4. RESULTS

4.1. SOME CLINICAL ASPECTS OF MALARIA INFECTED INDIVIDUALS

4.1.1. SURVEY OF MALARIA INFECTED INDIVIDUALS

The survey of malarial cases in the coastal areas of Thanjavur, Nagapattinam and Cuddalore districts of Tamil Nadu is shown in the table 1. Out of 120 samples collected, 72 cases were malarial cases. Among 72 malarial cases, 42 male and 30 female identified (figure 7). The maximum number (29) malarial cases were observed in Cuddalore than Thanjavur (24) and Nagapattinam (19) districts (figure 8). The samples contain only two species of parasites namely P. falciparum and P. vivax in different stages in the smears of malarial positive cases (figure 9). Among 72 malarial cases, 46 P. falciparum and 26 P. vivax were identified.
Table 1. The survey of malarial cases in some coastal pockets of Tamil Nadu.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>District</th>
<th>Sample Collection</th>
<th>Positive Cases</th>
<th>Male</th>
<th>Female</th>
<th>P. falciparum</th>
<th>P. vivax</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nagapattinam</td>
<td>37</td>
<td>18</td>
<td>12</td>
<td>6</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>2.</td>
<td>Thanjavur</td>
<td>42</td>
<td>25</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>3.</td>
<td>Cudalooire</td>
<td>41</td>
<td>29</td>
<td>18</td>
<td>11</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>120</td>
<td>42</td>
<td>30</td>
<td>46</td>
<td>26</td>
</tr>
</tbody>
</table>
Figure 7. Histogram shows the malarial cases in male and female.
Figure 8. Pictorial diagram shows the malarial cases in some coastal pockets of Tamil Nadu.
Figure 9. Histogram shows the number of positive cases of *Plasmodium* *spp.*
4.1.2. HAEMATOLOGICAL PARAMETERS

4.1.2.1. Identification of different stages of malarial parasites

The presence of malaria parasites in the blood has been examined. There are several types of parasites are found, but the samples contain only two species of parasites, they are *P. falciparum (PF)*, *P. vivax (PV)*. In the present study, the parasitic stages like ring stage, gametocyte stage and schizont stage of *P. falciparum* and *P. vivax* were observed (Figures 10-12) in the smears.

![Image of ring stage](image.png)

Figure 10. Ring Stage (RS) of *Plasmodium falciparum*. 
Figure 11. Gametocyte Stage (GS) of *Plasmodium falciparum*.

Figure 12. Ring Stage of *Plasmodium vivax*. 
4.1.2.2. Erythrocyte sedimentation rate

Erythrocyte sedimentation rate (ESR) was expressed in the nearest (mm) every half an hour interval and one hour. The normal level of erythrocytes sedimentation rates the range of 0-20 mm/hour in host. The Erythrocyte sedimentation rate was significantly decreased (table 2) as compare with normal. The Erythrocytes level was found to be reduced due to the differentiation of Erythrocyte sedimentation rate half and one hour time were shown in figure 13.

Table 2. Erythrocyte sedimentation rate in normal and malaria infected individuals in half and one hour intervals.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Subject</th>
<th>Mean blood erythrocyte sedimentation rate (mm/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal (n = 72)</td>
<td>24.4± 25.1</td>
</tr>
<tr>
<td>2.</td>
<td>Infected individuals in half hour (n =72)</td>
<td>22.9 ± 22.4</td>
</tr>
<tr>
<td>3.</td>
<td>Infected individuals in one hour (n =72)</td>
<td>50.9 ± 36.9*</td>
</tr>
</tbody>
</table>

* Significant at the probability level of P <0.05.
Figure 13. Erythrocyte sedimentation rate in normal and malaria infected individuals.
4.1.2.3. Total leucocyte count

The total leucocyte count was significantly higher in malaria infected individuals compared with normal. The mean blood results were shown in Table 3 and Figure 14.

Table 3. Total count of white blood cells in normal and malaria infected individuals.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Subjects</th>
<th>Total White Blood Cells (Cells / Cumm )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal individuals</td>
<td>9000 ± 3283.3</td>
</tr>
<tr>
<td>2</td>
<td>Infected individuals</td>
<td>9768.8 ± 962.6*</td>
</tr>
</tbody>
</table>

* Significant at the probability level of P <0.05.
Figure 14. Total count of WBC in normal and malarial infection.
4.1.2.4. Differential WBC Count

The differential WBC count for Neutrophils, Neutrophils and Neutrophils were higher in the malarial infected individuals than normal (table 4 and figure 15).

Table 4. Differential white blood cells counting in normal and malarial infected individuals.

<table>
<thead>
<tr>
<th>S</th>
<th>White blood cells</th>
<th>infected individuals</th>
<th>normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neutrophils</td>
<td>50.6</td>
<td>56.8</td>
</tr>
<tr>
<td>2</td>
<td>Neutrophils</td>
<td>0.47</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>Neutrophils</td>
<td>40.9</td>
<td>41.6</td>
</tr>
</tbody>
</table>
Figure 15. Differential WBC count in normal and malarial infected individuals.
4.1.3. BIOCHEMICAL ASPECTS

4.1.3.1. PROTEIN LEVEL IN MALARIAL INFECTED INDIVIDUALS

The result shows that the quantity of protein in normal and malaria patients. From this it could be understood that quantity of protein has been varied based upon the time interval of their infection. The protein level was significantly increased (13.67 (17.88) as compare with normal (7.13 (8.95) were shown in table 5. The quantity of protein has been higher (figure 17).

Table 5. Estimation of total protein in normal and malarial infected individuals.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Subjects</th>
<th>Mean serum of total protein (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal individuals</td>
<td>7.13 ± 8.95</td>
</tr>
<tr>
<td>2</td>
<td>Infected individuals</td>
<td>13.67 ± 17.88*</td>
</tr>
</tbody>
</table>

* Significant at the probability level of P <0.05.
Figure 16. Total protein in normal and malarial infected individuals.
4.1.3.2. SPARTATE TRANSAMINASE AND ALANINE TRANSAMINASE RATIO IN MALARIAL INFECTED INDIVIDUALS

Both AST and ALT percentage were higher in *P. vivax* infection patients samples. The percentage level was more in 40 to 50 age group than 20 to 30 age group (table 6). The percentage of AST and ALT was more in male than female of malarial infected patients. Male population has the highest AST / ALT ratio than female (figure 17). The percentage of AST and in male than female of the malarial infected patients (figure 18).

Table 6: Percentage of AST and ALT level in acute *Plasmodium vivax* malarial infection.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Type of Enzymes [U/L]</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20-30</td>
</tr>
<tr>
<td>1.</td>
<td>AST</td>
<td>42.8%</td>
</tr>
<tr>
<td>2.</td>
<td>ALT</td>
<td>39%</td>
</tr>
</tbody>
</table>
Table 7. Titre difference of AST / ALT in normal and malaria infected individuals.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Subjects (IU / L)</th>
<th>Mean Serum of aspartate and alanine amino transferase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal</td>
<td>33.4 ± 11.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 72)</td>
</tr>
<tr>
<td>2.</td>
<td>Infected Males</td>
<td>52.4 ± 13.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 40)</td>
</tr>
<tr>
<td>3.</td>
<td>Infected Females</td>
<td>42.8 ± 9.9*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n = 32)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 72 samples. Significantly differ from normal at P < 0.01
Figure 17. AST / ALT ratio in normal and malarial infection.
Figure 18. The percentage of AST and ALT in male than female of the malarial infected patients.
4.1.3.3. ALKALINE PHOSPHATASE ACTIVITY IN MALARIAL INFECTED INDIVIDUALS

Total Serum alkaline phosphatase activity in healthy and malaria infected patients are presented in the table 8 and figure 19. Serum Alkaline Phosphatase activity was significantly increased in male patients to the normal. Serum Alkaline Phosphatase activity was also higher in female patients.

Table 8. Total Serum alkaline phosphatase activity in healthy and Malaria infected patients.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Subjects (IU / L)</th>
<th>Mean Serum of aspartate and alanine amino transferase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Male Patients (n = 42)</td>
<td>80.5 ± 28.3</td>
</tr>
<tr>
<td>2.</td>
<td>Normal Males (n = 24)</td>
<td>75.4 ± 57.65</td>
</tr>
<tr>
<td>3.</td>
<td>Infected Females (n = 30)</td>
<td>71.9 ± 17.2</td>
</tr>
<tr>
<td>4.</td>
<td>Normal Female (n=20)</td>
<td>64.8 ± 30.53</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 72 samples.

*Significantly differ from normal at P < 0.01
Figure 19. Alkaline phosphatase activity in normal and malarial infection
4.2. PROTEIN PROFILE

4.2.1. ELECTROPHORETIC PROTEIN PATTERN IN SERUM SAMPLES OF MALARIAL INFECTED INDIVIDUALS

An unique bands were observed on 2,5,14, 21, 32 and 45 day samples (figure 20) but the 14th day samples was specially under taken in present study, because they formed more numbers of unique bands (figure21 ). So that bands were separated and then the protein was eluted from the gel, by using electro elution method. So, the slabgel was used for further target studies of protein profile.

Figure 20. Showing the marker and protein bands of infected samples of different time intervals.
Figure 21. Showing the marker and 51 kDa proteins.
Electro elution of protein from gel: In this electro elution method the 51 kDa protein was eluted from the gel (Figure 14). The total amount of protein finally recovered from the gel was used to study the sequencing and structural analysis of protein.

4.2.2. SEQUENCING AND STRUCTURAL ANALYSIS OF 51 KDA PROTEIN PLASMODIUM FALCIPARUM

The 51 kDa sequence was isolated by using electroblotting method. The structural prediction and sequence analysis of 51 kDa parasitic protein were made by using SOPMA and Ramachandran Plot. Finally that predicts the enzymatic protein such as acetyl co-enzyme A carboxylase, propionyl Co-enzyme A Carboxylase and methyl transferase A.

4.2.2.1. Structural prediction of accD3 and accD4 by SOPMA method

By the structural prediction of acetyl Co-enzyme A carboxylase was retrieved by 3 D Structural prediction method. A suitable homology was built by using the software MODELLER to retrieve the 3 D structure of the target protein that was given as input. The best method at minimum energy level obtained the best 3 D structure was viewed as shown (figures 22 and 23) with the help of SOPMA method were predicted. SOPMA correctly predicts 82.2 % of amino acid residues and 74 % of Co-predicted amino aids, in β-subunit structural protein.
Figure 22. Acetyl Co-enzyme A carboxylase (accD3).

Figure 23. Propionyl Co-enzyme A carboxylase (accD4).
Sequencing analysis of accD3 and accD4

The total sequence length having about 495, by the total length include -helix turn, extended strand and random coiling sequence. There are about 220 -helix (Hh) sequences 35 Beta turn (Tt) Sequence, 85 extended strands (Ee) and 155 random coiled (CC) sequences were analyzed.

```
  10  20  30  40  50  60  70
|    |    |    |    |    |    |    |
MSRITTDQLRHAVLDRGSFVSVDSEPLAVPVADSYARELAAARATGADESVTGEGRVFRVAVVACE
hhhechhhhhhhheectteeeetceeecccehhhhhhhhhhhhhhhhttechheecctceeeetceeeechh
FDLGGSGIGVAAAERITAAPERATAERLPPASPSGGRTRMQEGTVAFLQVKIAAIQHLNQARLPYLV
heeecccccehhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhtttccccceee
YLRHPTTGVFAWGSLGHLTVAEPGALIGFLGPRVYELLYGDPFSGVQTAENLRHGIIDGVVALDRL
eeeecccccccccehttteeeetcccccccccehheeecccccccccccccccccccccccccccccccc
RPMLRALTVLIDAPEPAPQTPAPVDPVTWDSVVASRRPDRPGVRQLRHGATDRVLLSGTDQGEAA
hhhhhhhhheeeeeeecccccccccccccccccccccccccccccccccccccccccccccccccccc
TTLLALARFGQVTQVLQQRAVGSGGTVIPLALREARRGMLAELCLPLVLVIDAAGPAALSAAEQG
hhhhhhhhhtttcccccccccehheeecccccccccccccccccccccccccccccccccccccccc
GLAGQIAHCLAELVTLDPTVSILLGQSGGPAALMLPADRLVLAALHGWLALPPEGASAIVFRDTAHAA

ELAAAQGIRSADLLKSGIVDTPEYPDAADEPIEFLRSAIAAEVHALRKRIPAPERLATNLQRYRRI

GLPRD
tccct

Sequence length: 495

SOPMA:

Alpha helix (Hh): 220 is 44.44%

Extended strand (Ee): 85 is 17.17%

Beta turn (Tt): 35 is 7.07%

Random coil (Cc): 155 is 31.31%

accD4

MTVTEPVLHTTAEKLAELRERLEAKEPGKEKAAXKRDKKGIPSARARIELVDPGFSMEIGALCRTPGD
eeeccechhhhhhhhhhhhhhhhhhhhttcehcccccccceeeecceehhhhhheecctteceehhhhhh

PNALYGDGVVTGHGLINGRPVGFSHDQTFGTVGEMFGRKVARLMEWCMVGCPIVGINDSGGARIQD
cccccccccccccctccccccccccccccccccccccccccccccccccccccccccccccccccccc

AVTSLAWYAELGRRHELSSGLVQISIILGKCAGAVSYPIQTDLVVAVRDQGYMFVTGPDVKVIDTGED
hhhhhhhhhhhhhhhhhhhttcccteeeeccccctccccccccctccccctccccccccccccccceehhh
VSLDELGADHQASYGNIHQVVEEAAAYQYVRDFLSFLPSNCFDKPPVNPGEITGHDLELDIVTP
cchhhccecechhccttecchhhhhhhhhhhhhhhhhhheccccccccccccccccccccchhhheccc
DSDNSAYDMHVEVLLRIFDDGDFLDVAQAQAIITGARYRDGRTVGVVANQPMHMSGAIDNEASDKAARF
cceccccchhhhhhhhhhttteeccccchhheeeeccttceeeeccccctccchhhhhhhhhhhhhh
IRFSDAFLTPLVFVDTPGFLPGVEQEKNIIKRGGRFLYAVVEADVPKVTTIRKSYGGAYAVMGSKQL
ecchhcceeecceeeecttceccccchhhhhhtthheecchhhhtccccceeeeccttceeeecccc
TADLNFAWPTARIAGADGAAQLMKRFDPNAEPAQAIIRKSVENYNLMAIPWIAERFIDAVIDP
cceeeecceceeeecctteehhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhhcccchhhhhhhhhheccc
HETRLLRKMHHLLRDKQLWWRVGRKHGLIPV
cchhhhhhhhhhhhhhhhccccccccccccce
Sequence length : 522
SOPMA :

Alpha helix (Hh) : 213 is 40.80%
Extended strand (Ee) : 90 is 17.24%
Beta turn (Tt) : 38 is 7.28%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 181 is 34.67%

Total carboxyase length having about 522. In this total length of the Propionyl CoA carboxyl’s having 213 (helix (Hh) Sequences, 90 extended (Ee) and then included 38 beta turn (Tt) Sequence, 181 random coil (Cc) Sequences were analysed.
4.2.2.2. Structural prediction of accD4 and mmA1 by Ramachandran Plot method

Ramachandran plot showed that the number of aminoacid residues present in the favored region. In this propionyl co A carboxylase acc D4 (figure 17) having 466 residues (92.3 %) present in favoured region. By the allowed region contain (5.9%) of residues and in the outside region contain only (1.8%) of residue were found ( i.e. 9 residues) suggesting that a good model. Among that favoured and allowed region contain rich amount preproline and glycogen residues region contain rich amount of preproline and glycogen residues.
Figure 17. Propionyl COA carboxylase (accD4).
Methy transfereas A1 (mm A1)

Only 270 residues were found (95.4%) in the favoured region. In Methy transfereas A (Figure 18), contain much amount of preproline and glycine residues in favored and allowed region. By the residues (3.5%) found in allowed region and lesser amount such as (1.1%) of residues found in outside region.

targetaccD4.pdb

Figure 18. Methyl Transeferase A1
4.2.2.3. Comparison of sequence with database by BLAST

Length of the Query sequence = 20 letters.

The peptide sequence submitted = MCDSKDNSGV SEKCGKKFTN

Color key for alignment scores

Sequences producing significant alignments:

H137Rv|sp|Plasmodium falciparum 51 kDa Parasite protein

Length=448  Score = 47.4 bits (111), Expect = le-04

Identities = 20/20 (100%), Positives = 20/20 (100%), Gaps = 0/20 (0%)

Query 1

MCDSKDNSGVSEKCGKKFTN 20

SBjct 1

MCDSKDNSGVSEKCGKKFTN 20
4.2.2.4. Rasmol Model

Fig -24. Rasmol Model.
Rasmol Model for accD4

Target protein were model using Swiss model, Swiss model automatically server for 3 Dimensional structure prediction it select templates and will generate 3 Dimensional. The trial model will contain templates as well as target. The target was segregate using Swiss view and trial structure validation using rampage.

Fig – 25. Rasmol model for mmaA1

Rasmol model for mmaA1

Target protein were model using Swiss model, Swiss model automatically server for 3 Dimensional structure prediction it select templates and will generate 3 Dimensional. The trial model will contain
templates as well as target. The target was segregate using Swiss view
and trial structure validation using rampage.

By the different aspects were carried out in this study, since some
of the proteins have been disappeared in different phases. But some
other proteins were persistent in some days, depends upon the type of
parasitic infection and time interval of the infection period. For this
study 14th day sample contain 51 KDa protein. Sequencing and
structural analyzing of 51 kDa protein revealed the presence of H137 RV
associated with membrane receptor protein in *Plasmodium falciparum.*