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EVALUATION OFENZYME (TRANSFERASE)IN PATIENTS WITH*PLASMODIUM FALCIPARUM* ATTENDING RIVERS STATE UNIVERSITY TEACHING HOSPITAL

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ABSTRACT

*Plasmodiasis*isanillness caused by intracellular protozoan parasites thataffectsbothhumansandotheranimalsandisspread by infectedfemale anopheles mosquitobites.*Plasmodiumfalciparum*ismostly responsibleforsevere illness. EvidencehasestablishedthatNigeriahasthehighestrateofmalariacasesinAfrica,makingitthe country mostat dangeroffailing tomeettheobjectiveftheMillenniumDevelopmentGoals.In the liver, *transferase* areproteins that speed upchemical reactions in thebody which are affected by infections.This study was aimed at the evaluationoftransferaseenzymeinpatients with*plasmodiasis*in Rivers StateTeaching Hospital.It wasacrosssectionalstudywherethesubjectswererandomlyselected.Thestudywascarried outamongpatientsattendingRiversStateUniversity TeachingHospitalinPortHarcourt,Rivers State.Onehundredandfifty(150)subjectswereincludedinthisstudy.Sixty-eight(68) subjectshadmalariaandwereregardedastestgroupwhileSixty-eightothershadnomalariawere regarded as thecontrolgroup. Blood samples were collected for malaria parasites using thick film diagnostic method while ALP, ALT, AST and GGT were collected in heparin bottle using standard procedure to analyze them.Theresultsreportedinthisstudy showedsomesignificantincreasesinactivitiesenzymesaspartate transaminase(AST),alaninetransaminase(ALT),andalkalinephosphatase(ALP)amongpatient s infectedwith *plasmodiasis* as compared to that ofthecontrol subjects were all significant as $P < 0.05$ except ALT which P value is 0.478.Our reportalsoshowedthatthere wasa significantelevationof theALPenzymesonthoseof the infectedindividualswithPlasmodiasiswhencomparedtothenon-infectedindividualsinbothmale andfemalesubjects which was significant as $P < 0.05$.Conclusively, the studyshowedthatPlasmodiasiselevates ALPlevelsinthebody andthiscan resulttoabone disorderor intrinsic liver diseasesand we hereby recommendedthatfurtherresearch shouldbe carriedoutonthe activitiesof Plasmodiasis,itstreatmentandroutine diagnosis in relation to transferase enzyme.

1.1 Backgroundofthe Study

*Plasmodiasis*isacontagiousillness thataffectsbothhumansandotheranimals andisspread by infectedmosquitobites.*Plasmodiumfalciparum*ismostly responsibleforsevere illness,but *Plasmodiumvivax,Plasmodiumovale*and*Plasmodiummalariae*are oftenresponsiblefor lesser andinfrequently lethalillness.Althoughitmayalsoinfecthumans,thezoonoticmalariaparasite *Plasmodiumknowlesi*causesmalaria inmacaques.These Plasmodiumspeciesdiffer intermsof appearance,immunology,geographicdistribution,relapse pattern, incubationduration,and therapeuticresponsiveness(Askira*et al.,* 2021).

EvidencehasestablishedthatNigeriahasthehighestrateofmalariacasesinAfrica,makingitthe country mostat dangeroffailing tomeettheobjectiveftheMillenniumDevelopmentGoals (Murray 2007).InNigeria,wheretransmissionofthediseaseissteady nationwide,malariaisaserioushealthconcern.Itisoneofthe*main*causesofmortalityandaccountsfor30%ofhospital admissions.A minimumof one incidentoccursfor 50%of the populationof Nigeriaeachyear (Asrani *et al.,* 2019).

Malaria can cause headaches,fever, weariness,muscle andbackpain,nausea,vomiting,spleen enlargement,dry cough,sweating,chillsontheskin,shivery,arthralgia(jointpain),anemia, haemoglobinuria, retinaldamage,andconvulsions.Typically,symptomsstarttoshow tento fifteendaysfollowingamosquitobite.Anindividualinfected withPlasmodiumfalciparum,ifnot treatedimmediately mayhavestarthavingcomplicationswhich may resultinexperiencingmoredevastating symptomslikekidneyfailure,seizures,mental confusion,coma,anddeath(Askira*et al.,* 2021).

A*transferase*isany oneofaclassofenzymesthatcatalysethetransferofspecificfunctionalgroups(e.g.amethylorglycosylgroup) fromonemolecule(calledthe donor) toanother(called the acceptor) (Tice *et al.,* 2013). They are involved in hundreds of different biochemical pathways throughout biology, andareintegral to some oflife's most important processes.

In the liver, *transferase* areproteins that speed upchemical reactions in thebody. These chemical reactionsinclude producing bileandsubstancesthathelpthebloodclot, breaking downfoodand toxins, and fightinginfection.

Aim

This research was aimed atevaluatingtransferase enzymein patients with*plasmodium falciparum*in Rivers State.

2.0

MATERIALSAND METHODS

2.1Study Design

Thiswasacrosssectionalstudywherethesubjectswererandomlyselected.Thestudywas carried outamongpatientsattendingRiversStateU

niversity TeachingHospitalinPortHarcourt,Rivers State(RSUTH).Onehundredandfifty(150)subjectswereincludedinthisstudy.Sixty-eight(68) subjectshadmalariaandwereregardedastestgroupwhileSixty-eightothershadnomalariawere regarded as thecontrolgroup.

2.2 Study Area

Thisstudy wascarriedoutinRSUTHPortHarcourtRiversStateNigeria.RiversStateissituated intheSouth-SouthregionofNigeriawithapopulationof5,198,716according to2006census report and islocatedat coordinates4.8396° N, 6.9112° E.

2.3Sample collection andpreparation

About 5mlofvenousbloodwasasepticallycollectedfromeachindividualinvolvedinthisstudy.Each sample wascollected anddispensedintoethylenediamine tetraaceticacid(EDTA)container bottles and mixed properlyformalaria testandheparin bottles.

2.5 Examination ofmalariaparasiteusing fields stainA andFieldstainB

A thick blood smear was madeon a cleanglass slide, and and was allowed to airdried.

The slide was fixed in methanolforoneminute and allowed to air dried. Thefixed smear was later placed inField Stain A (RedStain)for 5 to 6seconds, it was washed underrunningtapwater and thesmearwas dippedinto Field Stain b(Blue Stain) for 10to 30 seconds and rinse in water. The slide was allow to air dry and examined under the microscope using X100 0bjective.

3.6.1 Determination ofAspartateAminotransferase(AST)

ASTwasquantitativelydeterminedusingthe Reitman-Frankelmethod,asmodifiedbyRandox laboratoriesLimited (UK)

Procedure

0.1mlofeachplasmasampleand 0.5mlofASTreagent1wasmixedandincubatedat37^{0}Cfor exactly30 minutes.0.5mlof reagent 2 was added, mixed and allowed tostand for exactly30minutes at 25^{0}C.

5.0mlof0.4msodiumhydroxidewasaddedandmixed.Itwasallowedtostandfor5 minutes at room temperature. Absorbanceof thesamplewas read againstsampleblank at 550nm.TheactivityofASTintheserumwasobtainedfromtheactivitytableprovidedbythe manufacturer.

3.6.2 Determination ofAlanineAminotransferase(ALT)

ALTwasquantitativelydeterminedusingthe Reitman-Frankelmethod,asmodifiedbyRandox laboratoriesLimited (UK)

Procedure

0.1mlofeachplasmasample0.5mlofALTreagent1wasmixedandincubatedat37^{0}Cfor exactly30minutes 0.5mlof reagent 2 was added, mixed andallowed to stand for exactly30minutes at 25^{0}C5.0mlof0.4msodiumhydroxidewasaddedandmixed.Itwasallowedtostandfor5 minutes at room temperature. Absorbanceof thesamplewas read againstsampleblank at 550nm.TheactivityofALTintheserumwasobtainedfromtheactivitytableprovidedbythemanufacturer. Theresults wererecorded.

3.6.3 Determination ofAlkalinePhosphatase(ALP)

ALP was quantitativelydetermined usingthe colorimetricmethod, as modified byAgappeLaboratories

Procedure

	Blank	Standard	Sample
Doubledistilled water(µL)0.1mg/ml phenol	50	-	-
standard	-	50	-
Sample(µL)	-	-	50
Reagent 1 (µL)	500	500	500
Reagent 2 (µL)	500	500	500

Themixturewas mixedwelland incubatedat 37^{0}C for20 minutes

Reagent 3 (µL)	1500	1500	1500

Measurementwas madespectrophotometricallyata550nm wavelength

Calculation

Concentration ofALP = Absorbanceof Test x Concentration of Standard
Absorbanceof Standard

Statistical analysis

Allvalueswere expressedasMean±standarderrorofmean(SEM).Thedifferenceswere compared usingone-wayanalysisof variance(ANOVA)followed bystudent test.

RESULTS

4.1 Demographicscharacteristicsofthepartici pants

The socialdemographicsinformationofthe respondentswasstudied.Atotalofone hundredandfifty participants(150)whohadconsentedandwererecruitedinthisstudy, tookpartbycorrectlyfillingthequestionnaires andtheyalsosuccessfullyproducedwhole blood samplesfordetection ofplasmodiasis.

Ageofrespondents

Theparticipantsinthisstudywerebetweenth eagesof20and45yearsoldandtheage was stratified into nine(5) agegroups, namely;20-25years, 26-30years, 31-35years,36-40yearsand41-45years.Outof the 150participantsinthisstudy,thosewhowere inthe age group between20-25yearsand36–40yearshadthe lowernumbersof participants of eight(8)andthree(3) respectively.Those participantswhowere inthe agegroup betweentheagesof 26to 30years hadthe highest number ofmemberswho participated inthestudy atSeventy-four(74),asshowninTable 4.1;representationofagesofthe participants.

Table4.1:DemographicRepresentationofAges of theParticipants

Age(years)	Male(Freq)	Female(Freq)	Total(Freq)	Percentage(%)
20-25	3	5	8	5.3
26-30	32	52	84	56
31-35	29	16	45	30
36-40	7	3	10	6.7
41-45	3	0	3	2

4.2 Demography ofinfected Male and female subjects with plasmodiasis.

Thestudyresultsshowedthat,thoseparticipantswhowerebetweentheagesof26-30years hadthehighestnumberofpositivecasesof*plasmodiasis*infection,whichwas forty two(42) cases,translatingto52.0%,whiletheparticipantsbetweentheagesof20-25years and41-45yearshadthelowestnumberofpositivecases *plasmodiasis*infection, which was fourcases each translatingto 5%. Table4.2belowshowstheprevalence of*plasmodiasis*infectionbasedonvariousagegroupsofthe participants.

4.2 Demography ofinfected Male and female subjects with plasmodiasis.

Age(Group)	Male	Female	Total	Percentage(%)
20-25	2	2	4	5
26-30	17	25	42	52
31-35	14	11	25	31.3
36-40	4	1	5	6.3
40-45	3	1	4	5

4.3 Transferase enzymeinMale patients with*Plasmodiasis*.

Table4.3showsthemeanandstandarddeviationratingsamongthestudygroup(Male).Themean ±standarddeviation were (88.77±13.03),(45.10±5.36),(45.21±6.56)and 48.26±5.81for ALP, ALT,ASTandGGTrespectively forthepositively infectedgroup,whilethemean±standard deviationfor the negative group were(59.68±6.95), (33.65±5.07), (32.50±4.24) and 37.15±5.51 were ALP, ALT,AST and GGT.When the malegroupforthe fourparameters (GGT, AST, ALT andALP)werecompared,therewasnostatisticalsignificanceatP=0.7568andP=0.7441forGGT and ALTrespectively because P>0.05.WhileASTand ALPhadP=0.0114andP=0.001 respectively, showingastatistical significanceatP<0.05

Table 4.3 Transferase enzymeinMale patients with*Plasmodiasis*

Groups	ALP	ALT	AST	GGT
Infected	88.77±13.03	45.10±5.36	45.21±6.56	48.26±5.81
Non-infected P-	59.68±6.95	33.65±5.07	32.50±4.24	37.15±5.51

Value Remark	0.001	0.7441	0.0114	0.7568
	S	NS	S	NS

4.4 Transferase enzymeinFemale patients with*Plasmodiasis*.

Table4.3showsthemeanandstandarddeviationratingsamong thestudy group(Female).The mean ± standard deviation were(74.89±12.94),(40.77±5.96),(40.94±6.86)and (43.56±7.51)for ALP,ALT,ASTandGGTrespectivelyforthepositively infectedgroup,whilethemean± standarddeviationforthenegativegroupwere(52.43±8.69),(32.13±6.71),(29.54±6.65)and 37.47±8.11were ALP,ALT,ASTandGGTrespectively.Whenthefemalegroupforthefour parameters(GGT,AST, ALTandALP)werecompared,there wasnostatisticalsignificance for ALTatP=0.478becauseP>0.05.WhileGGT,AST,andALPhadP-valuesof0.0012,0.001and0.023 respectively, showingastatistical significance atP<0.05

Table 4.4 Transferase enzymeinFemale patients with*Plasmodiasis*

Groups	ALP	ALT	AST	GGT
Infected	74.89±12.94	40.77±5.96	40.94±6.86	43.56±7.51
Non-infected P-	52.43±8.69	32.13±6.71	29.54±6.65	37.47±8.11
Value Remark	0.023	0.478	0.001	0.0012
	S	NS	S	S

DISCUSSIONCONCLUSION ANDRECOMMENDATION

5.1 Discussion

Theresultsofthisstudyshowedtheevaluation oftransferaseenzymeinpatients with*plasmodiasis*in Rivers StateTeaching Hospital.Atotal of 150 participantswere recruitedforthisstudy inPortHarcourtlocatedwithinadeveloping country,Nigeria, theresultsprovethephenomenonthattheoccurrenceof*plasmodiasis*infectionisusually high on developingcountries.

In this study,Table4.3 and 4.4 showed the occurrenceof transferase enzyme in male and female subjectsrespectively.Therewasa statisticalsignificance inALPandASTinbothgenders.Also, ALTvaluesinbothgendersaswellwere statisticallysignificant.However,GGTinMalewasnot significant butGGT of thefemalegender was significant.

Theresultsreportedinthisstudy showsomesignificantincreasesinactivitiesenzymesaspartate transaminase(AST),alaninetransaminase(ALT),andalkalinephosphatase(ALP)amongpatients infectedwith *plasmodiasis* ascompared to that ofthecontrol subjects. Thesefindings are consistentwith otherstudies whichreported thatmajorityof thepatients show elevation in serumactivitiesof hepatic enzymes,(transaminases),whichare thebiomarkersof liver disordersand thereforeindicatingliverdamage(Oyewole *et al.,*2010; Onyesomand Onyemakono, 2011).

The increasedserumlevelsofhepaticenzymes,transaminases(ASTandALT),and ALParethebiomarkersofliverdisorders.Our resultsareconsistentwithotherstudieswhichreportedthat majorityofthepatientsshowelevationinserumactivities(AST, ALT,andALP)indicatingliver damage (Oyewole*etal*.,2010).The increasesinserumlevelof hepatic enzymes,transaminases (SGOTandSGPT),andalkalinephosphatasearethemarkersofliverdamage(Ignatius*etal*.,2008)reportedthattheliver enzymesleakage andbilirubinincreasedwithincrease inmalaria parasitedensity.

Our reportalsoshowedthatthere wasa significantelevationof theALPenzymesonthoseof the infectedindividualswithPlasmodiasiswhen comparedtothenon-infectedindividualsinbothmale andfemalesubjects.ThisshowsthatPlasmodiasiselevates ALPlevelsinthebody andthiscan resulttoabone disorderor intrinsic liver diseases,includingmetastaticliver diseaseandthe cholestaticautoimmunediseases,primarybiliarycirrhosisandprimarysclerosingcholangitis.All these are associated with elevatedalkaline phosphataselevel(Verma& Gorard 2012).

Ourfindingsfurtherrevealedthattherewasnosignificantdifferenceobservedfortheinfectedand non-infectedmaleandfemalesubjects.Thismeansthatplasmodiasisdoesn'taffectALTlevelsin infectedindividuals.However,thesefindings wereincontrary tothereportsofAl-Salahy*etal.,* (2016)whoreportedthatmalaria-inducedhepatocyteinjury may manifestsignificantelevated serumlevelenzymesofaspartatetransaminase(AST), alaninetransaminase (ALT),andalkaline phosphatase(ALP).

5.2 Conclusion

Thisstudy confirmedthepreviousliteraturesthatproved*Plasmodiasis*affectstheliver,thus resultingto possibleliverinjuryand if not moderated can lead to theraisein liver enzymes levels which might causevarious liver diseases or heartrelated issues.

5.3 Recommendation

Inviewofthefindingsobtainedinthisresearch, itishereby recommendedthatfurtherresearch shouldbe carriedoutonthe activitiesof Plasmodiasis,itstreatmentandroutine diagnosis.The futureresearchshouldbefocusedontheacti

vitiesofPlasmodiasis,thedrugsusedforitstreatment and theirpossible healthimplications in the various organs of thebodyespeciallythe brain.

5.4 Contribution to Knowledge

Theneedforinclusionofbiochemicalinvestigationsinplasmodiasisinfectiondiagnosis,treatment monitoringandcareisvery important,asthisisnecessary forearlyrecognitionofcomplication associatedwithacutemalariainfectionthat may requiremosturgentintensivecare toavoid mortalityduetocomplications. Thiswillenhancethedeterminationofseverityofmalariadisease atdiagnosisandaswellreducedelay inmedicalinterventionrequiredtoavoidmortality arising from malariacomplications

DISCLAIMER

All products used for this research are commonly used products in our area of research and country. There is no conflict of interest between the authors and producers of the products used becausewe intend to use the products only for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Individuals recruited as participants in this study were those who gave verbalinformed consent. Ethical approval was obtained from Rivers State Ministry of Health, Port Harcourt.

CONFLICTING INTERESTS

Authors have declared that there was no conflicting interests.

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