Electrophrotic Analysis of C - reactive protein with Body Mass Index

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Abstract: *The study was conducted at Harman Institute by Harman Institute of Science Education and Research, Thanjavur – 5. 64 obese and 61 non obese with Body Mass Index (BMI) 18.5 – 24.9 kg/m^2, 25 – 29.9 kg/m^2, 30 – 34.9 kg/m^2 and 35 – 39.9 kg/m^2 were selected. A parameter such as CRP was assessed with obese and non – obese subjects. The figure exhibits a relation of CRP with BMI. CRP levels with BMI ranges, suggesting a source of inflammation are more common among obese subjects than in non obese subjects.*
Key words: *Obesity, Body Mass Index, Gender, C – Reactive Protein.*

I. Introduction

Over the past several decades, the prevalence of obesity has been increasing both in developed and developing countries. It is currently estimated that as much as 20- 50 % of urban populations in Africa are classified as either overweight or obese, and that by 2025 three quarters of the obese population worldwide will be in non industrialized countries (Bourne *et al*., 2002). Obesity is a well recognized risk factor for various chronic diseases such as cardiovascular diseases, hypertension, and type II DM. These conditions lead to reduced quality of life and premature death. Cardiovascular disease is now the main cause of death in developing countries, being responsible for 42.5% of all deaths, while 20 years earlier they accounted for only 12.4% of mortality (Reddy and Yusuf, 1998).

C – reactive protein (CRP) is a member of the class of acute phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentrations of IL – 6, which is produced predominantly by macrophages (Martin and Dorf, 1991) as well as adipocytes (Susan *et al*., 2013). CRP binds to damaged tissue, to nuclear antigens and to certain pathogenic organisms in a calcium dependent manner. This binding activity the complement system and the interaction of CRP with Fc receptors leads to the generation of proinflammatory cytokines that enhance the inflammatory response. Thus, CRP is tough to act as surveillance molecular for altered self – antigens and certain pathogens. This recognition provides early defense and leads to proinfalmmatory signal and activation of the humoural, adaptive immune system (Szalai, 2002).

It is a well – known fact that the heart disease is the leading cause of deaths in North America. At the heart is a muscle that pumps nutrients and oxygenated blood throughout the body, heart disease can develop when the heart doesn't receive enough oxygen. According to recent studies, chronic inflammation and heart disease are actually more deeply linked than first believed. Heart disease, otherwise known as cardiovascular disease, is an umbrella term that refers to various disorders that prevent the heart from working properly. Some of these conditions include: heart failure, heart muscle disease, heart valve disease, congenital heart disease, coronary artery disease (http://earthingcanada.ca/chronic-inflammation-and-heart disease/).

II. Materials And Methods

The present study was conducted in Harman Institute of Science Education and Research, Thanjavur. The random sampling method was adopted for clinical study that is total n = 125. Obese subjects n = 64, non obese control subjects include n = 61.

2.1 Preparation of Samples
Sample buffer is prepared by mixing the following chemicals in appropriate quantities.

0.625 M Tris – HCL buffer (pH6.8)   - 20 ml
20% SDS (w/v)   - 20 ml
50% 2 - Mercaptoethanol (v/v)   - 20 ml
Glycerol   - 20 ml
0.02% - Bromophenol blue (w/v)   - 20 ml

10 – 50 µl of protein extract of the sample was mixed with equal amount of sample buffer to bring the sample to a final concentration of 0.0625 M Tris – HCL, pH 6.8, 2% SDS (w/v), 5% 2 – Mercaptoethanol (v/v), 10% glycerol (v/v) and 0.002% Bromophenol blue (w/v). The mixture was heated in a water bath at 100° C for 3 min and cooled to room temperature before loading onto the gel.

2.2. SDS PAGE Electrophoresis

SDS - polyacrylamide gel electrophoresis was carried out according to Weber and Osborn (1969).

2.3. Principle

SDS is an anionic detergent, meaning its molecules have a net negative charge. It binds to most soluble protein molecules in aqueous solutions over a wide pH range. Polypeptide chains bind amounts of SDS that are proportional to the size of the molecules. The negative charges on SDS destroy most of the complex (secondary and tertiary) structure of proteins, and are strongly attracted toward an anode (positively charged electrode) in an electric field. It is usually necessary to reduce disulfide bridges in proteins. This is done with 2- β Mercaptoethanol or dithiothreitol.

2.4. Buffers, Reagents and Gel Composition For SDS PAGE

10% Acrylamide
➢ Acrylamide 10g
➢ Bisacrylamide 0.8g
➢ Make it up to 100 ml volume
10% Ammonium per sulfate   - 100mg/ml
Resolving gel (10%) -   15 ml
➢ H_2O – 5.9 ml
➢ 30% acrylamide -5 ml
➢ 1.5M Tris (pH-8.8) -3.8 ml
➢ 10% Sodium Dodecyl Sulphate - 0.15 ml
➢ 10% AmmoniumPerSulphate - 0.15 ml
➢ TEMED (N,N,N',N'tetra methylene diamine) - 0.006 ml
  Stacking gel buffer (5%) - 5 ml
➢ H_2O - 3.4 ml
➢ 30% acrylamide -0.63 ml
➢ 1 M Tris (pH-6.8) -3.8 ml
➢ 10% Sodium Dodecyl Sulphate -0.05 ml
➢ 10% AmmoniumPerSulphate – 0.05 ml
➢ TEMED – 0.0005 ml
  Electrode /Running Buffer (1X):
➢ Tris -3.03 g
➢ Glycine -14.41 g
➢ SDS -1 g
➢ Make it up to 1000 ml using distilled water
  Sample Solubilising Buffer (SSB)
➢ TrisHCl (pH 6.8)   -   0.5M   -   1.0 ml
➢ SDS (w/v)   -   1%   -   0.8 ml
➢ Glycerol   -   25%   -   4.0ml
➢ β-Mercaptoethanol   -   02%   -   2.0ml
➢ Bromophenol blue   -   0.5%   -   50µl
➢ Make up with distilled water to 10ml.
  Stainer solution
➢ Coomassie Blue (R-250)   - 0.3g
➢ Methanol   - 80ml
➢ Glacial acetic acid   - 20ml
➢ dH_2O - 100ml
  De-staining solution
➢ Acetic acid   - 10ml
➢ Methanol   - 30ml
➢ Make up with distilled water to 100ml.

Procedure:

Prepare 10% resolving gel as mentioned above and pour the gel onto rectangular glass plates separated by spacer's up to ¾ the of the plate. The gel was over layered with water saturated Butanol (1 ml) to prevent meniscus formation of the gel. The gel was allowed to solidify. Prepare the stacking gel as mentioned above and place the comb so that the wells are formed. The sample buffer was mixed with the same volume of test protein sample and the mixture was incubated in a water bath at 100 ºC for 3 min and centrifuged at 14000 g for

10 min. Then, a volume of 25 µl protein samples was introduced at the top of the gel and electrophoresis was conducted at a constant 200 V for 1 h. Separated proteins were visualized. When the run was over, the gels were stained by the conventional Coomassie technique, i.e. 3 hours in 0.3 % Coomassie Brilliant Blue R-250, 80% methanol and 20% acetic acid. Destaining was carried out overnight in 10% acetic acid solution. The gels swell some 5% in the acidic solution used for staining and destaining. Gels with lower amount of cross- linker show more swelling.

Therefore the calculation of the mobility has to include the length of the gel before and after staining as well as the mobility of the protein and of the marker dye. Assuming even swelling of the gels, the mobility was calculated as

Mobility = $\dfrac{\text{distance of protein migration}}{\text{Length after destaining}}$ x $\dfrac{\text{length before staining}}{\text{distance of dye migration}}$

A linear regression curve was prepared using the plot of the mobility of standard proteins against the logarithms of their molecular weight. The apparent molecular weight of the sample proteins was determined from the regression curve using their Rm values.

III. Results And Discussion

Over the past several decades, the prevalence of obesity has been increasing both in developed and developing countries. It is currently estimated that as much as 20-50% of urban populations in Africa are classified as either overweight or obese, and that by 2025 three quarters of the obese population worldwide will be in non-industrialized countries (Bourne *et al.*, 2002). Plasma protein level display reasonably predictable changes in response to acute inflammation, malignancy, trauma, necrosis, infection, burns, and chemical injury. This so-called "acute-reaction protein pattern" involves increases in fibrinogen, alpha1-antitrypsin, Haptoglobin, ceruloplasmin, CRP, the C3 portion of complement, and alpha1- acid glycoprotein. Often, there are associated decreases in the albumin and transferrin levels (Kyle, 1999).

The present study compared the concentration of CRP in obese and normal weight subjects. Figure 1, lane 1, shows the BMI level of normal (18.5 – 24.9 kg/m^2) and overweight (25 – 29.9 kg/m^2) people. There were nearly 19 fractions observed in the normal blood serum out of which 11 fractions stained intensely and 8 fractions were found less stained. Results of blood serum obtained from lane 2 (BMI 25 – 29.9kg/m^2), showed nearly 11 fractions and all the fractions were more or less similar to that of normal blood serum. However, one particular band in the region of 130 kDa was found more prominent. The results of BMI with normal and moderate obese (30 – 34.5 kg/m^2) were presented in lane 2. Here, more or less similar pattern was observed with varying number of bands out of 12, six bands were stained intensely and six were found less stained. The fraction at 130 kDa was found stained intensely this lane 3. In lane 4 it contains totally 13 bands, seven were stained intensely and five were less stained. The similar result was observed in all the four figures in the region of 130 kDa.

The very clear relationship that we observed between CRP concentration with BMI and normal weight subjects confirms the role of adipose tissue in initiating and sustaining inflammation in obese persons, suggest that obesity is a state corresponding to sub minimal inflammation. This assumption is confirmed by Trayhurn *et al.* (2004) and Weisberg *et al.* (2003). Which showed a very clear relationship between CRP and BMI, which is an indicator of visceral obesity since, according to current knowledge, the principle places a production of inflammatory cytokines and proteins is visceral tissue. In addition, recent reports suggest that apart from visceral tissue, the perivascular adipose tissue, surrounding almost all blood vessels, may also be a source of inflammatory cytokines (Miao *et al.*, 2012).

Figure - 1
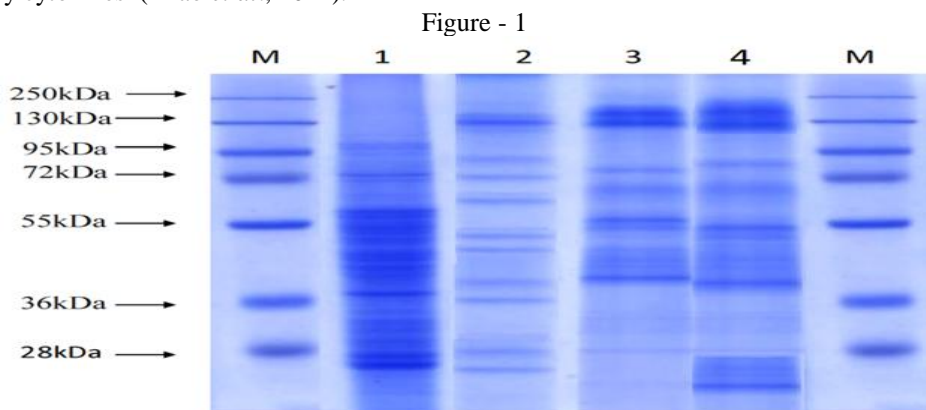
M – Protein marker, 1- 18 - 24.9 kg/m^2, 2 - 25- 29.9 kg/m2, 3 – 30 – 34.9 kg/m^2, 4 – 35 – 39.9 kg/m^2
FIG -1. SDS – PAGE of human serum from normal (1) 18.5 – 24.9 kg/m^2, over weight (2) 25 – 29.9 kg/m^2, moderate weight (3) 30 – 34.9 kg/m^2 and severe obesity (4) 35 – 39.9 kg/m^2 on a 10% gel. Lanes M is the

molecular weight protein markers. The protein load was 15 llg and staining was with Coomassie Blue R – 250. The more violet bands in the molecular weight region around 130 kDa indicate the C – Reactive Protein.

The association between measures of metabolic risk and CRP was mainly related to overall obesity (Festa *et al*., 2000). Recent guidelines suggest that if CRP levels of >10 mg/l is identified, there should be a search initiated for an obvious source of infection or inflammation, and that the result of the test should then be discarded (Pearson *et al*., 2003). Although only 7.6% of subjects had CRP values greater than the generally accepted cutoff for inflammation. Aronson *et al*. (2004) suggests that these values are most frequently associated with overweight and especially with obesity. Therefore, CRP results should always be interpreted in the context of the subject's BMI, as very high levels may be expected in severely obese subjects.

III. Conclusion

Obesity, especially visceral adiposity, up regulates various inflammatory cytokines and other bio molecules. Chronic elevation of these inflammatory mediators leads to cardiovascular morbidity and mortality. Exercise based lifestyle intervention can effectively prevent and retard the progression of cardiovascular and metabolic disorders. The mechanism of action of such benefit may be attributed to a reduction in weight and stress, networking in mind and body levels, thereby leading to a reduction in inflammation, and causation and progression of the disease. CRP is normally used as a marker for CHD and type II diabetes mellitus. CRP can be considered as a newer marker in obesity evaluation.

Acknowledgements

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