood groups exist: they include the ABO, Rhesus, Keel, Duffy, MNS, Lewis etc. (Austee 2010).

The importance of these antigen are mostly considered during blood transfusion. A blood antigen (mostly of the ABO system) has a corresponding antibody to the antigen it does not possess (Frances 2002). Reaction occurs when an antibody finds its corresponding antigen. Agglutination and lyzing of the red cell occurs. Of all the blood group antigens the Rhesus 'D' A and B are by far the most important (Frances 2002), Waseem *et al;* 2012)

During pregnancy the blood of the mother and her unborn child most at times mixes together across the placenta. Antigen-anti body reaction occurs which can be dangerous to the unborn child (Mollison *et al;*). The Rhesus 'D' antigen is very significant in this issue as antibodies produced from a Rhesus 'D' negative mother would cause the lyzing of the red cells of a Rhesus 'D' Positive fetus. This leads to a disease condition known as hemolytic disease of the new born (HDN) (Alaine arruabaureu 1977).

The ABO blood group antigens are also found in some body secretions such as Saliva (Horby and Gytrup 1989). These antigens are also found in many parts of the body and are most concentrated in the upper intestinal tract (Race and Sagner 1996).
There are strong evidences that a lot of disease are associated with the various blood groups (Horhy and Gyltrup 1989). A study in Peru

revealed that life threatening cholera is associated with blood group O (Platt *et al;* 1985). Hyperlyroid disease us associated with blood group O. In their research work, pathirana *et al;* (2005) discovered that individuals of group O are relatively resistant to several disease caused by *plasmodium falciparum* than individuals of group AB. Duodenal and stomach Ulcer is also strongly associated with individuals of blood group O (Jill 2003), cancer of the stomach is more common with individuals of group A than those of group O and B. Group A individual are also more prone to pernicious anaemia and diabetes mellitus (Harby *et al;* 1989, Jassim 2012).

The frequency of ABO and Rh.D phenotypes varies in different populations throughout the world (Aild *et al;* 1953). In the study carried out by Mollison *et al;* (1993), the commonest group in the Australian Arborigines are group O and A.

In Lapps and among the Europeans there is a higher frequency of group A. They also showed that blood group B is most predominant in African (Mollison *et al;* 1993). France (2002) gave the percentage distribution of whites in the United States as blood group O 46%, A 41%, B 9% and AB as 4%. That of the Negroes (New Yorkers) is given as blood group O 44.2%, A 39.3% B 21.8% and AB as 3.7% (Frances 2002).

In Saudi Arabia 52% of the individuals are of blood group O, 25% group A, 18% group B and group AB 5%. Mazban *et al;* (1998) gives the percentage frequency of Ahwaz (Iran) as group O being highest with 41.6%. The population in Parkistan Asia has blood group B 32.4%, A 22.6%, O 30.5% and AB 8.6% (Rahman *et al;* 2004). In the British population group O occupies 46.7%, A 41.7%, B 8.6%, and AB 3.0% (Hoffbrand 1981).

The African country of Kenya has a percentage distribution of blood group O as 47.4%, A 26.2%, B 22.0% and AB 4.4% (Lyko *et al;* 1992). The India population is dominated by blood group B (37.5%) (Talib 2000).
The European population is 95% Rh.D positive while 5% are Rh D negative (Mollison *et al;* 1993). The United States 85% of the

population are Rh D positive while 15% are Rh D negative (Frances 2002).

Marzban *et al;* give 90% Rh D positive and the remaining percentage as Rh D negative in Ahwaz region population.

The study of Bashwari *et al* (2001) gives the Saudi Arabian population as being 93% Rh D positive. The Rh D negative frequencies vary from 20-40% in Basques to 0-1% in many Asian countries such as Japan and China (Mollison 1993). Talib (2000) gives the summary of Rh.D positive distribution as Asians 90-98%, Africans 94-95%, Napalese 99-100% and the Caucasians as 85%.

This study is therefore, aimed at investigating the distribution of ABO and Rhesus D blood group antigens in Nembe community of Bayelsa State in Nigeria.

MATERIALS AND METHODS

RECRUITMENT OF PATIENTS

A total of two hundred and four (204) students in a coeducational institution in Nembe were used in this study. They were made up of one hundred and twenty four (124) males and eighty (80) females.

Collection of Samples

5.0ml of blood were collected from each of the recruited patients using venepuncture technique and transferred into a bottle containing ethylenediamine tetra acetic acid (EDTA). This was gently rocked to ensure thorough mixing of the blood sample with the anticoagulant.

Preparation of fresh red cell suspension

This was done using each of the samples to be grouped. Two ml of the samples were transferred from the EDTA bottle to a test tube. 3.0ml of normal saline solution was added to the tube, the contents mixed and centrifuged for 5 minutes. The supernatant was decanted and this was repeated three times.

20% suspension of known red cells containing the A,B, and Rhesus D antigen were prepared in the same way and used for the serum (reverse) grouping.

Procedures for ABO grouping

Tile agglutination technique was used in this determination. Microtitre wells were numbered 1-6 and one volume each of anti-A, Anti-B and Anti AB were added to wells 1, 2 and 3 respectively, while one volume each of A Cells, B cells and control 1 (patient cells and serum) were added to wells no. 4,5 and 6 respectively.To wells numbers 1-3 one volume of patients cells were added whereas in wells number 4 and 5 one volume of patients serum were added. One volume of low ionic strength solution (LISS) freshly prepared was added to each well.

The contents were mixed with separate applicator sticks. The tiles were rocked for four minutes making sure that the contents were not dried. Agglutination was observed, recorded and interpreted. The contents of wells 4 and 5 served as a check to the actual test carried out on wells 1 to 3, and this process is known as reverse grouping.

Rhesus D grouping:

Anti-D reagent and patient washed cells and serum were used. The procedure was the same as for the ABO grouping system except that only two wells were used. One well for the test while the other was used for the control. The result was interpreted as agglutination for Rh.D positive and no agglutination as Rh.D negative as in the ABO system.

RESULTS

The investigation of the distribution of ABO blood groups in the population studied showed that for group O there were 66 males and 28 females (total 94). Group A had 26 males and 28 females (total 54); group B had 26 males and 22 females (total 48) while group AB had 6 males and 2 female (total 8). Group O had the highest percentage frequency of 32.3% for males and 13.7% in females making a total of 46%. This was closely followed by group A, males having 12.8% and females 13.8% making a total of 26.6%. Males for group B was 12.7% and females 10.9% making a total of 23.6%. Group AB had the least

percentage frequency with males having 2.9% and females 0.9% making a total of 3.8% (Table 1).

Table 1: Table showing the distribution of number and percentage frequency of ABO blood groups in studied population.

	A	% Freq	B	% Freq	AB	% Freq	O	% freq
Males	26	12.8	26	12.7	6	2.9	66	32.3
Females	28	13.7	22	10.7	2	0.9	28	13.7
Total	54	26.6	48	10.9	8	3.8	94	46

The distribution of the numbers and percentage frequency of rhesus 'D' groups in the studied population showed that 116 males were Rh D positive making a percentage of 56.8% while 74 females were Rh 'D' positive making 36.2%. The total frequency of Rh 'D' positive individuals were thus 190 making 93%. In the Rh 'D' negative category, 8 males were Rh 'D' negative which was 3.9% while 6 females were Rh 'D' negative which was 2.9% of the total population. In all 14 individuals were Rh 'D' negative making a total percentage of 6.8% (Table II).

Table 2: Table showing the distribution of the number and Percentage frequency of Rh 'D' Blood Group in studied population

	No of Rh.D +	% freq.	No of Rh.D (-)	% freq
Males	116	56.8	8	3.9
Females	74	36.2	6	2.9
Total	190	93	14	6.8

The frequency distribution of Rh.'D' positive and Rh.'D' negative subjects in ABO blood groups in males showed that among group A, 26 males were Rh+ while none was Rh(-) making a frequency of 21% and 0% respectively. Group B males were made up of 22 Rh D + and 4 Rh D (-) making a frequency of 17.8% and 3.2% respectively. Among group AB males 4 were Rh D + while 2 were Rh D – being 3.2% and 1.6% respectively. Group O males were most prevalent being 64 Rh D + and

2 Rh d- making a percentage frequency of 51.6 and 1.6 of the total male population respectively.

Summarily the study showed that out of the 124 males subjects tested 116 were Rh D + making a percentage of 93.6% while 8 were Rh D- making 6.4% (Table III).

Table 3: Table showing the number and Frequency distribution of Rh D + and Rh D- Subjects in ABO Blood Groups in Males

Blood groups	No. of Rh D+	% Freq.	No. of Rh D-	% Freq.
A	26	21	-	-
B	22	17.8	4	3.2
AB	4	3.2	2	1.6
O	64	51	2	1.6
TOTAL	116	93.6	8	6.4

This study showed that out of the 80 female subjects tested 26 of them were of group A having the Rh.D+ antigen while 2 of group A female were Rh 'D'-. Their percentage frequency was 32.5 and 2.5 respectively. Group B females were 22 (27.7%), Rh.D+ while none in this blood group had the Rh D antigen. In blood group AB,2 females were of Rh.D positive with frequency of 2.5% while there was none that had Rh.D negative. In group O females, 24 were Rh D+ making 30% of the population and 4 Rh D- making 30% of the population and 4 Rh D- making a 5% frequency.

On the whole 74 female subjects were Rh D+ occupying 92.5% while 6 subjects were Rh D- making a total of 7.5% of the entire female subjects (Table 4).

Table 4: Table showing the number and frequency distribution of Rh D+ and Rh D- subjects in ABO Blood Groups in Females

Blood groups	No. of Rh D+	% Freq.	No. of Rh D-	% Freq.
A	26	32.5	2	2.5
B	22	27.5	-	-
AB	2	2.5	-	-
O	24	30	4	5
TOTAL	74	92.5	6	7.5

The frequency distribution of Rh D+ and Rh D- subjects in the ABO groups of both sexes showed that in the group A category 52 subjects were Rh D + occupying 25.5% of the total population while Rh D- subjects in that category were 2 making 1%. In group B subjects 44 were Rh D+ (21.5) while 4 were Rh D- (2%). In the AB blood group 6 subjects were Rh D+ (3%) while 2 were Rh 'D'- (1%). Group O had the highest number of subjects, 88 being Rh D+ (43.1%) while 6 were Rh D-(2.9%). Finally 190 of the total subjects tested were Rh D+ occupying a frequency of 93% while 14 subjects were Rh D- occupying 7% of the total population (Table 5).

Table5: Table showing the number and percentage frequency distribution of Rh D+ and Rh D- Subjects in ABO blood groups of total population

Blood groups	No. of Rh D+	% Freq.	No. of Rh D-	% Freq.
A	52	25.5	2	1
B	44	21.5	4	2
AB	6	2.9	2	1
O	88	43.1	6	2.9
TOTAL	190	93	14	6.9

DISCUSSION

The ABO and Rhesus blood group systems are by far the most commonly utilized blood group systems in blood transfusion. (Frances *et al;* 2002). According to Boyd (1950) these systems also play an

important role in transplantation, hereditary diseases, genetics and in the migration of races. The association of different blood groups with diseases is important as some of the blood groups are prone to developing certain diseases (Aird *et al;* 1953).

The particular type of blood group a person inherits depends on the genes that encode for blood grouping system. Within this system the frequency of distribution of these genes varies and their phenotype can reveal important information, including forensic evidence (Mollison *et al;* 1993, Bener *et al;* 2012).

This study found that blood group O has a percentage frequency of 47%, blood group A 26.6%, blood group B 23.6% and blood group AB 4% (Table 1). This work is in conformity with the works of Gaether *et al;* (1994). Gaetner *et al;*(1994) in their study of the Nigerian population gave their findings as blood group O 48.9%, group A 24.43%, group B 23.83% and AB as 2.7%. Other of their study has blood group O as 46.6%, A 23.05% and group AB as 4.4% when they studied the population of northern Nigerian. The agreement of this work with other works can be seen in the dominating nature of blood group O over other groups and blood group AB occupying only a small percentage of the total population. (Jaggi and Yadav 2014).

In the Rhesus D blood group system, this study also showed near conformity with other studies. This study gives Rh D+ individual as occupying as high as 93% while individuals with no Rh D antigen as 7%. These percentages are slightly lower in the Rh D+ finding of Gaetner 95% ad slightly higher in the Rh D- category of 5% in Gaetner. These results follows the global trend of Rh D- individuals being significantly rare than Rh D+ persons.

On the basis of the above findings, this study concludes that blood group O and Rh.D positive individuals are commonly found in Nembe community than other blood group antigens .

REFERENCES

Garrison R. Havlik R.J. Harkias R.B. Feinleb M. and Kanned W.B. (1976).ABO blood groups and cardiovascular disease- the farminghan study.*Atherosclerosis.* **25**:311 318

Frances T.F. (2002). Blood groups (ABO Groups). In common laboratory and diagnostic test 3rd edition Pp. 194-195 Philadelphia Lippincott.

Gaetner H. Lyko J. and Lyko S. (1994).The antigen ABO and Rh.D in Nigeria population. *Hamded Medicus.* **37(1):** 81 91.

BoyD W.C. (1950). Genetics and races of man pp. 335-342. Little Brown and Co Boston.

Anstee D.J. (2010). The relationship between blood groups and disease. Blood **115**:4635-4643.

Mollison P.L. Engelfrelt C.P. and Conteras M. (1993). Immunology of red cells in blood transfusion in clinical medicine. 9th edition Pp. 87-89 Blackwell scientific publication Oxford.

Alaine A. (1977).Causes of haemolytic disease of the newborn. English foundation course Pp 12-29 Black well publication oxford.

Horby J.and Gytrup H,J.(1989). A relationship of serum lipoproteins and lipids to the ABO blood group in patients with intermittent claudication.*Journal of cardiovascular surgery.* **30(4):** 533-537.

Race R.R. and Sagner R. (1996). Blood groups in man 6th edition oxford Blackwell scientific publishers.

Platt D., Muhlberg W., Kichi L., and Schmidt R.R. (1985). ABO blood group system, as age, sex risk factors and cardiac infarction. *Archive Gerontol Geriatric* **4(3)**:241-249

Pathiranna. S.L. Alles H.K. and Bandara S. (2005) Hyperthy road disease and blood group. *Annals of tropical Medicine and parasitology.* Vol **99(2)**:119-124.

Jill R. (2003). Human blood group: inheritance and importance in transfusion medicine. *Journal of nursing infusion.* **26(6)**:6367-372.

Aird I. and Bental H.H. (1953). A relationship between cancer of stomach and ABO blood group. *British Medical Journal.* **15**: 799-785.

Mazban M. Kamali M.S and Hosseinbasis T. (1998). Blood groups of the people of Ahaz. *Iran Anthropology ANZ* **46(1)**: 83-89.

Rahman M. and Ladhi V. (2004). Frequencies of ABO and Rhesus blood groups in blood donors in Punjab. *Pakistan medical Science Journals.* **20(4)**:20-27

Hoffbrand A.V (1981). Post graduate Haematology 2nd ed. London. Heineman professional publishing Ltd P. 270 350.

Lyko J., Gaetuer H. Kaiti J.N. (1992). Blood group antigens ABO and Rhesus in Kenyans. *Hamdard Medicus.* **35(3)**:59-67.

Tabib V. H. (2000). Handbook of Medical laboratory technology 2nd edition. New Delhi CBS Publishers p. 20-25.

Bashwari L.A. Mulhim A.A., Ahmad M.S. and Ahmad M.A (2001). Frequency of ABO blood groups in the eastern region of Saudi Arabia .*Saudi medical Journal.* **22(1)**:1008-1012

Jaggi S., Yadav A.S. (2014). Distribution of ABO and Rh.D allele frequency among the type 2 diabetic mellitus patients. *American International Journal of Research and Formal Applied Natural Science* **1**:24-26.

Dalisahi M. Aour Metri A., and Belmokhta R. (2011). The relationship between ABO /Rhesus blood groups and types 2 diabetics in Maghnia western Algeria. *South African family practice* **53**:568-572

Wazeem A.G., Igbal M. Khan O.A. and Tahir S..O. (2012) Association of diabetes mellitus with ABO and Rh blood groups. *Annals of Pakistan Institute of medical science.* **8**:134-136.

Jassim W.E. (2012). Association of ABO blood group in Iraquis with hyper cholesterolaemia, hypertension, and diabetis mellitus. Eastern mediterania Health Journal **18**:888 891