CHAPTER SIX

6.1 SUMMARY OF FINDINGS

On the basis of the objectives, the following were observed in this study:

1. All study subjects had knowledge of at least one of the classical symptoms of malaria and pattern of prescription in the study which shows a high preference for ACT as drug of choice

2. Packed Cell Volume (PCV) values decreased significantly across days 0, 7 and 14 relative to control

3. White Blood Cell (WBC) and lymphocyte values were not significantly changed across days 0, 7 and 14 relative to control

4. Eosinophil values increased significantly but neutrophil values decreased significantly across days 0, 7 and 14 relative to control

5. Platelet values decreased significantly at day 0 but tended towards the control across days 7 and 14

6. The mean AST, ALT and ALP values of the various liver function enzymes increased significantly across days 0, 7 and 14 relative to control

7. The multiplex PCR results showed an exclusive *Plasmodium falciparum* in the study population

8. K13 gene was highly expressed with only the wild type found in the study population. There was low *pfATPase 6* gene expression, with the wild type being far more expressed than the mutant type in the study population
9. K13 had 7 non-synonymous mutations (G496S, R539F, I543V, A557?, V566K, A578K and D584I) detected that are unique to the studied population

10. K13 also had SNP580 and this is the first time this will be reported in Nigeria

11. *pfATPase*6 had one (1) synonymous and two (2) non-synonymous mutations with the presence of S769 SNP which has been associated with delay in parasite clearance in individuals taking ACT

12. N86K and Y184F SNPs were detected for *pfmdr1* with very high prevalence in the parasite population under study

13. Cysteine-Valine-Isoleucine-Glutamic acid-Threonine(CVIET), specific for chloroquine resistance and associated with ACT resistance also had a very high prevalence in the parasite study population

### 6.2CONCLUSION

Malaria will remain a public health problem worldwide especially in Sub-sahara Africa since there is no fully protective vaccine available. The detection of resistance SNPs in this study (especially SNP580 for ACT resistance), is a clear indication that ACT resistance is eminent. 

Thou K13 mutant type gene was not expressed which might be due to lack of a complimentary or secondary SNP to SNP580, it is very important to continue to monitor expression of the K13 gene. Detection of parasite in patients on the third day but total clearance on the seventh day could be called delayed parasite clearance, which may be due to the presence of the *pfATPase*6 mutant gene expressed in this study. If this trend is not checked, it could lead to *P. falciparum* resistance to ACT.
In conclusion, the control of drug resistant in malaria parasites requires reducing the overall drug pressure, improving the ways the drugs are used and prescribing follow-up practices. The use of drug combination which are not likely to foster resistance like ACT is also a good measure of resistance control. Resistance has also been associated with low concentration of the active component. The low drug concentrations occur because of poor adherence taking the medication, substandard drugs, or unusual pharmacokinetics. The patient may be non-adherent to the medication because of the common side effects associated with the antimalarial pharmaceuticals. One of the common adverse effects of the medication and symptom of malaria is vomiting, which can also lower the drug concentration in the patient. The pharmacokinetics of the pharmaceuticals can be altered by the function of enzymes that metabolize the drug. An example is artemether, it is metabolized into an active metabolite by CYP450 3A4 and 3A5. If the patient is a poor metabolizer of one of these enzymes, the concentration of the active metabolite will be lowered. This lower concentration allows the parasite to develop resistance (White et al., 2009).

6.3 CONTRIBUTIONS TO KNOWLEDGE

1. The study observed seven (7) new variants of K13 SNPs in the studied parasite population suggesting a highly polymorphic target site implying potential resistance to ACT.

2. The study demonstrated that K13 wild type gene was highly expressed while pfATPase 6 had very low expression of both the wild and mutant type genes in the study population in Nigeria which suggest that the efficacy of ACTs will be greatly compromised.
3. The study showed the presence of SNP580 for the first time in Nigeria while the presence of SNPs in *pfcrt* and *pfmdr1* demonstrated potential resistance to ACT.

4. Activities of liver function enzymes in malaria infected patients treated with ACT were significantly elevated up to 14 days post treatment. This can predispose such patients to hepatic necrosis and hepatitis.

### 6.4 RECOMMENDATIONS

Continuous monitoring and surveillance of efficacy of ACT including identification of artemisinin resistant parasite is required for appropriate implementation of malaria control policy in Nigeria. Current works is driven by technology and by the massive amount of information in genomes. Molecular evolutionary analysis has now become a high-throughput industry. In light of these, I will recommend the following:

1. There is more need for sequencing of multiple isolates of a species and single-nucleotide polymorphism discovery, allowing broad genotyping approaches to be used to identify signatures of selection and associations with phenotypes.

2. Newer and more sophisticated assays and technical platforms are constantly becoming available; there is therefore increase need for technology transfer and the development of circumscribed scientific network schemes with those in most countries where malaria is endemic by the developed malaria free countries.

3. Availability of resources between leading research groups and those in most countries where malaria is endemic is very wide. Ministries of health needs to start taking ownership of control programmes by investing more in project that will help eliminate
malaria in their various countries. This will encourage foreign donors to support such programmes.

4. Local expertise is required to develop local strategies for the implementation of control programs; this can be achieved if local infrastructure and expertise is rebuilt. This will require selection and training of local personnel from countries with endemic infection who have a commitment to learning about malaria and who will remain and develop their careers locally.