EFFECT OF PERMETHRIN - IMPREGNATED BED NETS ON THE BIOSYNTHETIC FUNCTIONS OF THE LIVER

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ABSTRACT

The effect of the Permethrin-treated bed nets (PTNs) on some biosynthetic functions of the liver was studied by measuring plasma total cholesterol, total protein, albumin and globulin of four volunteers after sleeping under the PTNs for 28 consecutive days. Prior to sleeping under the nets, their mean total plasma cholesterol was 183.50+6.75 mg/dl. Within the first two weeks of sleeping under the nets, their mean total plasma cholesterol level had risen to 238.75+25.22mg/dl amounting to 30.11% increase. In the next fourteen days, that is, on the 28th day, the cholesterol level dropped to the near pre-PTN-use level of 187.50+6.775mg/dl, amounting to a decrease of 22.30%. Within the first fourteen days of sleeping under under the nets too. The mean total plasma protein level also rose from 7.00±10.90 g/dl before its use to 8.02±5.4 g/dl, that is, 15% in 14 days or 1.7% increase pay day. In the next 14 days, that is, the 28” day of the use of the nets, the mean total plasma protein level was lowered below its pre-PTN-use level.

The mean plasma albumin level dropped from 4.90+0.41 g/dl before the use of the nets to 4.68+0.40 g/dl within the period. On the 28th day, the mean plasma albumin level rose, but it turned out to be higher than the initial value, 5.10+0.20 g/dl instead of the 4.90+41 g/dl prior
to their sleeping under the nets. The mean total globulin level rose from pre-net use value of
2.10 +0.87 g/dl to 3.35 +0.30 g/dl after 14 days amounting to 1.25% of this substance. On the
28th day however, its mean level dropped drastically to 1.78+ 0.19 g/dl. These results
indicate that the biosynthesis of cholesterol, protein generally but not albumin and globulin
were elevated as the use of the nets progressed, the biosyntheses of all but albumin reduced
drastically and tended to normal. However the initial hypoalbuminaemia and subsequent
hyperalbuminaemia are sources of worry.

**KEYWORDS:** Permethrin-treated bed nets (PTNs), hypoalbuminaemia.

**INTRODUCTION**

Malaria, caused by infections by the protozoan Plasmodium, is one of the most serious diseases
in the world. According to the World Health Organization, about 500 million people are affected
by it at any given time, and about 2 million of them, mostly children die each year, (WHO, 1993).

Malaria kills most children under five years of age who contract it. In areas where malaria is
prevalent, most survivors more than five or six years of age do not become seriously ill again from
malaria infections. The symptoms, familiar throughout the tropics include severe chill, fever, and
sweating, an enlarged and tender spleen confusion and great thirst. Ultimately a victim of
malaria may die of anemia kidney failure, or brains damage. The disease may be brought under
control by the person's immune system or by drugs because some individuals are genetically
resistant to malaria, while other persons develop immunity against it.

Efforts to eradicate malaria are focused on how to eliminate the mosquito vectors, the
development of drugs to poison the parasites once they have entered the human body and the
development of vaccines. The wide scale application of DDT from the 1940s to the 1960s led to
the elimination of the mosquito vectors in the United States, Italy Greece and certain area of
Latin America.

For a time, the world wide elimination of malaria appeared to be possible, but this hope soon
-crashed by the development of DDT-resistant strains of malaria-carrying mosquitoes in many
regions, and no fewer than 64 resistant strains were identified in 1980 survey (WHO,1993). Even
though the worldwide use of DDT, long banned in the United State, its use nearly doubled from its
1974 level to more than 30,000 metric tons in 1984. Its effectiveness in controlling malaria
vectors has dropped. There have been documented concerns about environmental pollution about
the use of this long-lasting chemical anywhere in the world. In addition to the problem with
resistant strains of mosquitoes, strains of plasmodia have appeared that are resistant to the drugs that had been used to kill the vector (Curtis et al., 2000).

As a result of these problems, concern about the resurgence of malaria in the 1970s, led to the development of global malaria control strategy which was adopted by the World Health Organization by a resolution at its 31st session in 1978 which set an ambitious target to halve the malaria burden before 2010 (WHO, 1993). One of the most important strategies that will be used to reach this target is to promote the use of insecticide-treated bed nets (ITNs).

The metabolic function of the liver consists in the effective functioning of the liver in the metabolism of biomolecules, their interrelationships and the mechanisms that regulate the flow of metabolites through the metabolic pathways.

The national roll back malaria programme and medium-term beneficial effects of insecticide-treated nets are well documented, but concerns have been raised about the long-term effects, since the reduction in infections inoculation may affect both the development and the maintenance of malaria immunity.

**OBJECTIVE OF THE STUDY**

The study is designed to investigate the effects of permethrin-impregnated bed nets (ITNs) on some selected biochemical parameters, namely: total plasma cholesterol, total plasma protein, albumin and globulin using four volunteers.

**MATERIALS AND METHODS**

**PREPARATION OF REAGENTS FOR CHOLESTEROL ASSAY**

70 mM phosphate buffer, 6g of phenol, 0.2g of dichlorophenol, 0.5g of 4 aminoantipyrine, cholesterol esterase, 500 µ/L300 ii/l, of cholesterol oxidase, 1200 ku/k of peroxidase and non reactive stabilizers were added to about 800 ml of water under room temperature. This solution was then transferred to a volumetric flask and made up to 1 liters. It is stable for about 5 months at 2-8°C.

**PREPARATION OF BIURET REAGENTS FOR PROTEIN ASSAY**

0.47 ml of sodium hydroxide, 23.3 ml of potassium iodide, 6.5 ml of copper (II) tetracoxosulphate (VI), 22.1 ml of sodium-potassium tartrate, preservatives and stabilizers were added to 800ml of distilled water and mixed thoroughly under room temperature and then transferred to a volumetric flask and made up to 1 liter. It is stable at room temperature 20-25°C.
PREPARATION OF BROMOCRESOL GREEN REAGENTS FOR ALBUMIN ASSAY
50ml succinate buffer of pH 4.2, 0.75 g/L of bromocresol green, 10ml of surfactants, 10ml of preservatives and stabilizers were added and dissolved in 800ml of distilled water under room temperature, then transferred to a volumetric flask and made up to 1 liter. This solution when stored at 2-8°C keeps for about 6 oaths.

TREATMENT OF NETS
The net was treated by immersing it in a bucket containing 100ml of water and insecticide permethrin (1 x 20ml) for about an hour to absorb the mixture and removed, and spread on a flat surface under the sun to dry. The insecticide could last for 3-6 months depending on the frequency of washing the net.

TEST DIAGNOSIS COLLECTION OF BLOOD SAMPLE
5ml of venous was collected from each of the four volunteers before the use of the ITNs, on day 14 and on day 28 respectively, the sera were used in quadruplicates to assay for the selected biochemical parameters.

CHOLESTEROL ASSAY
This was carried out according to the CHOD -PAP method of Allain et al. (1974). The free and esterified cholesterol in the samples were assayed based on the coupled reactions shown below; a coloured complex was formed, and the optical density (O.D) read off at 520nm.

REACTIONS
Cholesterol esters + H₂O → Cholesterolase → Cholesterol + fatty acids
Cholesterol oxidase Cholesterol -H₂O₂ + O₂ → 4-Armnoantipyrine + 3,5-Dichrophenol
Peroxidase
Coloured quinone-imine derivative + 4H₂O

PROCEDURE
1. Six test tubes were set up, one test tube was used for serum blank, one for standard and the remaining four test tubes were used for the test.
2. 1.0 ml of the reagent was pipetted into each of the six test tubes and made up to 10 ml with distilled water left standing on the bench.
3. 0.01 ml of the serum sample was pipetted and added to each of the four test tubes for the test and was mixed and left to stand for 5 min at 37 C in a water bath.
4. 0.1ml of cholesterol standard was pipetted and added to the test tube labelled standard and left to stand for about 5 mins at 37°C.
5. The absorbance of each test solution was read at 520nm after setting the spectrophotometer reading to zero with the corresponding blank.

**CALCULATIONS**

\[
\text{O.D of sample} \times 200 = \text{mg/dl concentration}
\]

\[
\frac{\text{O.D of standard}}{1} = \text{optical density}
\]

Range of serum cholesterol levels

<table>
<thead>
<tr>
<th>Normal range;</th>
<th>100</th>
<th>200</th>
<th>mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>High value; 240 mg/dl and above</td>
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</tr>
</tbody>
</table>

**TOTAL PROTEIN ASSAY (DIRECT BIURET METHOD)**

*(Gomaller et al., 1949)*

**PRINCIPLE**

In alkaline pH 7.4, protein reacts with copper II ions and form a blue-coloured complex, which is measured spectrophotometrically at 520nm.

**PROCEDURE**

1. Six test tubes were set up, one test tube for the blank, one for the albumin standard, and the remaining four test tubes for the test.
2. 2.50 ml of the reagent was pipetted into the six test tubes and left to stand for 5 min at 20-25°C.
3. 0.01 ml of the serum samples were pipetted into the four test tubes for test.
4. 0.01 ml Bovine albumin standard was pipetted into the test tube labeled standard, mixed and allowed to stand for 5 min at 70 °F.V.
5. 10ml of distilled water was pipetted and put into the six test tubes to make up the volume and mixed.
6. After 5 min., the optical density of each sample was measured at 620nm against the blank.

**CALCULATIONS**

\[
\text{O.D of sample} \times 5 = \text{g of albumin/dl}
\]
O.D of standard Normal values, of serum albumin 3.5 - 5.0 g/dl

RESULTS

MEAN GLOBULIN (g/dl) (total protein-albumin) 2.1 + 0.87

Table 1: Levels of cholesterol, total protein, albumin and globulin before the use of the permethrin-treated bed nets (ptsns) (day-1).

<table>
<thead>
<tr>
<th>MEAN TOTAL CHOLESTEROL (mg/dl) (g/dl)</th>
<th>MEAN TOTAL PROTEIN (g/dl)</th>
<th>MEAN TOTAL ALBUMIN (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>183.50 ± 6.75</td>
<td>7.0 ± 0.90</td>
<td>4.90 ± 0.41</td>
</tr>
</tbody>
</table>

The mean cholesterol level of the volunteers was within normal range before the use of PTNs. The other parameters were also within normal ranges.

MEAN TOTAL ALBUMIN g/dl 7.0 ± 0.90

Table II: Levels of cholesterol, total protein, albumin and globulin two weeks after the use of permethrin treated bed nets (ITNs) (DAY-14)

<table>
<thead>
<tr>
<th>MEAN TOTAL CHOLESTEROL (mg/dl)</th>
<th>MEAN TOTAL PROTEIN (g/dl)</th>
<th>MEAN TOTAL ALBUMIN (g/dl)</th>
<th>MEAN TOTAL GLOBULIN (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>238.75 ± 25.22</td>
<td>8.030 ± 0.54</td>
<td>4.68 ± 0.40</td>
<td>3.33 ± 0.30</td>
</tr>
</tbody>
</table>

On day-14, the mean values of all the above parameters were elevated relative to day-1, except mean total albumin which decreased slightly from 4.90 to 4.68 g/dl.

Table III: Levels of cholesterol, total protein, globulins four weeks after the use. insecticide treated bed nets (ITNs)

<table>
<thead>
<tr>
<th>MEAN TOTAL CHOLESTEROL (mg/dl)</th>
<th>MEAN TOTAL PROTEIN (g/dl)</th>
<th>MEAN TOTAL ALBUMIN (g/dl)</th>
<th>MEAN TOTAL GLOBULIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>187.50 ± 18.4</td>
<td>6.97 ± 0.26</td>
<td>5.10 ± 0.20</td>
<td>1.78 ± 0.19</td>
</tr>
</tbody>
</table>

On day-28, the mean total cholesterol had been restored to its pre-PTN mean value. The mean values of other parameters also dropped when compared with their levels on Day-14.
DISCUSSION

In the study, the effect of prolonged use of one of the ingredients used to impregnate insecticide-treated bed nets, permethrin, was assessed in four volunteers who slept under permethrin-treated nets for twenty-eight consecutive days.

It is known (Walter and Talbot, 1985), that about 10% of plasma cholesterol is synthesized in the liver, while albumin and other proteins (including α- and p-Globulins but not the immunoglobulina) are made other proteins (including α and β). Indirectly, therefore, this work was designed to assess the effects of insecticides-treated bed nets (ITNs) on the biosynthetic functions of the livers of the volunteers used.

The volunteers had no clinically detectable liver problems prior to sleeping under the bed nets. The mean total cholesterol level of 183.50+ 6.75mg/dl before sleeping under the nets was within normal range (Allain et al., 1974). However, within the first two weeks (14 days) of sleeping under the nets, their mean total cholesterol level was elevated to the life-threatening level of 238.75+25.22mg/dl, amounting to 30.11% rise in the level of this clinically important substance. Although, cholesterol is also synthesized in other tissues, such as the intestines and the adrenal cortex, there is no doubt that hepatic synthesis of this amphipathic lipid was also elevated within this period because, 10% of it in plasma is of hepatic origin (Walter and Talbot, 1985).

In view of the well-know undesirable effects of hypercholesterolaemia, such as arteriosclerosis and its attendant cardiovascular problems, as much as 30.11% rise in its level within 14 days was considered unhealthy.

In the next fourteen days, that is, on the 28th day, however the cholesterol level dropped to the near-pre-ITN-use-level of 187. 50+18.4 mg/dl, amounting to a decrease of 22.30%. Since low density lipoprotein (LDL) is cholesterol-rich and its major transporter, and high density lipoprotein (HDL) is cholesterol-poor and a major scavenger of cholesterol, this scenario is suggestive of decreased LDL: HDL ratio within the last two weeks of the use of the ITNs. Since cholesterol is synthesized from acetyl-CoA, which in turn is derivable form carbohydrates, lipids and proteins, the mechanism by which the active principle in. The ITNs used precipitated this transient rise in cholesterol level which later tended to stabilizer remain to be investigated.
Within the first fourteen days of sleeping under the nets, the mean total plasma protein level also rose this time from 7.00 + 0.90 g/dl before its use to 8.025 + 0.536 g/dl, that is, 15% in 14 days, or 1.07% increase per day. The proportion of this rise in total plasma protein constituted by α-globulin, β-globulin, albumin or immunoglobulin, is not known, but it may be reasonable to argue that immunoglobulins (Igs) should constitute a significant proportion of it, following the uptake of permethrin, an antigen from the net.

In the next 14 days, that is, the 28th day of the use of net, the mean total plasma protein level was lowered below its pre-ITN-use level. This is suggestive of impaired protein synthesis by the liver and other tissues. If this impairment occurred in the liver, it is further suggestive of the inhibition of liver enzymes or liver cirrhosis or shortage in the supply of either amino acids or folate needed for the initiation of protein synthesis, or other factors.

The mean plasma albumin level dropped from 4.90g/dl ± 0.41g/dl before the use of the next to 4.68 ± 0.40g/dl within the 14 days. This amounted to 4.89% drop in albumin synthesis within the period. It is reasonable to suggest that albumin was not part of the 15% rise in the plasma protein level recorded within the period. Albumin is the principal protein synthesized by the liver secreted directly into the blood stream; hypoalbuminaemia is seen in starvation when protein reserves are metabolized, as well as in excessive loss, e.g., the nephrotic syndrome (Walter and Talbot, 1985). It is obvious that albumin synthesis was impaired within this period. It can be inferred that its usual functions, such as binding many substances and reducing their availability and toxic effects were impaired within this period.

On the 28th day, there appeared to be recovery as the mean plasma albumin. Level rose but it turned out to be higher than the initial values 5 to 10g/dl instead of the 4.90 ± 0.41g/dl prior to their sleeping under the nets. This tendency to hyperalbuminaemia is suggestive of haemoconcentration (Walter and Talbot, 1985), which is difficult to account for by the quantity of permethrin inhaled during the 28 days the volunteers slept under the nets.

The mean total globulin level rose from pre-net use value of 2.10 ± 0.87g/dl to 3.35 ± 0.3Qg/dl after 14 days. Which of the globulins accounted for this 1.25% rise is not certain. The only guess is that it could be due to the immunoglobulin which was raised against the active principle in the net that might have posed antigenic challenge.

On the 28th day however, its mean level dropped drastically to 1.78 ± 0.19g/dl, suggesting that the immunoglobulin (antibodies) rose against the antigen must have overcome it and
subsequently been metabolized, since it is protein.

**CONCLUSION**

Based on the results of this study, it can be concluded, that at the commencement of the use of PTNs, the biosyntheses of cholesterol, protein generally, but not albumin and globulins become elevated. As its use progresses, the biosyntheses of all but albumin reduce drastically and tend to normal values. However, the initial hypoalbuminaemia and subsequent hyperalbuminaemia are sources of worry.

**REFERENCE**