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# Relation between Lipid Profile and Total Antioxidant Status among Normal Population

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## Abstract

*This study was conducted to assess serum lipids in normal people in relation with their total antioxidant status. Altogether 50 fasting blood samples were collected for study in age between 20-50 years. Fasting lipid profile was determined with enzymatic method i.e. triglyceride (TG), high density lipo-protein (HDL) and low density lipo-protein (LDL) whereas total cholesterol (TC) by chemical method using $FeCl_3$ reagent. The antioxidant status was determined by Ferric Reducing Ability of Plasma (FRAP) assay using TPTZ solution. The statistical analysis was done by SPSS program using Pearson's correlation coefficient. TG showed negative significant correlation with total antioxidant status ($p<0.01$) and HDL showed positive significant correlation ($p<0.05$). Whereas TC and LDL showed non-significant correlation with total antioxidant status ($p>0.05$).*

***Keywords :*** *Antioxidant, Lipid Profile, Statistical Analysis, TG, HDL, LDL, TC*

## Introduction

Although oxidation reactions are crucial for life, they can also be damaging. Oxidation reactions produce free radicals which start chain reaction that damage the cells. To prevent the free radical damage, the body has a defense system of antioxidants. Antioxidants are a broad group of compounds that destroy single oxygen molecules, also called free radicals thereby protecting against oxidative damage. Antioxidants terminate these chain reactions by removing the free radical intermediate and inhibit the other oxidation reactions. The total antioxidant status has significant relation with lipid profile. Experimental studies have demonstrated that oxidation of LDL plays an important role in the development and progression of atherosclerosis in animal models[1-3]. Observational epidemiological studies have also documented an inverse association between increased intake of antioxidant vitamins such as vitamin E, ß-carotene, and vitamin C and reduced morbidity and mortality rates from coronary heart disease. Increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to atherosclerotic lesions[4].

The modern concept of medicine envisages that a subtle balance of redox homeostasis is necessary to maintain normal health and to avoid disease and that in this redox homeostasis free radicals and antioxidants play a crucial role, in many diseases especially age related ones. To

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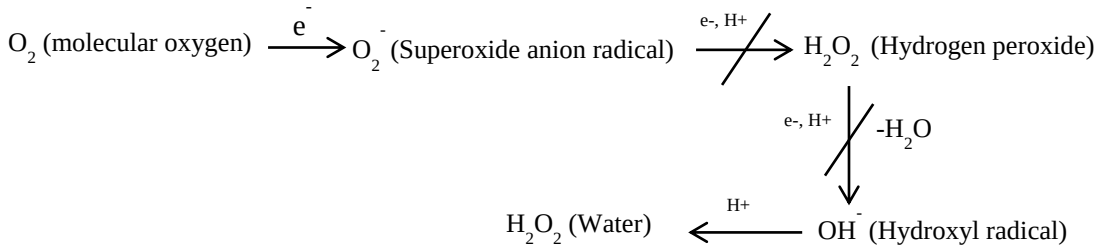
our knowledge, no work has so far been done in this regard on Nepali population and relationship, if any, with Body Mass Index (BMI) and Weight/Height (W/H) ratio, which are currently used as anthropometric markers for measuring overweight and obesity except some studies published from laboratory[8]. This study addresses relation of antioxidant status with lipid profile in normal population of different age groups. A lipid profile is possible because of the activity of lipids in the bloodstream. Essentially, lipids will adhere to proteins found in the system, creating what is known as lipoproteins. The lipid profile test helps to not only identify the total lipoprotein content in the blood, but also breaks down the components of the lipids in order to determine how much of each element is present.

Free radicals and antioxidants are considered so vital to our understanding of the origin of cancer, aging, illness, and disease that they have become a profound area of research. An important concern to human today is environmental hazards and its adverse effect to the health. The excess production of reactive oxygen species may initiate lipid peroxidation in cell membrane. These processes may result in a loss of contractile function of the heart and lead to severe myocardial cell damage, collectively termed as reperfusion injury[5]. The significant decrease in endogenous antioxidant in the patients could be due to overwhelming production and accumulation of superoxide anion causing inhibition of antioxidant activity. Whatever might be the cause of the decreased endogenous antioxidants, the net result is accumulation of $H_2O_2$, one of the most damaging products of the free radical metabolism. $H_2O_2$ can readily react with superoxide anion to produce the highly toxic hydroxyl radical and HOCl. Many findings suggest that antioxidants depletion has relevant impact to the precipitation of myocardial infarction and these findings are consistent with the notion that increased levels of antioxidants are protective[6].




Free radicals can be divided into 2 species: - ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species). ROS include superoxide ($\cdot O_2^-$) and its protonated form hydroperoxyl ($HO_2\cdot$), hydroxyl ($\cdot OH$), peroxyl ($HO_2^-$), alkoxy ($RO\cdot$) and non-radicals: hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), ozone ($O_3$) and singlet oxygen ($O_2$) that are agent or are readily converted to free radicals. Whereas, RNS includes nitric oxide ($NO^-$), peroxynitrite ($ONOO^-$) and peroxynitrous acid (ONOOH).

Molecular oxygen can be reduced to water. The intermediate step of oxygen reduction are the formation of the superoxide anion radical, hydrogen peroxide and hydroxyl radical by the addition of one, two and three electrons respectively[7].

RNS are produced with the reaction of nitric oxide with superoxide ($O_2$) to form peroxynitrite(ONOO).

$$\cdot NO \text{ (nitric oxide)} + O_2\cdot \text{(superoxide)} \rightarrow ONOO \text{ (peroxy nitrite)}$$

Additionally, peroxynitrite able to react with other molecules to form additional types of RNS including peroxynitrous acid, nitrogen dioxide($\cdot NO_2$) and dinitrogen trioxide($N_2O_3$) as well as other types of chemically reactive free radicals.

$$ONOO + H^+ \rightarrow \text{(peroxynitrous)} \rightarrow \cdot NO_2 + \cdot OH$$

$$ONOO^- + CO_2 \rightarrow ONOOCO_2 \text{ (nitrous peroxycarbonate}$$

$$ONOOCO_2^- \rightarrow \cdot NO_2 + O = C(CO\cdot)$$

## Experimental Methods

Fasting venous blood samples were collected from different places of Kathmandu valley (50 samples from normal people) aseptically by vein puncture into clean and dry glass vials. The blood was allowed to clot. Then sample was centrifuged at 3000 rpm for 5min and serum was collected for analysis. Total Cholesterol was estimated using chemical method (ZAK's method)[9] whereas TG,and HDL-cholesterol was estimated using the enzymatic kit method (GPO/PAP method[10,15.16] and PEG precipitation method[10,11,12,13,14] respectively) while LDL was estimated by using Friedewald's formula[10,11,12,13,14].

$$LDL = (TC) - (TG/5) - (HDL \text{ cholesterol})$$

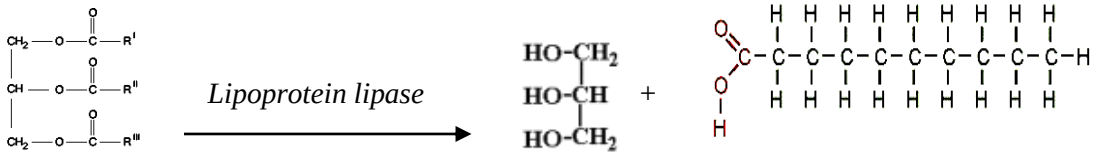
*Analysis of total cholesterol (by ZAK's method)*

$FeCl_3$ solution (0.05% w/v) was prepared in acetic acid. 3 dry and clean tubes were taken and labeled as Blank (B), Test (T) and Standard(S). 10 ml of ferric chloride reagent and 0.1 ml of serum sample was added in the test labeled tube. It was then mixed well, incubated at room temperature for 10 min and centrifuged at 2500-3000 rpm for 5 minutes to obtain clear supernatant. 5ml of supernatant was then taken in another test labeled tube and 5ml of cholesterol standard and $FeCl_3$ reagent was added in standard and blank respectively. 3 ml of conc. $H_2SO_4$ (18.38 M, 96% pure) was then added in each tube and was mixed and incubated at room temperature for 30 mins. Absorbance was then taken at 560 nm by Digital Photo colorimeter (model no. 312) and calculation was done.

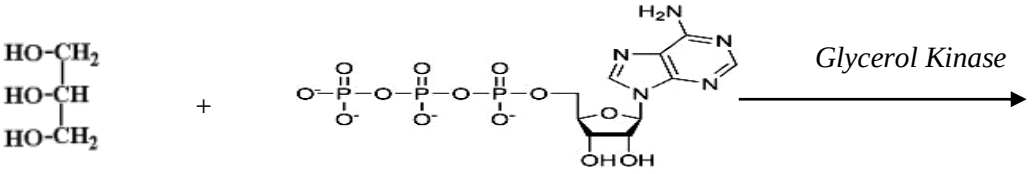
*Analysis of HDL (PEG Precipitation method)*

200 µl sample and 200 µl precipitating reagent was taken into the clean and dry tubes. They were mixed well and incubated at room temperature for 5 min and centrifuged at 2500-3000 rpm for 5 minutes to obtain clear supernatant. Supernatant was pipetted and kept into clean and dry tube and leveled as Blank (B), Standard (S), and Test (T) and one ml of working reagent was added to each tube. Similarly 50µl of supernatant was added to sample tubes, 50 µl of D/W to blank tube and HDL standard cholesterol to standard tube. Mixed well and incubated at 37$^o$C for 5

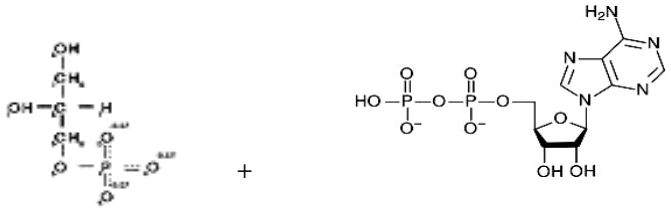
min Absorbance was measured at 505 nm by Digital Photo colorimeter and then calculation was done.



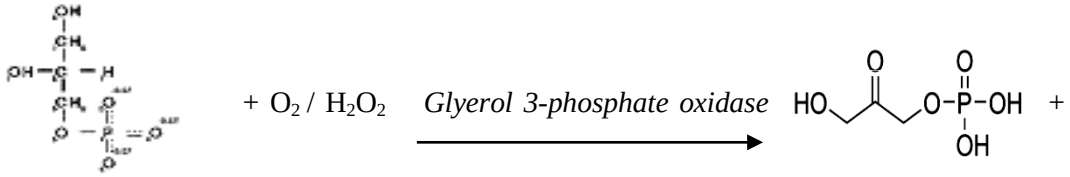

Triglycerides Glycerol free fatty acids



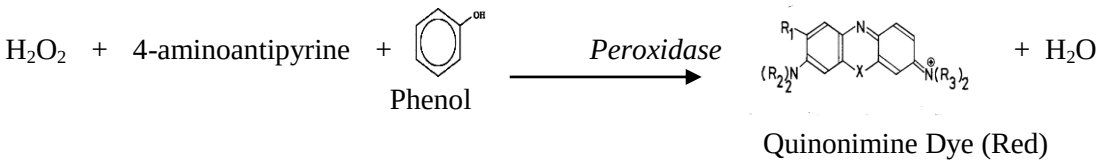

Glycerol
ATP



Glycerol 3-phosphate ADP




Glycerol 3-phosphate Dihydroxyacetone phosphate




Quinonimine Dye (Red)

*Analysis of Triglyceride (GPO/PAP method)*

Clean dry tubes were taken and 1ml of working reagent was added to each tube labeled as Blank (B), Test (T) and Standard (S). 10µl of D/W, 10µl of sample and 10 µl of standard TG was added simultaneously. Mixed well and was incubated at 37$^{o}$C for 5 min. Absorbance was made at 505 nm with Digital Photo colorimeter and calculation was done. Lipoprotein lipase hydrolyses triglyceride to glycerol and free fatty acids. The glycerol formed interacts with ATP in the presence of glycerol kinase forming glycerol 3-phosphate which is oxidized by glycerol phosphate oxidase to form hydrogen peroxide. Finally $H_2O_2$ further reacts with phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase to form a red-coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of TG present in the sample.

Similarly total antioxidant assay was estimated in blood serum using Ferric Reducing Ability of Plasma (FRAP) assay[7]. The FRAP reagent was used for the estimation of total antioxidant status. 300 mM acetate buffer was prepared by dissolving 0.82 g sodium acetate in 8.11ml glacial acetic acid and the volume was made 500ml by adding distilled water. 10 mm TPTZ solution was prepared by mixing 0.031 g TPTZ in 10 ml 40 mM HCl solution and dissolving it at 50 $^{0}$C in water bath. Similarly, 20 mM $FeCl_3$ was prepared by dissolving 0.032 g $FeCl_3$ in 10 ml distilled water.

The working FRAP reagent was then prepared by mixing 300mM acetate buffer, pH=3.6, 10 mm TPTZ solution and 20 mM $FeCl_3$ in the ratio of 10:1:1 at the time of use. The standard used was 100 µmol/L $FeSO_4.7H_2O$. The colorimeter was then set at zero by the blank i.e. 3 ml of FRAP reagent. 10µl sample was mixed with 3ml of working FRAP reagent. Then after, samples were allowed to stand for 4 min and absorbance was taken at 620nm. This process was repeated thrice for single sample.

Calculation:

Data were analyzed statistically by Pearson's Correlation coefficient using SPSS software.

$$Frap\ Value\ of\ Sample\ (\mu mol/L) = \frac{mean\ of\ absorbance\ of\ sample\ at\ 4\ min\ *\ concentration\ of\ standard}{mean\ of\ absorbance\ of\ Standard\ at\ 4\ min}$$

## Results and Discussion

*Correlation between TC and antioxidant status*

TC was determined using ZAK's method and total antioxidant was determined by FRAP assay method. The two parameters were then statistically analyzed by Pearson's correlation coefficient using SPSS software and are tabulated in Table 1.

The statistical analysis showed no correlation between TC and antioxidant status among normal population in the table 1 i.e. $p>0.05$ significant value.

***Table 1:*** *Statistical analysis between TC and antioxidant status*

| | | TC | Antioxidant |
|---|---|---|---|
| TC | Pearson Correlation | 1 | 0.085 |
| | Sig. (2-tailed) | | 0.557 |
| | N | 50 | 50 |
| Antioxidant | Pearson Correlation | 0.085 | 1 |
| | Sig. (2-tailed) | 0.557 | |
| | N | 50 | 50 |

*Correlation between TG and antioxidant status:*

TG was determined using enzymatic kit method and total antioxidant was determined by FRAP assay method. The two parameters were then statistically analyzed by Pearson's correlation coefficient using SPSS software. The result is summarized in table 2.

***Table 2:*** *Statistical analysis between TG and antioxidant status*

| | | Antioxidant | TG |
|---|---|---|---|
| Antioxidant | Pearson Correlation | 1 | -0.378** |
| | Sig. (2-tailed) | | 0.007 |
| | N | 50 | 50 |
| TG | Pearson Correlation | -0.378** | 1 |
| | Sig. (2-tailed) | 0.007 | |
| | N | 50 | 50 |
| **. Correlation is significant at the 0.01 level (2-tailed) | | | |

The statistical analysis showed negative significant correlation between TG and antioxidant status among normal population in the table 2 i. e. $p<0.01$ significant value. The statistical analysis shows that higher the level of TG, lower the antioxidant level and vice versa.

*Correlation between HDL and antioxidant status:*

Total HDL was determined by using PEG precipitation method and total antioxidant was determined by FRAP assay method. The two parameters were then statistically analyzed by Pearson's correlation coefficient using SPSS software.

***Table 3:*** *Statistical analysis between HDL and antioxidant*

| | | Antioxidant | HDL |
|---|---|---|---|
| Antioxidant | Pearson Correlation | 1 | 0.310* |
| | Sig. (2-tailed) | | 0.028 |
| | N | 50 | 50 |
| HDL | Pearson Correlation | 0.310* | 1 |
| | Sig. (2-tailed) | 0.028 | |
| | N | 50 | 50 |
| *. Correlation is significant at the 0.05 level (2-tailed). | | | |

The statistical analysis showed positive significant correlation between HDL and antioxidant status among normal population in the table 3 i.e. $p<0.05$ significant value. The statistical analysis shows that as the level of TG increases, the level of antioxidant also increases.

*Correlation between LDL and antioxidant status*

LDL was calculated by Friedewald's formula and total antioxidant was determined by FRAP assay method. The two parameters were then statistically analyzed by Pearson's correlation coefficient using SPSS software.

***Table 4:*** *Statistical analysis between LDL and antioxidant*

| | | Antioxidant | LDL |
|---|---|---|---|
| Antioxidant | Pearson Correlation | 1 | 0.234 |
| | Sig. (2-tailed) | | 0.103 |
| | N | 50 | 50 |
| LDL | Pearson Correlation | 0.234 | 1 |
| | Sig. (2-tailed) | 0.103 | |
| | N | 50 | 50 |

The statistical analysis showed that there is no correlation between LDL and antioxidant status among normal population in the table 4 i. e. $p>0.05$ significant value.

## Conclusion

The statistical analysis shows that there is significant correlation of TG and HDL with antioxidant status. TG and antioxidant status are indirectly proportional and HDL and antioxidant are directly proportional to each other. The elevated level of antioxidant is important to body as people grow older because it prevent from age related diseases. The higher the level of antioxidant inhibits oxidation of lipid which may cause coronary heart disease and also arthrosclerosis. The statistical analysis of LDL and antioxidant showed no correlation between them but literature review says significant relation between LDL and antioxidant. The increased intake of antioxidants reduces the oxidation of LDL that could lead to atherosclerotic lesions.

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