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Investigation Of hs-CRP Of Non Diabetic, Non Smoking And Non Alcoholic In Male And Female Of Salem District Of Tamil Nadu, India

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ABSTRACT
OBJECTIVE: *The serum hs-CRP level was investigated in the blood samples and were analysed by the using statistical methods to arrive at the normal level of hs-CRP in both male and female healthy individuals.*

METHODS*: We have collected 108 blood samples from the non diabetic, non alcoholic and non smoking healthy individuals. Among the 108 samples recorded 3 of them were cord blood, 57 and 48 were female and male respectively. From these individuals hs-CRP level were analyzed by the quantitative turbidimetric test. The data's were statistically measured the differences between the the male and female of different age groups .*

RESULT*: Among the 108 samples tested 34.2% has no detectable hs-CRP below0.005 mg/L. The highest value was found to occur at the age group of healthy individuals of 4.50 mg /L. The value observed in the present investigation revealed a mean hs-CRP level of 1.005mg/L with a standard deviation of 0.88mg/L and the median hs-CRP of 2.11 mg/L. The correlation of co-efficient 0.037 mg/L was recorded.*

CONCLUSION*: The present investigation reported that the levels of hs-CRP obtained in the different age group of the male and female were not differ significantly.*

KEY WORDS *: hs-CRP, High sensitive., C-reactive protein and Blood samples*

I. INTRODUCTION

C-RP is an acute phase inflammatory response of protein synthesized predominantly by the hepatocytes in response to tissue damage or inflammation and a major component of the body 's innate deference mechanism. It reacts promptly without specificity or memory upon exposure to different types of inflammatory stimuli. In patients with acute inflammation caused by the Streptococcus pneumonia. The C-RP has an affinity to carbohydrate (C) antigen on the cell wall of bacteria and fungi, hence the name C-reactive protein. The acute phase response to comprises the changes in the serum protein profile during the inflammation process. Generely CRP in the blood in very low concentration (<1mg/L), but during inflammatory process, this concentration increases significantly (2) (Kristin Kris et al., 2005). More than 20 epidemiological studies have demonstrated that low concentrations of CRP can be a marker of low level of infection and inflammation. The high sensitivity measurement of CRP (hs-CRP) can independently predict future risk in seemingly healthy individuals (3). (Wolfgang Koenig, et al., 1999). The measuring of CRP by ELISA (4,5)(Elizabeth, .M.E. Macy, et al., 1997) (Chang CP et al., 2002), Micro particle enhanced Immunoturbidometry (6) (William. L.Roberts, et al., 2001), Immunonephelometry have made in possible to accurately measure even very low levels of CRP which was not possible by methods such as capillary precipitation (7)(Sextad, 1970; Claus, 1976) and latex slide agglutination test(8). (Joshi, K.R., e al., 1975). Interpretation of results obtained by the newly introduced methodologies Immunoturbidometry requires to the basal or normal tested level of hs CRP in the population.. The present observation collecting the serum sample from apparently normal healthy individuals (Non smoking, non alcoholic, non diabetic) after interviewing them with a questionnaire and then measuring the hs-CRP by using the Immunoturbidimetric method

II. METHODS

The present investigation was conducted in Salem district of Tamil Nadu India from November 2013 to December 2013. The Blood samples were collected from three different villages Ayothiyaptinum (15Km), Pallipatti(10) and Valasaiyur(15) away from Salem. The other samples were also collected from the school students and staff of Vysya college of Salem and 6^{th} to 12^{th} standard students of Government Higher Secondary School of Valasaiyur, and 1^{st} Standard to 5^{th} standard students of Primay School at Pallipatti and also from adults of Pallipatti village and Ayothiyapatinum village, umbilical cord blood samples from normal delivery cases from the Vijaya hospital of Salem city. The Healthy volunteer were personally interviewed. The samples were collected only from apparently healthy individuals without any of the conditions listed Fever, cold/cough, sorethroat, skin or wound infection, Diabetics, Smoking , alcoholic, Pregnancy, Recent physical activity like exercise or games. Apart from these data on the age, sex, body weight and dietary habits of the individuals were also collected. The Blood samples were collected by veni puncture by using sterile, disposable syringe and needle. 2-3ml of blood was collected and immediately put in plain disposable test tube and allowed to clot. The collected blood samples were aseptically transported to the laboratory within 24 hours. The clotted blood was centrifuged at 850 to 1000 g form 10 minutes to separate serum. Then the serum was transferred and stored in a small plastic vials. The separated serum samples were either tested immediately or were stored at 2-8C in refrigerator for maximum of 48 hours before testing.

The Commercially available hs-CRP kit from SPINREACT COMPAY, SPAIN was used in this study. The manufacturer's instructions given along with the kit were followed. The hs-CRP kit is a quantitative turbidimetric test for the measurement of low level of C-reactive protein (C-RP) in human serum. Latex particle coated with specific goat IgG anti-human C-RP are agglutinated when they mixed with serum samples containing C-reactive protein. The agglutination caused on absorbance change, depending on the CRP concentration of the patient's samples. The C-RP contents in the sampling quantified by comparison from a calibrator of known CRP concentration. Reagents are diluent (Tris buffer 20 mmol/L, pH 8.2, sodium azide 0.95g/L), Latex particles coated with goat IgG anti-human C-RP, CRP calibrator-Human serum, CRP concentration was 10.5 mg /L stated on the vial label.The sensitivity of the assay and the target value of the calibrator have been standardized against the reference material CRM 470/PPHS (Institute for Reference materials and Measurement, IRMM). The latex vial was gently shaken before use. 1ml of Latex reagent was taken and mixed with 14ml of diluents

The CRP calibrator was reconstituted with 2.0ml of distilled water. Before using the preparation was mixed and brought to room temperature for 10 minutes. CRP calibrator dilution were prepared by using sodium chloride (Nacl) 9gm/L as diluents. The concentration of the CRP calibrator were multiplied with the corresponding factor values were given in the **Table 3.** Vital 21 chemistry analyzer was used to quantify (hs-CRP) high sensitive C-reactive protein level of the blood samples. The working reagent , samples, flow cell of photometer was brought to 37 ^{o}c before the work was started. The assay condition used are Wave length 546 nm (530-550), temperature 37^{o} C . Using distilled water the instrument was adjusted to zero. 1ml of working reagent and 10 micro liter of serum were pipette out into a cuvette. The working reagent and the serum sample were mixed and readed the absorbance immediately (A1) and after 4 minutes (A2) of the sample addition. Calculate the absorbance difference (A2-A1) of each point of the calibration curve and plot the value obtained against the CRP concentration of each of calibrator dilution. C-RP concentration in the sample is calculated by interpolation of its (A2-A1) in the calibration curve

III. RESULT

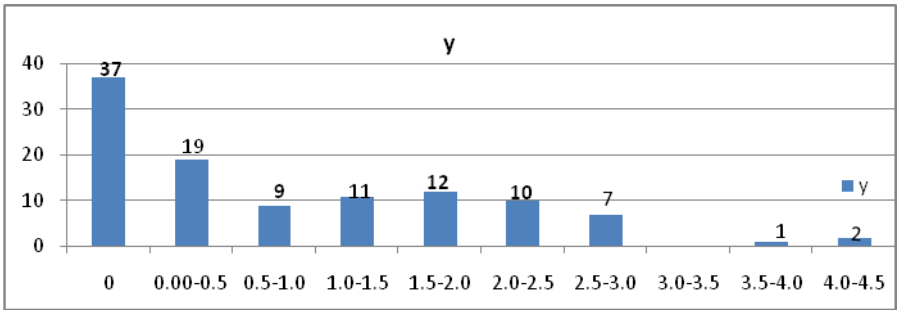
Among the 108 blood samples collected 57 female and 48 male and 3 cord blood were analyzed . The age group and sex details of healthy individuals from whom these samples were collected are summarized in the Table 4. From the collected samples 52 samples in the age group of 20-40 years, 13 samples in age group of 40-60 years. 6 sample of children's below the age of 10 years and 34 samples from the age group of 10-20 years were recorded.. From the 108 samples, a total of 39 samples were collected from 28 students and 11 staffs of V ysya College at Salem city. Eleven samples were collected from sixth to twelth standard students of Government higher secondary school at Valasaiyur, Salem and 10 samples from first standard to fifth standard students of Primary School at Pallipatti. A total of 45 samples were collected from adults of Pallipatti Village and Ayothiyapatinum village, alem. Three umbilical cord samples were collected from Vijaya hospital of Salem The Figure 1 shows the Age and sex distribution of subjects tested .

Table I The Different Age and sex distribution tested

AGE GROUP	*MALE*	*FEMALE*	*TOTAL*
CORD BLOOD	-	-	3
0-10	03	03	03
10-20	15	19	34
20-30	07	17	24
30-40	12	16	28
40-50	08	02	10
50-60	03	-	03
	48	57	108

The label in the vial of the calibrator mentions the concentrations of hs-CRP in the calibrator as 10.5mg/L. Using this standard curve the healthy individuals samples CRP values were calculated according. None of the cord blood samples tested had any detectable hs-CRP. The highest hs-CRP concentration seen in this age group was 0.64 mg/ L. Among the 10-20 years age group there were 19 females and 15 male. Eighteen of the 34 people in this age group did not have any detectable hs-CRP. The highest concentration of hs-CRP detectable among males in this age group was 2.64 mg/L and among the female it was 2.67mg/ L. Among the 20-30 age groups there were 7 males and 17 females. Six of these 24 people did not have any detectable hs-CRP and the highest concentration of hs-CRP among male was 4.50 mg/L and among female it was 2.55mg/L. In the 30-40 age group among the 38 individual tested 4 did not have any detectable hs-CRP and the highest level of hs-CRP detected among male was 2.96mg/L. Among the 40-50 years age group (10 subjects) 3 did not have any detectable hs-CRP and the highest level of hs-CRP detectable among male was 3.8mg/ L and among female it was 2.96 mg/L. There were only 3 subjects in the age groups. One of them did not have any measurable hs-CRP. The highest level recorded was 4.08 mg/L.The Over all among the 108 samples tested 34.2% has no detectable hs-CRP below 0.05 mg/L. The highest value found in this age group of healthy individuals was 4.50 mg/L. Figure 3 shows the hs-CRP levels in study subjects.

Figure 3



The statistical analysis of the values of hs-CRP obtained in this study revealed a mean hs- CRP level of 1.005 mg/L with a Standard Deviation (SD) of 0.88 mg/L and the median of hs-CRP was 2.11 mg/L and the correlation co-efficient in this study was 0.037 mg/L. The mean, median, Standard deviation (SD) , correlation co-efficient level of study subjects are given in the Table **2.**

Table II M**edian and mean levels of hs-crp**

MEDIAN	**2.11 mg/L**
MEAN	**1.005 mg/L**
STANDARD DEVIATION	**0.88 mg/L**
CORRELATION CO-EFFICIENT	**3.7 %**

To check on the precision of methodology used in testing these samples two individual samples one with low hs-CRP concentration and another one with a high hs-CRP concentration were repeatedly tested on 5 different occasions. The results are presented in Table 3 and 4.Statistical analysis of these results revealed a standard deviation (SD) of ±0.039 for the low value (Sample 1) and 0.2 for the high value (Sample 2) . The co-efficient variation for the high value sample was 0.025 and for the low value(Sample 1) it was 0.026%.

Table III hs-crp immuno turbidimetry intra assay-precision

ASSAY NO	***SAMPLE I (LOW VALUE) mg/L***	***SAMPLE II (HIGH VALUE) mg/L***
1	1.51	8.03
2	1.45	7.97
3	1.54	8.10
4	1.49	7.92
5	1.43	7.89

Table IV mean and standard deviation levels of hs-crp in sample 1and sample 2

	SAMPLE I (LOW VALUE)	***SAMPLE II (HIGH VALUE)***
MEAN	1.484	7.982
STANDARD DEVIATION(SD)	0.039	0.2
CO-EFFICIENT VARIATION (CV)	0.026%	0.025%

The Statistical analysis revealed that the levels of hs-CRP obtained in this study in the different age group as well as among the male and the female did not differ significantly.

IV. DISCUSSION

Generally CRP measurement in clinical laboratory was carried out by Capillary precipitation test and latex particle slide agglutination test which had a detection limit>6ml/L. This was useful and convenient to detect cases of acute inflammatory conditions such as Rheumatic fever, Sepsis, Arthritis etc., The development of methodologies such as Immunoturbidometry, ELISA, Immunoluminometry, Immunonephelometry etc lowered the detectable level hs-CRP in blood to as low as 0.05 mg/L. Results obtained with these methodologies reveals the CRP concentration not only raises dramatically in acute disease condition but raise even chronic inflammatory conditions such as atherosclerosis(9).(Agarwal, et,al., 2005).

There are many factors which influence the hs-CRP serum concentration including age, gender, ethnicity, body mass index, smoking status, pregnancy, level of physical activity, stress level, duration of disease, type of infection and tissue involvement(10). (de matt.M.P et.al., 2001). When carrying out CRP analysis, the overall health of patient, the suspected disease and the sampling procedure must also be taken into consideration when interpreting the test results(2).(Kristin kriz, et.al., 2005). Hence , in the presence study care was taken to exclude individual who gave a history of smoking, diabetes, obesity, pregnancy, and general or localized inflammation conditions such as fever, cold/ cough, sore throat, skin or wound infection.

The main focus of hs-CRP measurement in recent years is its utility in detection future Cardio Vascular Disease (CVD) complication in apparently healthy individuals. CVD is a leading cause of death is not only in the Western world, but also in many developing countries. In the United States Cardio vascular disease is the cause of approximately as many deaths each year as the next five leading causes of combined Cancer, Chronic lower respiratory disease, Accidents, Diabetes mellitus and Influenza, pneumonia(11).(American Heart Association/Stroke 2005). More than 20 perspective epidemiological studies have demonstrate that hs-CRP can be a marker of low level of inflammation and an independent predictor of Vascular risk in seemingly healthy individuals.

There are not many studies on the hs-CRP levels in healthy individuals among the Indian populations. If clinicians and patients are to benefit from the use of hs- CRP measurement knowledge about the basal or normal level of hs-CRP in the local populations is a must. Interpretation of hs-CRP level will be based on the normal level in the populations. To the best of our knowledge this is the first study the level of hs-CRP in South Indian subjects. Our findings in apparently healthy individuals are compared with those of other studies updated from different parts of the world in table 5. The median level of hs-CRP in various populations varies from 0.64ml/L to 3.75mg/L.

Table V hs-crp level in healthy subjects reports from other studies

S.NO	***NO OF STUDY SUBJECTS***	***GEOGRAPHICAL AREA***	***MEDIAN hs-CRP LEVEL mg/L***	***REFERENCE***
1	>5000	AMERICA	1.69	Paul Ridker.et.al., 2001
2	161	CALIFORNIA	1.13	Heidi Witherell et.al., 1999
3	143	BURLINGTON	0.64	Elizabeth.M.Macy, et.al., 1997
4	244	U.S	3.75	Paul Ridker.et.al., 1998
5	31	ITALY	2.1	Punzi, et.al., 2005
6	40	EUROPE	1.9	Loose.et.al., 1993
7	PRESENT STUDY	INDIA	2.1	

The basal median hs-CRP level of 36.75 mg/L reported in apparently healthy subjects by Paul Ridker et.al., included individuals who were current smokers or had a past history of smoking & also individual who had diabetes. This could be a reason while the basal median hs-CRP level is high in this particular study. However, Paul Ridker in a latter large scale prospective study reported in 2001, found a median hs-CRP level of 1.6 mg/L among >5000 apparently healthy American men and women(12). Loose, et, al., (1993) during their study on hs-CRP in osteoarthritis patients found that among 40 healthy control subjects, the median hs-CRP was 1.9 mg/L whereas it was 5 mg/L in osteoarthritis patients(13). Similarly, Heidi Witherell, et.al., (1999) in their study involving health check up on patients with myocardial infarction found that among 161 age and sex matched control subjects, the median hs-CRP level was 1.13 mg/L (14). Elizabeth .M. Macy, et.al., (1997) conducted a study in 143 healthy individuals. Citrated plasma samples from these 143 blood donors ,ages 18 to 67 (69 women and 74 men) were acquired from the Vermont new Hampshire Red Cross center, Burlington. Each subject was questioned briefly as to state of health. The hs-CRP concentration estimated by ELISA method. The median hs-CRP concentration was 0.64 mg/L(4).

According to Paul M. Ridker,et.al., hs-CRP testing must have prognostic usefulness for patients with acute phase response associated with ischemia (12). Liuzzo, found that inflammation is critical component in determination of plaque stability assay system for CRP measurement were found to have predictive value for individual admitted to hospital with acute coronary ischemia (18). Several studies from both the U.S and Europe indicate that elevated levels of hs-CRP among apparently healthy individual are a strong predictor of future cardiovascular events (20,21,22,23)(Ridker. P.M.et.al., 2000; Koenig.E. et.al., 1999; Daniesh .J. et.al., 2000; Mendall, et.al., 2000) The f levels of hs-CRP less than 1 mg/L; 1-3/mg/L ;>3mg/L have corresponded respectively to low, moderate and high risk of future coronary events in individuals with metabolic syndrome (24).(Pearson. T.A. et.al., 2003). Measurements of lowering the blood hs-CRP level include adoption of a healthy diet, exercise, cessation of smoking, statin therapy and improved glycaemic control. Apart from Cardio vascular disease(CVD), hs-CRP measurements also seem to be value in determining severity or activity of erosive osteoarthritis (EOA) (25).The increases of hs-CRP in EOA confirm the presence of inflammatory arthritis in the form of arthropathy and the possibilities that a severe local injury such as osteoarthritis also has a systemic component. Owing to its high sensitivity, hs-CRP will probably being suitable for intra patients evaluation in outcome study.

V. FUTURE THRUST

The findings were established in the basal or normal hs-CRP level as 1.005 mg/L and the median hs-CRP level 2.11 mg/L among 108 subjects (including 3 cord blood samples). This knowledge can be utilized in interpreting hs-CRP level in patients at high risk of developing (Cardio Vascular Disease) CVD. Osteoarthritis or peripheral vascular disease complications. This will also help us to study the usefulness of hs-CRP measurement along with factors such as lipid profile, serum homocystein etc., in risk assessment for coronary artery disease.

VI. SUMMARY

The Healthy subjects were undertaken for the sample collection by questioning them about their current status of health and recent health history. Based on the data collected for hs-CRP measurement. An imported commercial kit for measuring hs-CRP was used in this study (sensitivity =0.05mg/L). Findings established a mean hs-CRP level of 1.005 mg/L with a standard deviation (SD) of $\pm$ 0.88mg/L, the median hs-CRP level in this subject was 2.11 mg/L. The findings in this study are in line with those reported from study in South India. This study also reveals that age and sex do not play any it should be possible to interpret with confidence the results of hs-CRP measurement in patients suspected if Cardio Vascular Disease (CVD)/risk for Cardio Vascular Disease, COPD (Chronic Obstructive Pulmonary Disease), osteroarthritis or peripheral vascular disorders.

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