RESEARCH DISSERTATION

SERUM HIGH-SENSITIVITY C-REACTIVE PROTEIN CONCENTRATION IN HEALTHY BLOOD DONORS AT THE NATIONAL BLOOD TRANSFUSION CENTRE, NAIROBI, KENYA.

A research dissertation submitted in part fulfillment for the degree of Master of Medicine in Internal Medicine, University of Nairobi.

By

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2011

DECLARATION

I hereby declare that this is my original work and has not been presented for a degree in any other university.

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ACKNOWLEDGEMENTS

I would like to offer my sincere gratitude to the following people, without whose input, this work would not be complete.
My supervisors, Prof M. D. Joshi and Dr A. Amayo, for all their input, guidance, prodding, and patience.

Dr Deanna Cettomai and Dr Anna-Claire Meyer, for their technical support in defogging the realm of statistical analysis.

Dr Ahmed Kalebi and Eunice of Lancet Kenya, for your support and subsidy in running the laboratory samples

Elizabeth Mutie and James Muriithi, my research assistants, who diligently and efficiently helped in the recruitment, sometimes without lunch.

My Wife, Judy, and sons, David and Tiren, for all their encouragement and support.

STUDY OUTLINE

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ABBREVIATIONS

AFCAPS/TexCAPS- Air Force/Texas Coronary Atherosclerosis Prevention Study

AHA- American Heart Association

ARIC- Atherosclerosis Risk in Communities study

A to Z- Aggrastat to Zocor study

BMI- Body mass index

CDC- Centre for disease control

CRP- C reactive protein

CVD- Cardiovascular disease

DALYs- Disability adjusted life years

EPIC- European Prospective Investigation of Cancer study

HDL- high density lipoprotein

HIV- Human Immunodeficiency virus

hsCRP- high sensitivity C-reactive protein

IVUS- intravenous ultrasound

JUPITER- Justification for the use of statins in prevention: an intervention trial evaluating Rosuvastatin

LDL- low density lipoprotein

MI- myocardial infarction

MONICA- Monitoring of trends and determinants in cardiovascular disease study

MRFIT- The Multiple Risk Factor Intervention Trial

NBTC- National Blood Transfusion Centre

NHANES- National Health And Nutrition Examination Survey

MESA- MultiEthnic Study of Atherosclerosis

PROVE IT-TIMI- Pravastatin or Artovastatin and Infection Therapy:

Thrombolysis In MI

REVERSAL- Reversing Atherosclerosis with Aggressive Lipid Lowering

UON- University of Nairobi

USA- United States of America

WHO- World Health Organization

WHS- Women Health Study

ABSTRACT

BACKGROUND

According to the WHO, an estimated 17 million persons die annually from cardiovascular disease (CVD). The burden of CVD is rising rapidly in the developing countries but stabilizing or declining in the developed nations. This is partly due to targeting modifiable risk factors and identifying at-risk persons for close follow-up for CVD. High Sensitivity C-Reactive Protein (hsCRP) is a novel CVD risk factor, and its inclusion in global risk stratification for CVD led to reclassifying 20% - 30% of persons at intermediate risk into clinically higher or lower risk categories for CVD. Current hsCRP risk stratification data is drawn from Caucasian populations and at the time of the study, there was no local data.

METHODS

This was a cross-sectional descriptive study to determine the normative hsCRP data from 219 healthy Kenyan adult blood donors. Basic anthropometric data was obtained and the serum hsCRP level was determined using a photometric immunoturbidimetric assay method. Mean and median values were calculated and the distribution of hsCRP level was explored. We explored the effect of various exclusion criteria on the mean and median, to cater for outliers. We chose hsCRP>5mg/L exclusion criteria (n=205) to develop a normal range of values for a healthy Kenyan population. We defined a normal range as within 2.5% - 97.5% percentiles of the

distribution. Bivariate and multivariate linear regression models were used to explore the effect of gender, age, and BMI on hsCRP.

RESULTS

The sample was 71% (155/219) male with a median age of 26 (IQR=22-33) years. The mean BMI was 24 kg/m^2 (SD=3.8), with 1% being underweight, 68% having normal weight, 22 % overweight, 8% obese and 0.5% classified as morbidly obese. The median hsCRP level was 0.6 mg/L (IQR 0.3 - 1.7mg/L, Min=0.1, Max=65.3). Excluding hsCRP >5mg/L, the median hsCRP was 0.5mg/L (IQR 0.3 – 1.2 mg/L). The normal hsCRP level among Kenyan adults was calculated to lie between 0.1-3.3mg/L. Bivariate and multivariate regression analysis found a significant association between hsCRP levels and gender (95% CI -0.68, 0.00 [p=0.05]), age (95%CI 0.01, 0.04 [p=0.01]) and BMI (95%CI 0.03, 0.11 [p=<0.001] for multivariate models). In terms of hsCRP risk categorization, 68%(140/205) of blood donors had levels < 1 mg/L, 26% (53/205) with 1-3 mg/L, and 6% (12/205) with >3 mg/L.

CONCLUSION

The median hsCRP in healthy Kenyan blood donors is 0.5 mg/L (IQR 0.3 – 1.2 mg/L) with a normal range between 0.1 and 3.51 mg/L. Gender, age, and BMI were significantly associated the hsCRP levels. Majority of our blood donors are in the low- risk category of hsCRP stratification for CVD events.

1.0 INTRODUCTION AND LITERATURE REVIEW

The burden of cardiovascular disease (CVD), particularly ischemic heart disease and stroke, varies remarkably between regions of the world and ethnic groups. Whereas CVD is declining or stabilizing in Europe, North America, Australia and New Zealand, it is increasing in developing countries [1]. An estimated 17 million people die of CVD every year, with an estimated disability-adjusted life years (DALYs) of 153 million by 2010 and 187 million by 2030. CVD is responsible for 10% of DALYs in developing countries and 18% in developed countries, but more than 60% of the global burden of coronary heart disease occurs in developing countries.[2]

CVD has multiple risk factors, of which the major risk factors are modifiable. According to the World Health Organization (WHO), the major risk factors are hypertension and dyslipidemia. The other modifiable risk factors are tobacco use, physical inactivity, obesity, unhealthy diets, diabetes mellitus, low socioeconomic status, mental ill-health, psychosocial stress, medications (oral contraceptives and HRT), and left ventricular hypertrophy. The non-modifiable risk factors include advancing age, family history of CVD, gender, ethnicity/race.

C-reactive protein (CRP), an indicator of inflammation, has recently been identified as a novel CVD risk factor.

Measurement of hsCRP augments risk assessment in the identification of persons who should be considered for statins, antiplatelets, or other cardioprotective drugs or lifestyle changes. Another, but untested, use is to motivate persons with moderate to high risk to improve their lifestyle or comply with their treatment.

A major and urgent need is for population-based high sensitivity CRP (hsCRP) data as CVD risk predictor in sub-Saharan Africa. Current "normal values" of hsCRP are derived almost exclusively from European or European American reference population.[3] It is possible that a difference exists on the "normal values" among our Kenyan population and thus the standardized use of hsCRP to determine risk of future CVD may not be applicable.

A local study to determine the range of CRP concentration in our population was warranted.

1.1 History of C Reactive Protein

CRP was described by Tillet et al in 1930 at the Rockefeller University, who described a serologic fraction that could be isolated from patients infected with pneumococcus that was distinct from previously known capsular polysaccharide and nucleoprotein fractions[1]. In 1940, Oswald Avery and Maclyn McCarty described CRP as an "acute phase reactant" that was elevated in patients with inflammatory conditions such as rheumatic fever or myocarditis[5, 6]. A decade later, Irving Kroop and N Shackman described CRP elevation after coronary ischemia and myocardial necrosis in a case series[7]. In the 1980s, Mark Pepys identified CRP as a hepatically derived, non-glycosylated, circulating pentraxin composed of five identical subunits arranged with pentameric symmetry that had dense calcium-dependent binding to specific ligands, including LDL-cholesterol[8].CRP is produced by the liver under regulation of cytokines such as interleukin-6 and tumor necrosis factor, and has a half-life of 17hrs; thus plasma concentration is determined by the synthetic rate [9, 10].

Interest in CRP increased with consistent reports of association with coronary disease[11]. The study, Elevation of CRP in 'active' coronary heart disease, showed that concentrations of CRP rise with myocardial infarction (MI), in individuals with known vascular disease, however, it was not informative regarding whether CRP concentrations are increased in-advance of disease progression. The next step was to determine CRP levels among healthy individuals and follow them over time to see if baseline CRP increases associated with future vascular events.

1.2 Studies investigating utility of CRP

Prospective cohort studies of initially healthy individuals who had baseline CRP measurements and were then followed longitudinally have been performed to investigate whether an elevated baseline CRP was associated with future CVD. In the first study to use this design, The Multiple Risk Factor Intervention Study (MRFIT), investigators found an association, but only for fatal events among high-risk populations who were predominantly smokers. Tobacco use in itself raises levels of CRP, and, as such, these data could not differentiate whether increased CRP was a result of the disease process or a significant biomarker of risk preceding onset of disease itself [12].

The Physician Health Study, a large scale prospective cohort of healthy American men in 1997, found CRP was significantly higher in men who went on to develop MI (relative risk = 2.9, P <0.001) and stroke (RR=1.9, P <0.02) than those who did not[13]. This data provided a strong basis of support for the inflammatory hypothesis of atherosclerosis.

The Women's health study (WHS) is an ongoing evaluation of aspirin and vitamin E for the primary prevention of cardiovascular events among women 45 years of age or older. The initial analysis included a head-to-head comparison of ten lipid and non-lipid biomarkers of vascular risk and found hsCRP to be the single most powerful predictor of future vascular events. The WHS investigators then evaluated a full lipid profile and baseline hsCRP levels among 27,939 initially healthy women and followed them over a 10-year period for future vascular events. The relative risks of first cardiovascular events according to increasing quintiles of CRP, as compared

with the women in the lowest quintile, were 1.4, 1.6, 2.0, and 2.3 (P<0.001), where as the corresponding relative risks in increasing quintiles of LDL, as compared with the lowest, were 0.9, 1.1, 1.3, and 1.5 (P<0.001): the worst outcomes observed for those with increased levels of both LDL and hsCRP and those with low LDL but high hsCRP, and the best outcomes observed with low levels of both LDL and hsCRP. Overall, 77% of all events occurred among women with LDL below 4.14mmol/L, and 46% occurred among those with LDL below 3.36mmol/L. It showed the importance of understanding interactions between lipid-lowering treatment and inflammation, especially as statins lower the risk of stroke despite fact that LDL-cholesterol is not a major risk marker for stroke [14].

The Cholesterol And Recurrent Events (CARE) trial in 1998 showed Pravastatin reduced cardiovascular events, especially in those with increased CRP, than placebo (RR=1.29,*P*=0.5, and RR=2.11, P=0.048 respectively) [15]. Statins also reduced CRP levels in a largely independent manner of LDL-cholesterol. These observations were subsequently confirmed for all statins, but more so for Rosuvastatin, which showed the greatest decrease in LDL-cholesterol and CRP [16, 17, 18, 19, 20]

By 1999, evidence was rapidly accruing that CRP was not only a novel biomarker of vascular risk, but that CRP may merit consideration as a tool to monitor pharmacologic interventions used to prevent and treat cardiovascular disease.

In 2001, the Air Force/Texas Coronary Atherosclerosis Prevention study (AFCAPS/TexCAPS) showed that statin therapy decreased vascular events among those with high CRP and low LDL-

cholesterol, but not those with low CRP and low LDL-cholesterol [21]. In 2002, the Women's Health Study showed that persons with low cholesterol and high CRP represented a high risk population outside the current guidelines for statin therapy [22].

By 2003, clinical guidelines for use of hsCRP as an adjunct to global risk prediction was endorsed by the American Heart association (AHA) and the Centre for Disease Control (CDC). The guidelines formalized the reporting of hsCRP, where levels of <1mg/L, 1-3mg/L, and >3mg/L represents low, moderate, and high relative risk of vascular disease within the context for global risk evaluation [23].

Several studies have confirmed hsCRP as an independent predictor of future cardiovascular events in more than 30 diverse population cohorts, including the Monitoring of Trends and Determinants in CVD (MONICA) study [24 25], the Atherosclerosis Risk in Communities study (ARIC) study [26], and the European Prospective Investigation of Cancer (EPIC) study, among others. [27 28 29 30 31 32 33 34 35].

Based on consistency of these data, clinical risk algorithms known as the Reynolds Risk Scores for men and women were developed and validated that, in addition to traditional risk factors, incorporate information on both inflammation (hsCRP) and genetics (parental history of MI before age 60 years). This has reclassified 20% to 30% of those at intermediate risk into clinically relevant higher or lower risk categories [36, 37]. The utility of hsCRP for risk reclassification has been confirmed by the Framingham Heart Study.

Additional observations by Pradhan, Manson, Rifai et al. were made in the WHS in 2001 that hsCRP predict incident diabetes [38]. Several investigators reported on the relationship between CRP, insulin resistance, and metabolic syndrome. Among them are Laaksonen, Sattar N, Festa, Haffner, and Dandona whose data indicate that hsCRP is a predictor of metabolic syndrome and diabetes even after adjusting for insulin concentrations and that insulin itself may regulate CRP expression [39, 40, 41]

These observations of hsCRP provided necessary, but not sufficient evidence, to warrant its routine use as a target for intervention: several studies have now addressed this. Among patients with acute coronary ischemia, both the Pravastatin or Artovastatin and Infection Therapy: Thrombolysis In MI 22 (PROVE IT- TIMI 22) trial in 2005 [42] and the Aggrastat to Zocor (A to Z) trial in 2006 [43] demonstrated that the best clinical outcomes after starting statins accrue among those who not only reduce LDL-cholesterol to less than 1.8 mmol/L, but also reduce hsCRP to < 2mg/L. Similarly, in the Reversing Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, atherosclerotic regression with statins as detected by intravenous ultrasound (IVUS) was only observed among those who reduced hsCRP and LDL-cholesterol [44]. These effects are particularly robust for the prevention of stroke, where LDL-cholesterol reduction alone following statin therapy has not been found sufficient to prevent stroke, but those subjects who achieve low LDL and hsCRP after statin therapy have a lower incidence of stroke [45]. On this basis, it has been proposed that physicians consider dual goals for statin-treated patients that include both low concentrations of LDL-cholesterol and low concentrations of hsCRP [46].

As data on AFCAPS/TexCAPS was a post-hoc analysis, a trial to test the effects of statins on persons with low LDL but increased CRP was required, and the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER), was launched in 2003 [47]. By May 2008 the trial was stopped by its Independent Data and Safety Monitoring board due to emergence of a statistically significant benefit of Rosuvastatin among apparently healthy men and women with low concentration of LDL (<130mg/dL) but increased concentration of hsCRP (> 2mg/L). Specifically, among 17,802 enrolled participants in the JUPITER trial followed for 5 years, random allocation of Rosuvastatin resulted in a 54% reduction in MI (P= 0.0002), a 48% reduction in stroke (P=0.002), a 47% reduction in need for arterial revascularization (P=0.00001), and a 20% reduction in all cause mortality (P=0.02) compared with placebo [48].

The effects among patients with increased hsCRP were consistent in all subgroups, including those traditionally assumed to be at low risk, such as female, low Framingham scores, and low native LDL (< 2.59mmol/L). In patients with optimal lipid concentrations according to current guidelines (median LDL at study entry 2.80mmol/L), median HDL 1.27mmol/L), a 37% reduction in primary end points in the subgroup with increased hsCRP but no major risk factor other than increased age was demonstrated. The number needed-to-treat for the primary five year projected endpoint for individuals with increased hsCRP was twenty five. All reductions were evident within months of starting statin therapy. It was also effective in defining high risk population among women and minority populations [55].

However, it remains controversial if hsCRP is only a biomarker of risk or whether it has a causal relationship. Only through direct experimentation, such as through trials of targeted anti-inflammatory therapies, will it be possible to ultimately clarify whether hsCRP is a direct participant in the atherosclerotic process or not [49].

1.3 Factors influencing serum hsCRP concentrations

Evidence currently supports the role of inflammation in the etiology of atherosclerosis and coronary heart disease [50]. An individual risk to develop CVD may be influenced by difference in lifestyle factors and genetics. These factors may affect plasma hsCRP concentrations including smoking, adiposity, genetics, hormone replacement therapy, age and gender.

Smoking:

Smoking is strongly associated with CHD and that by removing the exposure, rates of heart diseases decline. Smokers have a higher baseline hsCRP levels. Whether smoking and hsCRP are causally linked to CHD is not completely understood, but smoking is linked to inflammatory process, atherosclerosis, and CHD[51].

Body mass index:

Measures of total and abdominal adiposity are both highly correlated with hsCRP levels in men and women, but there appears to be a stronger association among women. Weight loss, among overweight and obese persons, is associated with significant and sustained reductions in hsCRP[52].

Genetics:

In the background of accumulating data that baseline CRP is indicative of persons prone to cardiovascular disease and that 40-50% of the serum concentration is heritable. Eklund et al examined the effect of CRP nucleotide polymorphism on CRP baseline concentration. 336 healthy blood donors were genotyped and found that CRP gene polymorphism influences baseline CRP values and might be predictive of persons prone to cardiovascular disease.[53]

Hormone replacement therapy (HRT):

Women on HRT have increased level of hsCRP. HRT increases liver production of hsCRP during first-pass metabolism. It is not known, however, whether the increase translates to adverse clinical vascular outcomes. While cessation of HRT leads to reduced levels of hsCRP[54].

Age and Gender:

Several studies have reported conflicting reports on difference in distribution of hsCRP in men and women, but the cut-point proposed by the CDC/AHA for assessing risk of CVD are valid for all populations examined. There is, however, an increase of hsCRP with age[55].

1.4 Population differences in serum hsCRP levels

Distribution of hsCRP in samples representative of the general population are highly skewed to the left among males and females. hsCRP concentrations increase with age, BMI, smoking, pre-existing illnesses, and modified by medications (statins), nutrition, and genetics.

In the National Health and Nutrition Examination Survey (NHANES), USA of 1999-2000, the median hsCRP concentrations were 1.6 mg/L for all men, 1.6 mg/L for white men, 1.7 mg/L for African Americans, 1.5 mg/L for Mexican American men. In women, after exclusion of those on HRT, the hsCRP concentrations were 2.5 mg/L for all women, 2.3 mg/L for white women, 3.5 mg/L for African American women, and 3.6 mg/L for Mexican American women [56]

In the Women's Health Study, the median hsCRP levels (Fig. 1) were significantly higher among Black women (2.96mg/L, interquartile range 1.19- 5.86mg/L) than white women (2.02mg/L, IQR 0.81- 4.37mg/L). BMI was a significant predictor of increased levels of CRP in all ethnic/racial groups ($P < 0.001$), but when controlled for BMI, the difference among races was attenuated, particularly among black women [57].

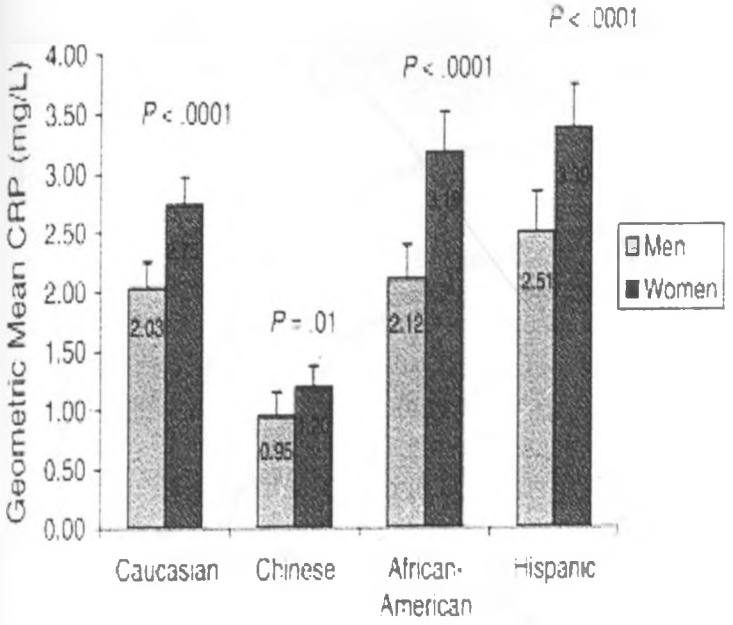

Figure 1 hsCRP among US ethnic groups from Women's Health Study.

A Canadian study on CRP as a screening test for cardiovascular risk in a multiethnic population found varying levels between the groups (Fig. 2). The age- and sex- adjusted median hsCRP was 4.2 mg/L among Aboriginals, 1.8 mg/L among South Asians, and 0.69 mg/L among Chinese compared with 1.24 mg/ L among Europeans (overall P < 0.0001). The differences in CRP concentrations between ethnic groups were substantially diminished, but not abolished, after adjustment for metabolic factors (BMI, waist circumference, triglycerides, and systolic blood. The authors concluded that prospective validation of CRP as a risk factor for cardiovascular disease among nonwhite ethnic groups is required [58].

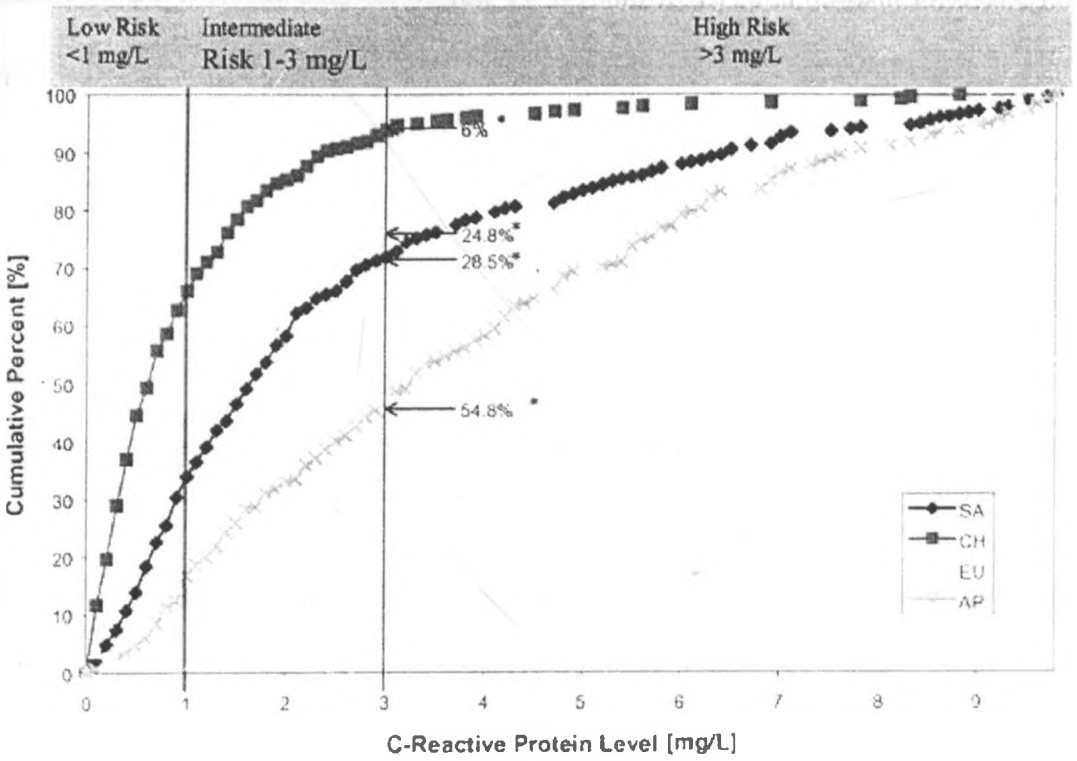

Figure 2. Ethnic comparisons of CRP. (CRP as a screening test for cardiovascular risk in a multiethnic population, Anand et al. Arterioscler Thromb Vasc Biol 2004)

(SA, South Asians, CH, Chinese, EU, Europeans, AP, Aboriginal people)

Data from the MultiEthnic Study of Atherosclerosis cohort (MESA), found CRP to be higher in women than men, even after accounting for BMI (1.85mg/L vs 1.33mg/L, $P < 0.001$), and this was maintained across all racial groups [59].

A European study on the distribution of hsCRP in healthy men and women, found no difference between European men and women, with the median for those aged 20-44 years 0.6- 1.1mg/L and those > 44 years 1.2-1.7mg/L [60].

In view of the conflicting data on distribution of hsCRP among in men and women, the cut-off points for risk stratification by the CDC/AHA remain valid for all populations.

1.5 Local Data

Currently there is no local data on the distribution of hsCRP among the healthy population.

A study on hsCRP in type 2 diabetic patients with and without the metabolic syndrome published in 2008, studied 197 patients and the data reported the hsCRP mean of 4.33mg/L and a median of 2.53mg/L (Fig 3)[61].

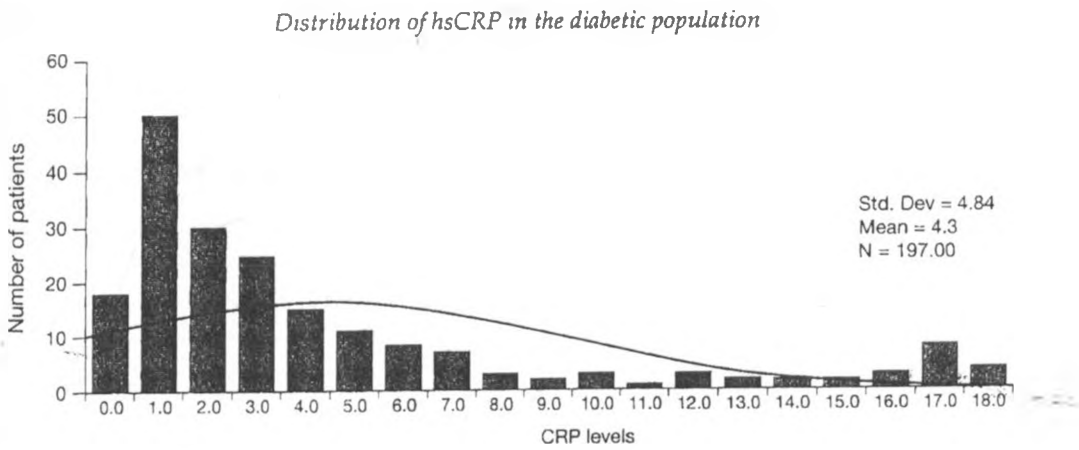

Figure 3. Distribution of hsCRP in the diabetic population *(hsCRP in type 2 diabetic patients with and without metabolic syndrome, East African Medical Journal, 2008)*

From all these studies, there appears to be differences in the population distribution of hsCRP. Local data on the distribution of hsCRP concentrations in healthy populations currently lacks and with the emergence of hsCRP as a biomarker for future cardiovascular risk, a study is warranted to establish the hsCRP levels locally.

1.6 Studies using blood donors

Blood donors are often used as proxies for the general population, but are inappropriate in detecting abnormalities or certain diseases as blood donors are an apparently healthy population. Several studies have been undertaken to determine reference ranges and validate assay methods with the use of blood donors as a study population in evaluating CRP.

In Denmark, a study sampled 268 healthy blood donors aged 20 years to 65 years to determine reference range of hsCRP using a latex mono assay. The reference interval for serum hsCRP for both genders was comparable to reported European studies in the general population.[62]

2.0 RATIONALE AND JUSTIFICATION

Cardiovascular disease (CVD) is rapidly emerging as a major cause of morbidity and mortality in developing nations, while it is on the decline or stabilizing in developed countries. Of the total global CVD burden, developing nations accounts for 60% and further contributes to10% of DALYs. Interventions targeted at reducing its impact are urgently required in our country to prevent it becoming the leading cause of death, as occurred in developed countries.

C-reactive protein is a novel biomarker that may predict those at risk of cardiovascular disease. Its measurement augments risk assessment in the identification of persons who should be considered for statins, antiplatelets, or other cardioprotective drugs or lifestyle changes.

Current risk stratification incorporating hsCRP relies on data drawn mainly from European or European American populations, with no local reference range data available in normal healthy persons. Various studies report different hsCRP profiles as per ethnic/racial groups and a trend toward higher hsCRP concentrations among the Black population. Previous local data on hsCRP targeted groups with preexisting diseases at significantly increased risk of CVD.

This study was done in attempt to fill this void and establish the hsCRP profile in this country by sampling normal healthy population and controlling for confounding variables.

3.0 OBJECTIVES

The broad objective of this study was to determine the serum hsCRP concentrations in healthy persons donating blood to the National Blood Transfusion Center, Nairobi.

The Specific Objectives were:

- To determine the distribution of serum hsCRP level among healthy adults donating blood to the National Blood Transfusion Centre, Nairobi.

- To determine if the following have an effect on hsCRP levels among healthy adult Kenyan blood donors:

    1. BMI.
    2. Gender.
    3. Age.

4.0 STUDY DESIGN AND METHODOLOGY

4.1 STUDY DESIGN

This study was a cross-sectional descriptive study in which adult healthy blood donors were sampled for hsCRP, age, gender, and BMI to determine normative data.

4.2 STUDY AREA

The study was conducted at Nairobi central business district donor sites for the National Blood Transfusion Center, Nairobi.

4.3 STUDY POPULATION

This comprised of healthy adults who had self-selected to donate blood at the National Blood Transfusion donor sites.

4.4 INCLUSION CRITERIA

- Adults 18 years or older
- Blood donors to the NBTC
- Negative for HIV, Hepatitis B and C, and syphilis as per NBTC screening protocol

4.5 EXCLUSION CRITERIA

- Age less than 18years
- HIV, hepatitis B, hepatitis C, and syphilis positive
- Any recent (previous two weeks) illness
- Known chronic illness
- Drugs (immunosuppressive, statins, oral contraceptives)

4.6 SAMPLE SIZE

The sample size was 208 as calculated by the formula (Stata 10.0)

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2}{\Lambda^2}$$

Where:

n = sample size.

σ = Within group standard deviation.

Λ = detectable difference.

$Z_{\alpha/2}$ = Z-statistic corresponding to the assumed alpha level.

Z_{β} = Z-statistic corresponding to the assumed power (power = $1 - \beta$).

Within group standard deviation = 4

Detectable difference = 0.9

Alpha level (P) = 0.05

Power = 0.9

(Reference: Anand SS, Razak F, Yi Q, et al. C-reactive protein as a screening test for cardiovascular risk in a multiethnic population. Aterioscler Thromb Vasc Biol. 2004;24:1509-1515.)[50]

4.7 SAMPLING METHOD

Each week, the principal investigator and two research assistants accompanied staff of the NBTC to their blood donor recruitment sites in the NCBD and selected volunteers for blood donation. If consent was obtained, they were considered recruited if inclusion criteria were met. This sequential sampling continued until the desired sample size was achieved.

4.8 CLINICAL METHODS

4.81 SUBJECT RECRUITMENT

The study was explained to every blood donor and if consent obtained, were subjected to the screening questionnaire (see appendix 4). If the results of the screening questionnaire were acceptable, a signed consent obtained and subject considered recruited. A study proforma then completed (see appendix 5). This was repeated until the sample size was achieved.

4.82 CLINICAL EVALUATION

History and physical data was obtained from all recruited patients as outlined by NBTC and study proforma. The NBTC staff obtained a history targeted to eliminate risk of blood

transmissible diseases. Data on anthropometric measures (height and weight for BMI) was obtained in the following manner:

Height was obtained with the participant facing the investigator/assistant, against a wall, with feet together, legs straight, and no foot/head wear. Height was measured with measuring stick to the nearest 0.5cm.

Weight was obtained using a standard bathroom scale with the participant wearing light clothing and no shoes to nearest 0.5kg. The scale was calibrated weekly to determine zero setting against a beam balance and thereafter used the investigator's own weight as a reference before each weighing.

4.83 LABORATORY METHODS

Five milliliters (5mls) of blood was drawn from a peripheral vessel, preferably the antecubital fossa, using an aseptic technique. The serum was used to obtain serological tests as per WHO/NBTC safe transfusion protocol and later for hsCRP measurement using photometric immunoturbidimetric assay done at 37 degrees Celsius.

Two serum vials (aliquots of 1.5mls each) were obtained from the specimen used for serological screening of transfusion transmissible infections as per WHO safe transfusion protocol.
Serum vials were then stored at -20^{0}C until the period for analysis of hsCRP.

Samples from participants found to have HIV, Hep B/C, or syphilis were discarded.

Analysis was done in a batch after the specimens were thawed to room temperature.

The hsCRP was measured using photometric method on the Olympus A400 Analyzer.

Quality control samples were run before the specimens were analyzed. Specimens were only run when the Quality Control materials met the quality standards to minimize preanalytical errors.

4.84 QUALITY ASSURANCE

Blood was obtained and stored at -20 degrees Celsius as per recommended procedure to minimize pre-analytical errors.

All equipment was calibrated according to the manufacturer's specifications.

Commercial controls were used to validate the calibrations. The results were only accepted if the control values were within the expected ranges.

Results were recorded onto data sheets which were checked by two people to minimize post analytical transcriptional errors.

4.85 DATA ANALYSIS

Statistical analysis of data was undertaken using the Stata10 (Stata Corp. College Station. Texas). Data was presented in the form of tables, graphs and histograms.

Descriptive statistics such as means, medians, proportions and standard deviation were determined where applicable.

Primary objective: The mean and median serum hsCRP level among the study participants was reported along with standard deviation and interquartile range.

Secondary objective: Multiple linear regression models were performed to explore the association between age, gender and BMI on serum hsCRP levels among study participants.

5.0 ETHICS AND CONFIDENTIALITY

Permission to carry out this study was obtained from Kenyatta National Hospital Scientific/University of Nairobi-Ethical Review Committee and Director of National Blood Transfusion Centre, after approval from the University of Nairobi, Department of Clinical Medicine and Therapeutics.

Participants were enrolled in the study only after giving informed consent.

All information obtained from the study was handled in confidence and used only for the intended purpose.

All laboratory and BMI outcomes were communicated to the subjects for relevant action.

6.0 RESULTS

A total of 250 subjects were screened between January 20th and 28th 2011. of these 31 subjects were excluded for various reasons: 19 had transfusion transmissible infections, 5 refused consent, 5 met exclusion criteria, and 2 did not complete study procedure. A sample of 219 subjects was enrolled into the study. All study subjects had anthropometric data obtained (age in years, weight in kg, height in meters) and serum hsCRP biochemical analysis done.

6.1 Demographics

The demographic characteristics are shown in table 1.

Table 1. Study Population Characteristics

	n=219
Male Gender *[%(n)]*	71% (155)
Age (years) *[median (IQR)]*	26 (22-33)
Height (m) *[mean (SD)]*	1.7 (0.1)
Weight (kg) *[mean (SD)]*	70 (11)
Body Mass Index (kg/m^2) *[mean (SD)]*	24.0 (3.8)
Body Mass Index (BMI) Categories *[% (n)]*	
Underweight (BMI < 18.5)	1% (3)
Normal Weight (18.5 ≤ BMI < 25)	68% (149)

Overweight (25 ≤ BMI < 30)	22% (48)
Obese (30 ≤ BMI < 40)	8% (18)
Morbidly Obese (BMI ≥ 40)	0.5% (1)

Gender

The population comprised 71% (155/219) male and 29 % (64/219) female.

Age

The overall population median age was 26 yrs with IQR 22-33. The age distribution is shown in Figure 4 below.

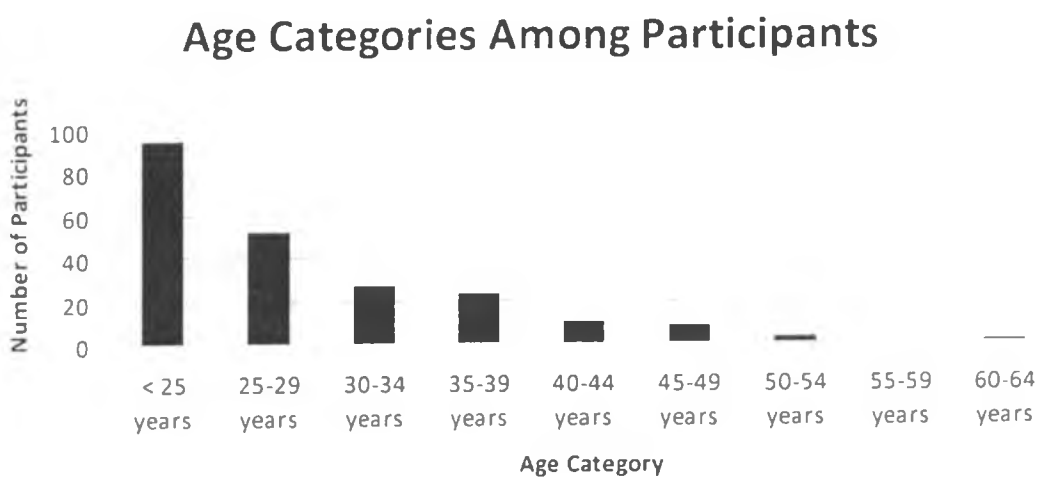

Figure 4. Age categories among participants

Body mass index (BMI)

The mean weight was 70 kg (SD 11) and mean height 1.7 meters (SD 0.1). The calculated mean BMI was 24 kg/m^2 (SD 3.8). When categorized by BMI definition: 1% (3) underweight, 68% (149) normal weight, 22% (48) overweight, 8% (18) obese, and 0.5% (1) morbidly obese as shown in figure 5 below.

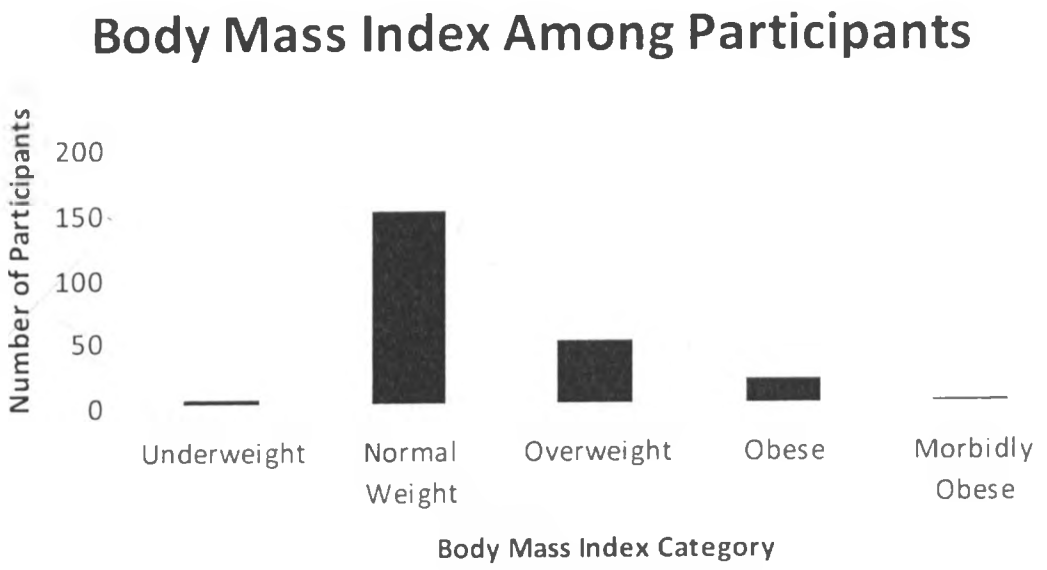

Figure 5. Body Mass Index of participants.

6.2 hsCRP Results

The median hsCRP was 0.6 mg/L , IQR 0.3 – 1.7 mg/L, and range of 0.1mg/L 65.3 mg/L., with a mean of 1.7 mg/L (SD 5). The distribution was non-gaussian and heavily skewed to the left (see figure 6 below).

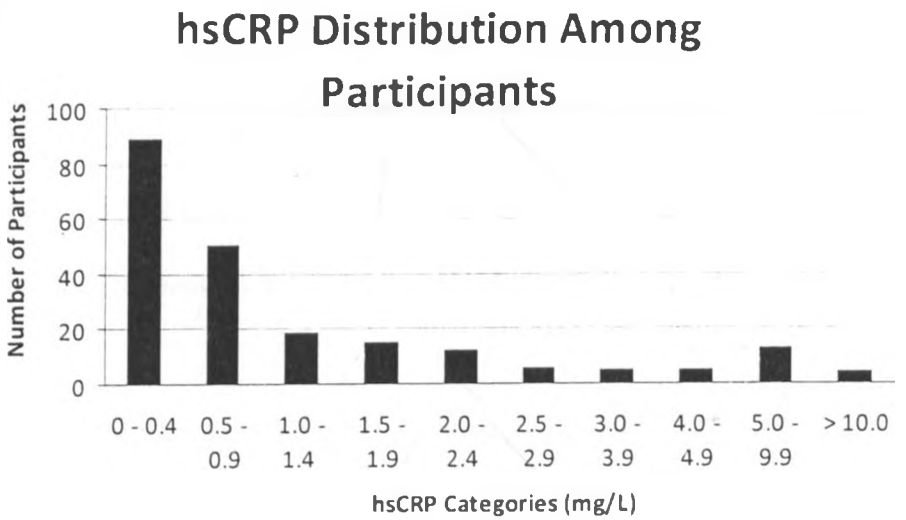

Figure 6. hsCRP distribution among participants.

We calculated the mean and median, and explored the distribution of hsCRP levels. We explored the effect of various exclusion criteria (hsCRP < 10 mg/l and < 5 mg/L) on the mean and median. The mean changed significantly with each cutoff but the median was little affected. Finally based on previously validated data of using hsCRP of 5 mg/L or less for cardiovascular disease risk stratification, we chose exclusion of hsCRP of greater than 5 mg/L to develop a normal range for hsCRP for a healthy Kenyan blood donor population (See table 2 below). We defined a normal range as 0.1 mg/L – 3.3 mg/L within 2.5% - 97.5% percentiles of the distribution.

Table 2: hsCRP Results with Different Exclusions

	n	Mean	SD	Median	IQR	Min/Max
No Exclusions	n=219	1.7	5	0.6	0.3-1.7	0.1-65.3
Excluding hsCRP > 10mg/L	n=215	1.2	1.7	0.6	0.3-1.5	0.1-9.2
Excluding hsCRP > 5mg/L	n=205	1	1.1	0.5	0.3-1.2	0.1-5.0
Excluding 2SD above mean	n=216	1.3	1.8	0.6	0.3-1.6	0.1-10.2
Excluding 3SD above mean	n=217	1.3	2	0.6	0.3-1.6	0.1-14.6

Effect of gender, age and BMI on hsCRP

Gender

The female population had a significantly higher hsCRP as compared to males as shown in table 3 below.

Table 3. hsCRP Stratified by Gender

	Male (n=151)	Female (n=53)	p
hsCRP (mg/L) *[mean (SD)]*	0.9 (1.0)	1.3 (1.2)	0.02

Age

There is a trend of hsCRP to rise with age that was statistically significant as shown in table 4 below.

Table 4. hsCRP Stratified by Age (10 year intervals)

	< 30 yrs (n=139)	30-39 yrs (n=44)	40-49 yrs (n=17)	50-59 yrs (n=3)	60-69 yrs (n=1)	p
hsCRP (mg/L) *[mean (SD)]*	0.9 (1.0)	0.9 (0.9)	1.7 (1.4)	1.1 (0.9)	1.7	0.049

BMI

The study shows a consistent rise of hsCRP with rise in the BMI as shown in table 5 below.

Table 5. hsCRP Stratified by Body Mass Index (BMI)*

	Underweight (n=3)	Normal Weight (n=146)	Overweight (n=41)	Obese (n=14)	Morbidly Obese (n=1)	p
hsCRP (mg/L) *[mean (SD)]*	0.7 (0.4)	0.8 (1.0)	1.4 (1.1)	1.5 (1.4)	2	0.006

**Underweight: BMI < 18.5 kg/m^2; Normal Weight: 18.5 kg/m^2 ≤ BMI < 25 kg/m^2; Overweight: 25 kg/m^2 ≤ BMI < 30 kg/m^2; Obese: 30 kg/m^2 ≤ BMI < 40 kg/m^2; Morbidly Obese: BMI > 40 kg/m^2*

Linear regression modeling

Bivariate and multivariate linear regression models were to explore associations between hsCRP levels and gender, age and BMI. Sensitivity analysis of multivariate models was conducted to explore the effects of excluding outliers.

Gender, age and BMI each showed a significant association with hsCRP on bivariate regression (β [95%CI] of -0.41 [-0.74, -0.08], 0.03 [0.01, 0.05] and 0.10 [0.06, 0.13] respectively). The significance was maintained for each on multivariate regression (β [95%CI] of -0.34 [-0.68, -0.00], 0.03 [0.01, 0.04] and 0.07 [0.03, 0.11] respectively), as shown in table 6 below.

Table 6. Regression Modeling using hsCRP ≤ 5mg/L

	Bivariate Linear Regression		Multivariate Linear Regression	
	β (95% CI)	p	β (95% CI)	P
Male gender	-0.41 (-0.74, -0.08)	0.02	-0.34 (-0.68, 0.00)	0.05
Age in years	0.03 (0.01, 0.05)	0.001	0.03 (0.01, 0.04)	0.01
Body Mass Index (BMI) (kg/m^2)	0.10 (0.06, 0.13)	<0.001	0.07 (0.03, 0.11)	<0.001

Risk stratification of hsCRP

Using the AHA/CDC risk stratification for hsCRP: 68% (140/205), 26% (53/205), and 6% (12/205) fell into the low risk, moderate risk, high risk categories, respectively (see table 7 below).

Table 7: hsCRP Risk Stratification using previously validated cut-offs

	n=205
hsCRP Low risk (<1mg/L)*[%(n)]*	68% (140)
hsCRP Moderate risk (1-3mg/L)*[%(n)]*	26% (53)
hsCRP High risk (>3mg/L)*[%(n)]*	6% (12)

7.0 DISCUSSION

This was a cross-sectional descriptive study that was conducted at the National Blood Donor Transfusion Center donor sites located in the central business district of Nairobi. The overall population was mainly males, constituting 71% of the recruited subjects. It was a young population with a median age of 28 years (IQR 22-33 yrs). Volunteers aged less than 35 yrs comprised 79% of the population. These reflect the characteristics of the donor population worldwide. Veldhuizen et al in Holland conducted a study on the demographics of blood donors and found that men donate five times more than women (odds ratio 5.27). Zuller N et al noted that Chinese blood donors were mainly men aged thirty six years or less[63 64]

A large proportion (68%) of the volunteers had a normal body mass index (BMI) and 22% were overweight. The mean BMI was 24 kg/m^2 (SD 3.8). A contributory factor being the population was mainly males, and men have lower BMI than women.

In this study we attempted to develop the normal reference range for hsCRP for a presumed healthy Kenyan population. The mean hsCRP was 1.7 mg/L (SD 5.0), median 0.6 mg/L (IQR 0.1-1.7 mg/L), and range of 0.1 – 65.3 mg/L. The distribution was non-Gaussian and skewed to the left. This is consistent with other blood donor and general population hsCRP studies [58 60 62 65].

Macy E et al, at the University of Vermont, USA, conducted hsCRP study on 143 blood donors aged 18 to 67 with equal representation of gender and found the hsCRP median at 0.64 mg/L (IQR 0.08 – 3.11 mg/L)[65]. A general population study in Canada on hsCRP in different ethnic groups with mean age 47.7 years was done by Anand S et al between 1996 and 2002 and found varying hsCRP among them. The Chinese population compared closely to this study with hsCRP median at 0.69 mg/L (IQR 0.3 – 1.5 mg/L). The highest was reported among South Asians and Aboriginals: median of 1.80 mg/L (IQR 0.86-3.90 mg/L) and 4.2 mg/L (1.8-7.6 mg/L). This was postulated to be due to higher abdominal adiposity among this latter group [58]. Erlandsen E et al, did hsCRP in Danish blood donors aged 20 – 65 years old and found a median of 0.98 mg/L (range 0.2 – 17.3 mg/L) [62]. Price et al did a similar study in British blood donors in London and found a median hsCRP of 2.4 mg/L (IQR 0.4 – 5.4 mg/L) [66].

An analysis done on hsCRP level to exclude outliers did not alter the median greatly, changing from 0.6 mg/L to 0.5 mg/L. The mean changed by a larger margin when the various exclusion criteria were applied, as would be expected, due to the skewed hsCRP results. The cutoff for hsCRP level in detecting future cardiovascular event is 5 mg/L[23], and using this cutoff to exclude outliers from our sample, the normal reference range for a healthy Kenyan population was defined as lying between 0.1 – 3.51 mg/L.

This reference range compares well to the CDC definition of a normal hsCRP range of 0.08 – 3.1 mg/L, with > 95% of populations from more than 15 populations with more than 40,000 persons

having hsCRP < 10 mg/L [23]. The hsCRP distribution among a healthy Kenyan population is therefore comparable to that used by the CDC to define normal hsCRP levels.

Bivariate and multivariate regression models showed a significant association between gender, age and BMI on level of hsCRP in our sample. The gender effect was slightly attenuated but remained independently significant on multivariate modeling. Age and BMI also remained significant. Our study showed that: males had hsCRP 0.34 mg/L lower than females (p 0.05); with each one-year increase in age, the hsCRP rose by 0.03 mg/L, and for each unit rise of BMI, the hsCRP rose by 0.07 mg/L ($p < 0.001$). The NHANES study of 1999-2000 found hsCRP to be higher in women, particularly African -American Women: 1.6 mg/L in men and 2.5 mg/L in women and 3.5 mg/L in African-American women [56]. Anand S et al found similar association with gender, but slightly attenuated by controlling for metabolic factors [58]. The MultiEthnic Study of Atherosclerosis (MESA) found hsCRP significantly higher in women than men: 1.85 mg/L and 1.33 mg/L respectively, $p < 0.001$ [59]. Winston et al found a rise of hsCRP in his cohort aged 24 years to 74 years, with hsCRP median doubling from 1mg/L in the youngest group to 2 mg/L in oldest group[67]. These four studies [56 58 59 67] all found a rise of hsCRP with increase in BMI.

Using the AHA/CDC [23] hsCRP risk stratification for future cardiovascular events, among our donors, those in the low risk category of < 1 mg/L were 68% (140/205), moderate risk category of 1 -3 mg/L were 26% (53/205), and high risk > 3 mg/L 6% (12/205). This is comparable to findings by Anand et al for the Chinese population in whom 65.7% had low risk, 28% moderate risk, and 6.3% in high risk [58].

The limitations of this study are: blood donors may not be fully representative of the Kenyan general population ; and the population studied was skewed to young males who are the majority of blood donors.

The strength of this study is that the data is validated by several studies on hsCRP. This is the first Kenyan study attempting to generate a reference range and can be used to build a larger data base in the future.

CONCLUSION

The reference hsCRP in a healthy Kenyan blood donor population is 0.5 mg/L (IQR 0.3 – 1.2 mg/L). This falls into the CDC hsCRP reference range validated in over 40 populations. Gender, age, and BMI were associated significantly with the levels of hsCRP. Sixty eight percent of Kenyan blood donors are categorized as low risk as per AHA/CDC hsCRP risk classification for future cardiovascular event.

8.0 REFERENCES

[1]Jamison TD, Breman G, Meassham AR et al. Disease Control Priorities in Developing Countries. Forgaty International Center of National Institutes of Health, the World Health Organization, and the Population Reference Bureau. 2nd Edition, Oxford Press). Pages 645 – 662.

[2] The World Health Organization: Cardiovascular disease resources.

[3] Mosca Lori. C- reactive protein. To screen or not to screen ?. NEJM 2002; 347 : 1615-1617

[4] Tillet WS, Francis T. Serologic reactions in pneumonia with a non-protein somatic fraction of pneumococcus. J Exp Med 1930; 52 : 561-571

[5] Abernathy T, Avery O. The occurrence during acute infections of a protein not normally present in blood. I - Distribution of the C reactive protein in patient's serum and the effect of calcium on the flocculation reaction with C polysaccharide of pneumococcus. J Exp Med. 1941;73:182-90.

[6] MacLeod C, Avery O. The occurrence during acute infections of a protein not normally present in blood. IV- Crystallization of C-reactive protein. J Exp Med 1947;85: 491-98

[7] Kroop I, Shackman N. Levels of C-reactive protein as a measure of acute myocardial infarction. Proc Soc Exp Biol Med 1954;86:95-97

[8] Pepys MB. C-reactive protein fifty years on. Lancet 1981;1 (8221):653-657

[9] Pepys MB, Hutchinson WL, Noble GE. The pentraxins, C-reactive protein, and serum amyloid P component, are cleared and catabolized by hepatocytes in vivo. J Clin Invest 1994;94:1390-96

[10] Virgushin DM, Pepys MB, Hawkins PN. Metabolic and scintigraphic studies of radioiodinated human CRP in health and disease. J Clin Invest 1993;91:1351-57

[11] Berk BC, Weintraub WS, Alexander RW. Elevation of C-reactive protein in "active" Coronary heart disease. Am J Cardiol 1990;65:168-172

[12] Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. Am J Epidemiol 1996;144:537-547

[13] Ridker PM, Cushman M, Shaten J. Inlammation, aspirin, and risk of CVD in apparently healthy men. NEJM 1997; 336: 973-979

[14] Ridker PM, Buring JE, Hennekens CH, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. NEJM 2000;342:836-843

[15] Ridker PM, Rifai N, Pfeffer MA et al. Inflammation, Pravastatin, and the risk of coronary events after MI in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigation . Circul 1998; 98: 839-844.

[16] Ridker PM, Rifai N, Lowenthal SP. Rapid reduction in CRP with cerivastatin among patients with primary hypercholesterolemia. Circul 2001; 103(9): 1191-1193.

[17] Albert MA, Danielson E, Rifai N et al. Effect of statin on C - reactive protein The Pravastatin Inflammatory /C-reactive protein Evaluation (PRINCE) study. JAMA 2001; 286: 64-70.

[18] Jialal I, Stein D, Balis D et al. Effect of Hydroxymethyl Glutaryl CoEnzyme A Reductase Inhibitor Therapy on hsCRP levels. Circul 2001;103:1933-1935.

[19] Balk EM, Lan J, Grondas LC et al. Effects of statins on nonlipid serum markers associated with cardiovascular disease, a systemic review. Ann Intern Med 2003; 139:670-682.

[20] . Plenge JK, Hernandez TL, Weil KM et al. Simvastatin lowers CRP within 14 days: an effect independent of LDL-cholesterol reduction. Circ 2002;106:1447-1452

[21] Ridker PM, Rifai N, Clearfield et al. Measurement of CRP for targeting of statin therapy in the primary presentation of acute coronary events. NEJM;2001: 344:1959-1965.

[22] Ridker PM, Rifai N, Rose L et al. Comparison of CRP and LDL-cholesterol levels in the prediction of first cardiovascular event. NEJM 2002;347:1557-1565.

[23] Pearson TA, Mensah GA, Alexander RW et al. Markers of inflammation and CVD: application to clinical and public health practice. A statement for healthcare professionals from the CDC/AHA. Circ 2003;107:499-511.

[24] Koenig W, Sund M, Frohlich M et al. CRP, a sensitive marker of inflammation, predicts future risk of CAD in initially healthy middle-aged men: results from the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study. Circ 1999;99:237-242.

[25] Koenig W, Lowel H, Baumert J et al. CRP modulates risk prediction based on Framingham score: implications for future risk assessment: results from a large cohort study in southern Germany. Circ 2004;109:1349-1353.

[26] Ballantyne CM, Hoogeveen RC, Bang H et al. Lipoprotein associated phospholipase A2, hsCRP, and risk for incident coronary heart disease in middle aged men & women in the Atherosclerosis Risk In Communities (ARIC) study. Circ 2004;109:837-42

[27] Boekholdt SM, Hack CE, Sandhu MS et al. CRP levels and CAD incidence and mortality in apparently healthy men and women. The EPIC-Norfolk prospective population study 1993-2003. Atherosclerosis 2006;187:415-422.

[28] Pai JK, Pischon T, Ma J et al. Inflammatory markers and risk of CHD in men and women. NEJM 2004;351:2599-2610.

[29] Danesh J, Whincup P, Walker M et al. Low grade inflammation and CHD. Prospective study & updated meta analysis. BMJ 2000;321:199-204.

[30] Danesh J, Wheeler JG, Hirschfield GM et al. CRP & other circulating markers of inflammation in the prediction of CHD. NEJM 2004;350:1387-1397.

[31] Curb JD, Abbott RD, Rodriguez BL et al. CRP & the future of thromboembolic stroke in healthy men. Circ 2003;107:2016-2020.

[32] Cushman M, Arnold AM, Psaty BM et al. CRP & the 10 year incidence of CHD in older men and women: the cardiovascular health study. Circ 2005;112:25-31.

[33] Best LG, Zhang Y, Lee ET et al. CRP as a predictor of cardiovascular risk in a population with a high prevalence of diabetes: strong heart study. Circ 2005;112:1289-1295.

[34] Laakson DE, Niskanen L, Nyyssonen K et al. CRP in the prediction of cardiovascular & overall mortality in middle aged men: a population-based cohort study. Eur Heart J 2005;26:1783-1789

[35] Wilson PWF, Pencina M, Jacques P et al. CRP & reclassification of cardiovascular risk in the Framingham Heart Study. Circ Cardiovascular Qual Outcomes 2008:1:92-97.

[36] Ridker PM, Buring J, Rifai N et al. Development & validation of improved algorithms for the assessment of global cardiovascular risk in women. The Reynolds risk score. JAMA 2007;297:611-619.

[37] Ridker PM, Paynter NP, Rifai N et al. CRP & parental history improve global cardiovascular risk prediction: the Reynolds score for men. Circ 2008;118: 2243-2251.

[38] Pradham AD, Manson JE, Rifai N et al. CRP, IL6, & risk of developing type 2 diabetes mellitus. JAMA 2001;286:327-334.

[39] Laaksonen DE, Niskanen L, Nyyssonen K et al. CRP & the development of the metabolic syndrome & diabetes in middle aged men. Diadetologia 2004;47:1403-1410.

[40] Sattar N, Gaw A, Scherbakova O, Ford I et al. Metabolic syndrome with or without CRP as a predictor of CHD and diabetes in the West of Scotland Coronary prevention study. Circ 2003;108:414-419.

[41] Festa A, D'Agostino Jr, Tracy RP et al. Elevated levels of acute phase proteins & plasminogen activator 1 predict the development of type two diabetes: the Insulin resistance Atherosclerosis Study. Diabetes 2002;51:1131-1137.

[42] Ridker PM, Cannon CP, Morrow D et al. CRP levels & outcomes after statin therapy. NEJM 2005;352: 20-28

[43] Morrow DA, de Lemos JA, Sabatine MS et al. Clinical relevance of CRP during follow up of patients with ACS in the A to Z Trial. Circ 2006;114:281-288.

[44] Nissen SE, Tuzcu EM, Schoenhagen P et al. Statin therapy, LDL, CRP, & CAD. NEJM 2005;352:29-38.

[45] Mega JL, Morrow DA, Cannon CP et al. Cholesterol, CRP, & cerebrovascular events following intensive & moderate statin therapy. J Thromb Thrombolysis 2006;22:71-76

[46] Ridker PM, Morrow DA, Rose LM et al. Relative efficacy of Artovastatin 80mg & Pravastatin 40mg in achieving dual goals of LDL < 70 & CRP < 2mg/dL. PROVE IT- TIMI 22. J Am Coll Cardiol 2005;45:1644-1648.

[47] Ridker PM. Rosuvastatin in the primary prevention of cardiovascular disease among patients with low LDL & elevated hsCRP: rationale & design of the JUPITER trial. Circ 2003;108:2292-2297.

[48] Ridker PM, Danielson E, Fonseca FAH, et al. for the JUPITER study group et al. Rosuvastatin to prevent vascular events in men and women with elevated hsCRP. NEJM 2008;359:2195-2207.

[49] Ridker PM. The time for cardiovascular inflammation reduction trials ahs arrived: how long to go hsCRP ? Aterioscler Thromb Vasc Biol 2008;28:1222-1224.

[50] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105:1135-43.

[51] Bazzaro LA, He J, Muntner P, et al. Relationship between cigarette smoking and novel risk factors for CVD in the United States. Ann Intern Med. 2003;138:891-7

[52] Saiyo Y, Kiyota N, Kawasaki Y, et al. Relationship between CRP and visceral adipose tissue in healthy Japanese subjects. Diab Obes Metab. 2004;6:249-58.

[53] Eklund C, Lehtimaki T, Hurme M. Epistatic effect of CRP single nucleotide polymorphism (SNP) + 1059 and interleukin-1B SNP + 3954 on CRP concentration in healthy male blood donors. Int J Immunogenet 2005 ;32(4):229-232.

[54] Pradham AD, Mason JE, Rossouw JE, et al. Inflammatory biomarkers, HRT,and incident coronary artery disease: prospective analysis from the Womens Health Initiative observational study. JAMA 2002;288:980-7

[55] Rifai N, Ridker PM. Population distribution of CRP in apparently healthy men and women in the US. Implication for clinical interpretation. Clin Chem. 2003;49:666-9.

[56] Ford ES, Giles WH. Serum CRP and self reported stroke: findings from the third NHANNES. Arterioscl Thromb Vasc Biol 2000;20:1052-1056.

[57] Michelle AA, Glynn RT, Buring J et al. CRP among various ethnic groups living in the USA from the WHS. Am J of Cardiol 2004;93;10:1238-1242.

[58] Anand SS, Razak F, Yi Q, et al. CRP as a screening test for cardiovascular risk in a multiethnic population. Aterioscler Thromb Vasc Biol 2004;24:1509-1515.

[59] Lakoski SG. Cushman M, Criqui M et al. Gender and CRP: data from the MultiEthnic Study of Atherosclerosis (MESA) cohort. Am Heart J 2006;152:593-598.

[60] Armin Imhof, Frohlich M, Loewel H et al. Distribution of CRP mean by hsCRP assays in apparently healthy men and women from different populations in Europe. Clin Chem 2003;49:669-672.

[61] Joshi MD, Wala J, Amayo A et al. hsCRP in type 2 diabetic patients with and without the metabolic syndrome. East African Med J 2008 April; 85(4):178-186.

[62] Erlandsen EJ, Randers E. Reference interval for serum CRP in healthy blood donors using the Dade Behring N Latex CRP mono assay. Scand J Clin Lab Invest 2000 Feb;60(1):37-43.

[63] Veldhuizen IJ, Doggen CJ, Atsma F, De Kort WL, Donor profiles: demographic factors and their influence on the donor career.Vox Sang 2009 July;97(2):129-138.

[64] Zuller N, Nelson KE, Ness P, Wen G. Demographic characteristics and risks for transfusion transmissible infections among blood donors in Xingjian autonomous region, People's Republic of China. Transfusion 2006 Feb;46(2):265-71.

[65] Macy EM, Hayes TE, Tracy RD. Variability in the measurement of CRP in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997;43:52-58.

[66] Price CP, Calvin J, Walker SA, Trull A, Newman DJ. A rapid and sensitive automated light scattering immunoassay for serum CRP and the definition of a reference range in healthy blood donors. Clin Chem Lab Med 1999 Feb;3792):109-113.

[67] Winston L, Wolfgang K, Margil F et al. Immunoradiometric assay of circulating CRP: Age-related values in the adult general population. Clin Chem 2000;46:934-938.

9.0 APPENDICES

APPENDIX 1

CONSENT EXPLANATION

The participants will be counseled individually and given an explanation on the importance of the study and its relevance as follows:

1. This study is part fulfillment of my postgraduate programme
2. The study has been approved by the Ethics Committees
3. The participant has a right to choose to enter the study or not
4. The participant has the right to withdraw from the study for whatever reason at any time.
5. The participant and population will benefit from knowledge of CRP levels, and individual results will be communicated to the participant and referred appropriately to medical personnel if in high-risk group category.

The participant shall then be explained to in detail what the study involves. This will involve explaining that 3mls of the sample to be used for screening of transfusion transmissible infections will be taken to determine hsCRP level concentration. The procedure does not involve additional direct sampling from the participant.

The participants who agree to enter study shall be required to sign a consent form.
All results shall be communicated to the participants.

If I (PI) find a patient with high-risk hsCRP levels (3- 5 mg/L) and/or obese (BMI > 30), I shall counsel the participant on lifestyle modifications and refer to the nearest health facility for further management.

At the end of the study, the results will be presented to the internal medicine department of clinical medicine and therapeutics, UON.

Any questions or information, you may contact myself, Dr Chebii Kipkulei Tel 0722 331 391
Contacts of my supervisors or the Ethics Chairperson:

- Prof. K.M. Bhatt, Chairperson, KNH/UON- Ethics Review Committee: Tel 726300-9. Fax 725272.
- Dr. M. D. Joshi, Dept. of Clinical Med. & Therapeutics, UON: Tel 0722 516904
- Dr. A. Amayo, Dept. of Clinical Chemistry, UON: Tel 0733 617678

APPENDIX 2

CONSENT FORM

Study No:_____ Sex_____ Age_____

I..... of..... Hereby agree to participate in the study on " serum hsCRP concentration in apparently healthy blood donors at the NBTC".

It involves having weight, height measurements and taking a sample of 3mls of blood for measurement of hsCRP concentration from the serum to be used to test for transfusion transmissible diseases by the NBTC as per WHO safe transfusion protocol. I understand the nature of the study and that my participation in this study is on voluntary basis and has willingly agreed to take part in it.

Signed..... Date.....

Witness..... Date.....

APPENDIX 3

INVESTIGATOR'S STATEMENT.

I, the investigator, have educated the research participant on the purpose and implications of this study.

Signed..... Date.....

APPENDIX 4

SCREENING QUESTIONNAIRE

Date.....

Name.....

Contact (Telephone number).....

Age..... Gender: ☐ Male ☐ Female

Are you known to have or be (Please tick):

1. Are you below 18 years of age as of today?

   ☐ Yes ☐ No **(If yes, exclude)**

2. Have you had any illness within the past two weeks?

   ☐ Yes ☐ No **(if yes, exclude)**

   If yes, please specify:

3. Do you have any of the following conditions?

   ☐ Cancer ☐ Lupus ☐ Scleroderma ☐ Inflammatory bowel disease

   ☐ Other autoimmune condition (Please specify:_____)

   ☐ Diabetes ☐ Hypertension ☐ HIV ☐ Gingivitis ☐ Bronchitis

   ☐ Other chronic condition (Please specify :_____)

   (if have any condition, exclude)

   ☐ None

4. Do you take any of the following medications?

   ☐ Statin (e.g. atorvastatin) ☐ Prednisolone ☐ Dexamethasone

   ☐ Cyclophosphamide ☐ Hydroxychloroquine

   ☐ Other immunosuppressive medication (Please specify:_____)

   ☐ Oral contraceptives

   (If on any of the medications, exclude)

   ☐ None

If any of the above questions are not answered or meets exclusion requirements, please EXCLUDE subject from study and screen another subject.

APPENDIX 5

STUDY PROFORMA

Study Number……..

Gender: ☐ Male ☐ Female

Date of Birth: __/___/_____ Age: _____yrs

Height:_____ (Meters) Weight _____ (Kgs)

hsCRP level: _____ mg/L

APPENDIX 6

LABORATORY METHODS

hsCRP: Determined in serum by immunoturbidimetric assay at 37 degrees Celsius.
Requires 1.5 ml of serum x 2
Fasting not required
Blood collection in red top vacutainers
Analysis by Olympus A400
Reference range: Adults < 5 mg/dL

APPENDIX 7

CLINICAL OUTCOMES

$$\text{BODY MASS INDEX (BMI)} = \frac{\text{WEIGHT (KGs)}}{\text{HEIGHT}^2 \text{ (meters}^2\text{)}}$$

Classification of BMI

Underweight	< 18.5
Normal weight	18.5 – 24.9
Overweight	25 – 29.9
Obesity (class 1)	30 – 34.9
Obesity (class 2)	35 – 39.9
Extreme Obesity (class 3)	≥ 40