Int J Pharma Res Health Sci. 2017; 5 (2): 1659-1663
DOI:10.21276/ijprhs.2017.02.11

CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

# Plasma Levels of Haptoglobin, Complement C3 and C4 in Plasmodium Infected Children Presenting with Hypoglycaemia

Mathew Folaranmi Olaniyan

Department of Medical Science, Achievers University, Owo, Nigeria.

ARTICLE INFO

Received: 21 Mar 2017
Accepted: 05 April 2017

ABSTRACT

Haptoglobin (Hp) is an acute phase protein and also an immune parameter produced by the liver which binds free haemoglobin in the blood. Complement 3 (C3) and complement 4 (C4) are innate immune materials but could be involved in adaptive immunity. They protect the body from the invasion of pathogens by being a trigger of phagocytosis and opsonization. Low blood glucose indicates hypoglycaemia which has been associated with Plasmodium infection in children. Liver cells producing Hp, C3 and C4 are involved in the pathophysiology of Plasmodium infection and glucose metabolism. **Aim and Objective:** This work was therefore designed to determine plasma levels of Haptoglobin, Complement C3 and C4 in Plasmodium infected children presenting with hypoglycemia. **Materials and Methods:** Twenty six (26) Plasmodium Infected children with hypoglycaemia, Thirty (30) Plasmodium Infected children with normal blood glucose level and Fifty (50) Plasmodium NON- Infected children with normal blood glucose aged 5- 9 years were recruited as test and control subjects respectively. Anti-HIV, anti-HCV and HBsAg, plasma Hp, C3 and C4 were determined in all the subjects by ELISA technique while plasma glucose was determined spectrophotometrically. Children who were seronegative to HIV, HCV and HBV were studied. **Results:** Results obtained showed a significantly lower plasma value of C3 in Plasmodium infected children with hypoglycaemia than the control children free of Plasmodium infection and had normal glucose level with $p<0.05$. There was a significantly lower plasma value of Hp, C3 and C4 in plasmodium infected children with hypoglycaemia than plasmodium infected children with normal blood glucose and plasmodium non-infected children with normal plasma glucose level with $p<0.05$. There was also a significant decrease in the plasma value of Hp, C3 and C4 in plasmodium infected children with normal blood glucose compared with the children not infected with plasmodium having normal plasma glucose level with $p<0.05$. There was a significantly lower plasma value of glucose in plasmodium infected children with hypoglycaemia than plasmodium infected children with normal blood glucose and children not infected with plasmodium having normal plasma glucose level with $p<0.05$.**Conclusion:** Decrease in Hp, C3 and C4 was obtained in Plasmodium infected children and was more intense in hypoglycemic plasmodium infected children.

**Keywords:**Plasma, Haptoglobin, C3, C4, Plasmodium. Children. Hypoglycaemia.

## 1. INTRODUCTION

Complement C3 and C4 are innate immune materials but could interplay between adaptive and innate immunity [1]. Their major functions include: opsonization, lysis and

**Corresponding author ***
Mathew Folaranmi Olaniyan
Department of Medical Science, Achievers University, Owo, Nigeria
E-mail: olaniyanmat@yahoo.com

clumping of target cells and chemotaxis. Complement is a complex biological system which works in conjunction with antibodies to protect the body from invasion of pathogens [2]. Upon activation by classical or alternative pathway , complement acts on biological membranes and may cause cell death [3]. Complement C3 and Complement C4 levels are important in determining inherited or acquired deficiencies. The complement system has the potential to be extremely damaging to host tissues [4].

Complement triggers phagocytosis by opsonizing antigens; inflammation by attracting macrophages and neutrophils and attack on membrane by rupturing membranes of foreign cells. Most of the proteins and glycoproteins that constitute the complement system are synthesized by the liver cells though significant amounts are also produced by tissue macrophages, blood monocytes, and epithelial cells of the genitourinal tract and gastrointestinal tract [2, 5, 6].

Haptoglobin binds hemoglobin, inhibiting microbe iron uptake. Complement factors complement factors are positive acute phase proteins. Haptoglobin ( Hp) in humans is encoded by the HP gene [7, 8]. In blood plasma, haptoglobin binds free hemoglobin (Hb) released from erythrocytes with high affinity and thereby inhibits its oxidative activity that can cause cellular damage Haptoglobin is produced mostly by liver cells but also by other tissues which include skin, lung and kidney [7, 8].

Glucose is the primary source of energy for the body. The body obtains glucose through the digestion of carbohydrates. Glucose is vital and interacts with the digestive and endocrine system. Due to this Hypoglycaemia has been reported Plasmodium infected children [9, 10].

This work was designed to determine plasma levels of Haptoglobin, Complement C3 and C4 in Plasmodium infected children presenting with hypoglycemia.

## 2. MATERIALS AND METHODS

### Materials

### Study area

This work was carried out at the Out Patient Department of Baptist Medical Centre, Saki- Nigeria. It is afaith-based teaching hospital training health professionals at tertiary and postgraduate level in addition to providing healthcare services.

### Study Population

Twenty six (26) Plasmodium Infected children with hypoglycaemia, Thirty (30) Plasmodium Infected children with normal blood glucose level and Fifty (50) Plasmodium NON- Infected children with normal blood glucose aged 5-9 years were recruited as test and control subjects respectively.

### Biological sample

Venous blood sample was collected into Fluoride-Oxalate anti-coagulated bottle for measurement of blood glucose and Lithium heparinized bottle for Haptoglobin and complement assay. The plasma was extracted through by centrifugation.

### Research Design

Case control-observational and cross sessional

### Assay Methods

### Glucose Assay usingRandox kit

**Principle:** Glucose in the plasma is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase and hydrogen peroxide in the presence of peroxidase enzyme is converted to water and nascent oxygen. The nascent oxygen in turn react with 4-antipyrine to give a pink coloured complex measured at 500nm on spectrophotometer. The intensity of the coloured complex is directly proportional to the concentration of glucose in the plasma.

### Human Haptoglobin ELISA test using abcam kit

**Principle:** A Haptoglobin specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently biotinylated Haptoglobin is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Complex is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is inversely proportional to the amount of Haptoglobin captured in plate.

### Human Complement C3 ELISA using abcam Kit

**Principle:** A Complement C3 specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a Complement C3 specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Complement C3 captured in plate.

### Human Complement C4 ELISA using abcam Kit

**Principle:** A Complement C4 specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently a Complement C4 specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of Complement C4 captured in plate.

**Anti-HIV, Anti-HCV and HBsAg test by ELISA using Bio-Rad kit**

Anti-HIV, Anti-HCV and HBsAgtests were carried out by ELISA using Bio-Rad kit. The manufacturers instruction was followed strictly.

**Ethical Consideration**

The proposal of this woek was reviewed and approved by the Research and Ethical Committee of Baptist Medical Centre, Saki-Nigeria before the commencement of the work. All parents of the subjects conbcent and volunteered their children for the work

**Competing Interest**

There is no competing interest

**Method of Data analysis**

The plasma value of the parameters obtained was subjected to statistical analysis using SPSS 18.0 to determine level of significance of the differences at 0.05.

## 3. RESULTS

The results obtained showed a significantly lower plasma value of C3 in Plasmodium infected children with hypoglycaemia than the control children free of Plasmodium infection with normal glucose level with $p<0.05$(Table 1,2 and Figure 1). There was a significantly lower plasma value of Hp, C3 and C4 in plasmodium infected children with hypoglycaemia than plasmodium infected children with normal blood glucose and plasmodium non-infected children with normal plasma glucose level with $p<0.05$(Table 1,2 and Figure 1). There was also a significant decrease in the plasma value of Hp, C3 and C4 in plasmodium infected children with normal blood glucose compared with the children not infected with plasmodium having normal plasma glucose level with $p<0.05$(Table 1,2 and Figure 1). There was a significantly lower plasma value of glucose in plasmodium infected children with hypoglycaemia than plasmodium infected children with normal blood glucose and children not infected with plasmodium having normal plasma glucose level with $p<0.05$(Table 1,2 and Figure 1).

However, there was no significant difference in the plasma value of C3 in plasmodium infected children with normal blood glucose level compared with plasmodium infected children having hypoglycaemia and plasmodium non-infected children with normal glucose level with $p>0.05$(Table 1,2 and Figure 1).

Also, there was no significant difference in the plasma value of glucose in plasmodium infected children with normal blood glucose level compared with plasmodium non-infected children with normal glucose level with $p>0.05$(Table 1,2 and Figure 1).

**Table 1: Mean and Standard deviation of the blood plasma values of C3, C4, Hp and Glucose obtained in the subjects**

| | Plasmodium Infected children with hypoglycaemia (n=26) | Plasmodium Infected children with normal glucose level (n= 30) | Plasmodium NON-Infected children with normal glucose level (n=50) |
|---|---|---|---|
| **C3 (mg/dl )** | 46±5.0 | 55±6,0 | 70±5.0 |
| **C4 (mg/dl )** | 7.0 ±2.0 | 12 ± 1.0 | 26 ± 2.0 |
| **Hp (mg/dL)** | 23 ± 4.0 | 43 ±3.0 | 68 ± 2.0 |
| **Glucose (mg/dl)** | 55 ± 3.0 | 82 ± 3.0 | 86 ± 4.0 |
| **Anti=HIV** | Neg | Neg | Neg |
| **Anti=HCV** | Neg | Neg | Neg |
| **HBsAg** | Neg | Neg | Neg |

**Table 2: Comparative analysis of the blood plasma values of C3, C4, Hp and Glucose obtained in the subjects**

| | | Plasmodium Infected children with hypoglycaemia VS Plasmodium Infected children with normal glucose level | Plasmodium Infected children with hypoglycaemia VS Plasmodium NON-Infected children with normal glucose level | Plasmodium Infected children with normal glucose level VS Plasmodium NON-Infected children with normal glucose level |
|---|---|---|---|---|
| **C3 (mg/dl )** | 't' | -1.15233 | -3.39411 | -1.92055 |
| | 'p' | 0.184 | 0.04* | 0.097 |
| **C4 (mg/dl )** | 't' | -4.9029. | -9.45285 | -6.26099 |
| | 'p' | 0.02* | 0.006** | 0.012* |
| **Hp (mg/dL)** | 't' | -4 | -10.06231 | -6.93375 |
| | 'p' | 0.03* | 0.005** | 0.010* |
| **Glucose (mg/dl)** | 't' | -6.36396 | -6.2 | -0.8 |
| | 'p' | 0.012* | 0.013* | 0.25 |

*Significant

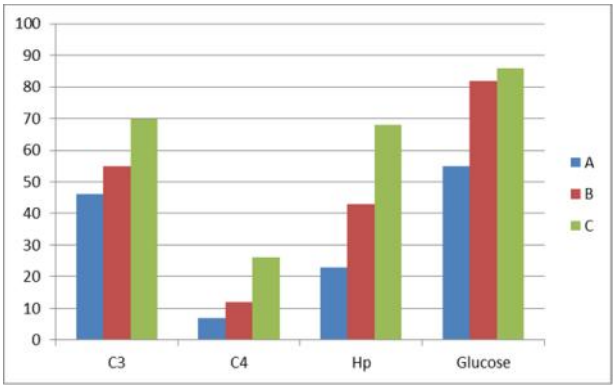


**Fig 1: Comparative description of the blood plasma values of C3, C4, Hp and Glucose obtained in the subjects**

**Note:**

**A-** Plasmodium Infected children with hypoglycaemia (n=26)
**B-** Plasmodium Infected children with normal glucose level (n= 30)
**C-** Plasmodium NON- Infected children with normal glucose level (n=50)

## 4. DISCUSSION

Results obtained showed a significantly lower plasma value of C3 in Plasmodium infected children with hypoglycaemia than the control children free of Plasmodium infection and had normal glucose level. There was a significantly lower plasma value of Hp, C3 and C4 in plasmodium infected children with hypoglycaemia than plasmodium infected children with normal blood glucose and children not infected with plasmodium and with normal plasma glucose level. There was also a significant decrease in the plasma value of Hp, C3 and C4 in plasmodium infected children with normal blood glucose compared with the children not infected with plasmodium having normalplasma glucose level.

Significant decrease in the plasma value of Hp, C3 and C4 in plasmodium infected children could be an indication of liver inflammation as a result of the pathophysiology of plasmodium infection which involves liver cells which could affect the normal physiological functions of the liver cells which include synthesis of Hp, C3 and C4 thereby diminishing their plasma level. Decrease in plasma Hp, C3 and C4 in the plasmodium infected children could also be due to the fact that the parameters are immune materials that fight against invading pathogens and are also work with antibodies to provide adaptive immunity. These parameters could be excessively used in plasmodium infection to provide immunity [2, 5-8].

Low plasma haptoglobin may be as a result of destruction of red blood cells in plasmodium infection leading to release and accumulation of free haemoglobin, as a result more haptoglobin will be utilized to bind with the free haemoglobin thereby depleting the plasma level of Haptoglobin [7, 8].

Decrease in C3 and C4 in plasmodium infection has been reported by Phanuphak*et al.,* [11].which is consistent with the findings of this work.

There was a significantly lower plasma value of glucose in plasmodium infected children with hypoglycaemia than plasmodium infected children with normal blood glucose and children not infected with plasmodium having normalplasma glucose level.

Hypoglycaemia is a complication of plasmodium infection possibly because of the excessive metabolism of glucose to provide energy as a result of plasmodium infection and has also been reported by other authors [9-10]. Again, glucose is readily utilized by cells of the immune system and is used to generate energy and biosynthetic precursors [12].. Activation of immune cells for body to respond to the invasion of Plasmodium is associated with increased glucose utilization and this is facilitated, in part, by increased expression of glucose transporterswhich could cause hypoglycaemia [12]. Both hypo- and hyperglycaemia impair immune-cell functions and promote inflammatory responses. Excessive lowering of blood glucose concentration may also be harmful to the immune response [12].

## 5. CONCLUSION

This work has been used to determine Plasma levels of Haptoglobin, Complement C3 and C4 in Plasmodium infected children presenting with hypoglycemia which revealed a decrease in haptoglobin, C3 and C4 in plasmodium infected children with either hypoglycaemia or normal plasma glucose level and was found to be more intense in plasmodium infected children with hypoglycaemia.

## 6. RECOMMENDATION

Routine evaluation of plasma glucose, haptoglobin, C3 and C4 in children infected with plasmodium is recommended for effective management.

## 7. REFERENCES


1. Murphy, Kenneth; Weaver, Casey. "Innate Immunity: the First Lines of Defense". Janeway's Immunobiology (9th ed.). Garland Science.2017; p. 49.
2. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology (6th ed.). Elsevier. 2010; pp. 272–288..
3. Nesargikar PN, Spiller B, Chavez R. "The complement system: history, pathways, cascade and inhibitors". European Journal of Microbiology & Immunology.2012; 2 (2): 103–11.
4. Chaplin H. "Review: the burgeoning history of the complement system 1888–2005". Immunohematology / American Red Cross. 2005; 21 (3): 85–93.
5. Klos, A.; Wende, E.; Wareham, K. J.; Monk, P. N. "International Union of Pharmacology. LXXXVII. Complement Peptide C5a, C4a, and C3a Receptors". Pharmacological Reviews. 2013; 65 (1): 500–43.
6. Phillips, C.M., and Perry, I.J. Does inflammation determine metabolic health status in obese and nonobese adults? J.Clin. Endocrinol. Metab. 2013.; 98: E1610-E1619
7. DobryszyckaW . "Biological functions of haptoglobin--new pieces to an old puzzle". Eur J ClinChemClinBiochem. 1997; 35 (9): 647–54.
8. Wassell J. "Haptoglobin: function and polymorphism". Clin. Lab. 2000; 46 (11–12): 547–52.
9. Olaniyan, M.F. (2005) The pattern of packed cell volume, plasma electrolytes and glucose levels in patients infected with Plasmodium falciparum Afr. J. ClinExper. Microbiol. 2005; 6(2):87-90.
10. Alphonsus N. Onyiriuka, Olasimbo O. Peter, Louis C. Onyiriuka, Patience O. Awaebe, Fidelis U. Onyiriuka Point-of-admission hypoglycaemia among under-five Nigerian children with plasmodium falciparum malaria: Prevalence and risk factors Aristotle University Medical Journal, 2012Vol. 39, Issue 2.
11. Phanuphak P, MattanaHanvanich, ReutaiSakulramrung, Moollaor .P, Sitprija . V & Phanthumkosoli .D. Complement changes in falciparum malaria infection Clin. exp. Immunol. 1985; 59, 571-576.

12. Calder PC, Dimitriadis G, Newsholme P. Glucose metabolism in lymphoid and inflammatory cells and tissues. Curr Opin Clin Nutr Metab Care. 2007 ;10(4):531-40.

**Conflict of Interest: None**

**Source of Funding: Nil**