ELSEVIER

Clinica Chimica Acta xx (2004) xxx–xxx

www.elsevier.com/locate/clinchim

Hyperhomocysteinemia in acute *Plasmodium falciparum* malaria: an effect of host–parasite interaction

R. Chillemi[a], B. Zappacosta[b], J. Simporè[c], S. Persichilli[b], M. Musumeci[d], S. Musumeci[e,*]

[a] *Department of Chemical Sciences, University of Catania, Catania, Italy*
[b] *Department of Clinical Chemistry, Catholic University of Rome, Rome, Italy*
[c] *Centre Medical Saint Camille, Ouagadougou, Burkina Faso*
[d] *Department of Biomedical Sciences, University of Catania, Italy*
[e] *Department of Pharmacology, Gynecology and Obstetrics, Paediatrics, University of Sassari and Institute of Population Genetics, CNR, Alghero (SS), Italy*

Received 9 January 2004; received in revised form 4 May 2004; accepted 5 May 2004

Abstract

Background: *Plasmodium falciparum* utilises the polyamine pathway, essential in proliferation and differentiation, and imposes an oxidative stress on host cell, enhancing the loss of glutathione. *Methods*: Standard hematological parameters were determined in 40 black African subjects with acute *P. falciparum* malaria, 30 aged 5–24 months, 5 aged 4–10 years and 5 aged 19–35 years. Plasma homocysteine, cysteine, glutathione and cysteinylglycine levels were measured by HPLC method. Twenty-eight healthy black children (15 aged 6–24 months and 13 aged 3–10 years) and 20 healthy black adults (aged 20–40 years) were also included as controls. *Results*: Plasma homocysteine levels were higher in all subjects with *P. falciparum* malaria and correlated positively with the disease severity and number of parasites, but negatively with Hb levels and patient ages. Cysteine level was found higher in all patients and markedly higher in 4–10 year old patients. Cysteinylglycine level was found lower particularly in 19–35 year old patients. Glutathione level was significantly lower in all patients. *Conclusions*: The elevated level of homocysteine during acute *P. falciparum* infection suggests an imbalance in the folate cycle, which could be a consequence of the reduced availability of NADPH and Vit B_{12}, caused by increased oxidative stress. This may suggest a selection for the C677T MTHFR allele, driven by *P. falciparum* in sub-Saharan regions. Hence Hcy level could be useful as a predictive parameter of severity, as well as of treatment efficacy.

Keywords: Hyperhomocysteinemia; *Plasmodium falciparum* malaria

Abbreviations: Hcy, homocysteine; SAM, *S*-adenosylmethionine; SAH, *S*-adenosyl homocysteine; CBS, cystathionine beta synthase; ODC, ornithine decarboxylase; AdoMetDC, *S*-adenosylmethionine decarboxylase; SAHH, *S*-adenosylhomocysteine hydrolase; MTHFR, methylentetrahydrofolate reductase; Hb, haemoglobin; HPLC, high-pressure liquid chromatography; CMSC, Centre Medical Saint Camille.

* Corresponding author. Cattedra di Pediatria Sociale e Puericultura, Università di Sassari, Viale San Pietro 12, 01700 Sassari, Italy. Tel.: +39-360-285505; fax: +39-95-7179690.

E-mail address: smusumeci@tiscalinet.it (S. Musumeci).

0009-8981/$ - see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.cccn.2004.05.007

1. Introduction

Homocysteine (Hcy) is a sulphur-containing amino acid that it is not found in structural proteins. The biological role of Hcy has been extensively studied in human [1]. In the methionine cycle, Hcy constitutes the intersection of two pathways, known as remethylation and trans-sulphuration [2].

In remethylation, Hcy acquires a methyl group from N^5-methyltetrahydrofolate or, alternatively, from betaine, to generate methionine (Fig. 1). Methionine synthase, which requires vitamin B_{12} as a cofactor, catalyses the reaction with N^5-methyltetrahydrofolate, while the reaction with betaine is catalysed by the enzyme betaine-homocysteine methyltransferase, which is vitamin B_{12}-indepen-

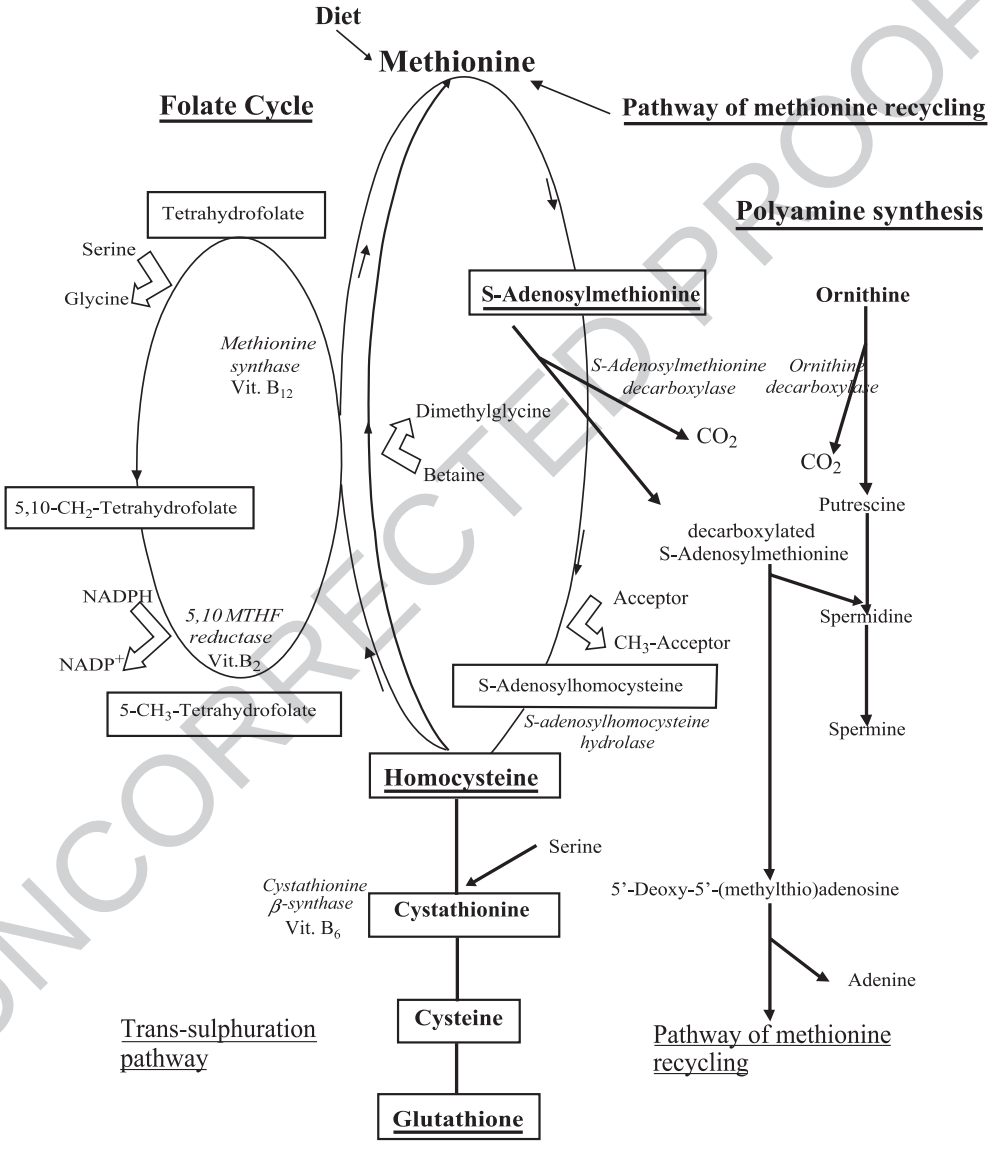

Fig. 1. Homocysteine and polyamine pathways in *P. falciparum* malaria.

dent. A considerable amount of methionine is then activated by ATP to form *S*-adenosylmethionine (SAM), which represents a universal methyl donor to a variety of DNA, RNA, protein, phospholipid and neuromediator substrates. *S*-Adenosylhomocysteine (SAH), produced in these transmethylation reactions, is hydrolysed to generate Hcy, which becomes available to start a new cycle. Since the hydrolysis is a reversible reaction that favours the synthesis of SAH, elevated levels of Hcy are always associated with elevated SAH concentrations [3].

In the trans-sulphuration pathway (Fig. 1), the cystathionine beta-synthase (CBS) mediates the condensation of Hcy with serine to form cystathionine, which is then hydrolyzed to cysteine. This pathway provides an effective route to transform the excess Hcy that can be utilised for glutathione synthesis.

The methionine metabolism has, through SAM, a link to the metabolism of polyamines, ubiquitous compounds, which play an important role in cell proliferation and differentiation. In fact SAM, by a methyl group transfer, generates SAH. In an alternative pathway, SAM undergoes decarboxylation and then, transferring an aminopropyl residue to putrescine, gives rise to spermidine and then spermine (Fig. 1). Ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC) are the enzymes involved in the above-mentioned decarboxylation processes and are the rate-limiting enzymes in the polyamine biosynthesis [4].

The complexity of the Hcy and methionine metabolic pathways and the perfect balance required for the correct function of this metabolic cycle may be strongly influenced by the presence of *Plasmodium falciparum* malaria. In fact, the parasite utilises the polyamine metabolism, which is essential for its growth, and causes drastic changes in the glutathione level of its host cell [5].

The aim of this study was to investigate if *P. falciparum* malaria affects the levels of plasma Hcy and of other three thioles (cysteine, cysteinylglycine and glutathione) and if the levels of plasma thioles correlate with the severity of the malaria infection.

2. Materials and methods

2.1. Place of study

The study was made in the city of Ouagadougou (Burkina Faso), which is situated in a meso-endemic area of *P. falciparum* malaria, at the centre of the country, with an intense seasonable occurrence of malaria, from July to October. In this country, *P. falciparum* malaria is a major cause of morbidity and mortality in children <5 years (38.5%, all causes). Hyperendemic, perennial transmission peaking from August to October (>90% *P. falciparum*, 1% *P. malariae*). Primary malaria vectors are *A. gambiae* and *A. funestus* (150 infective bites/year). Children with *P. falciparum* malaria and control subjects were evaluated and enrolled at the local Centre Medical Saint Camille (CMSC) of Ouagadougou, the capital of Burkina Faso. The protocol of this study was reviewed and approved by the Ethic Committee of the CMSC. Informed consent was obtained from parents of study participants, if younger than 10 years or from study participants themselves.

2.2. Diagnosis and classification of cases

Thirty children affected by acute malaria, aged 5–24 months, 5 boys, affected by acute malaria, aged 4–10 years, 5 adults, affected by acute malaria, aged 19–35 years were enrolled during the month of October 2002 at the CMSC of Ouagadougou, 18 were males and 22 females. Inclusion and classification of each patient were based on the symptoms, physical signs and laboratory findings of malaria at the time of the first presentation.

Severe malaria (complicated) was established by microscopic confirmation of asexual parasites in the peripheral blood and at least one of the following clinical and physical signs, according to the WHO criteria: evidence of neurological involvement (prostration, lethargy), gastrointestinal symptoms, severe anemia (Ht $<20\%$, Hb <6 g/dl), hyperparasitemia corresponding to $E_p > 2.5 \times 10^5$ or >2.5% in non-immune subjects, hypoglycemia (serum glucose less than 2.2 mmol/l corresponding to 40 mg/dl), acidosis with respiratory distress, oliguria, cardiovascular shock, jaundice, diffuse hemorrhages.

Mild malaria (uncomplicated) was established by microscopy confirmed parasitemia of $E_p < 2.5 \times 10^5$ or <2.5% with fever, headache, myalgias, without any finding of severe malaria.

Patients with mild malaria were treated with chloroquine at the dosage of 10 mg/kg for 2 days, then 5 mg/kg for another day. Patients with severe malaria were placed under intravenous treatment with glucose 5%, including quinine dichlorhydrate at the dosage 20 mg/kg in 4 h as charge dose then, after an interval of 4 h of saline infusion, the dosage was reduced at 10 mg/kg for 2 cycles with an interval of 4 h with saline, according to the official therapeutic protocol of local "Programme National de Lutte contre le Paludisme" (PNLP). At the end, a treatment with quinine for 7 days or chloroquine for 3 days was started.

Twenty-eight healthy black children (15 aged 6–24 months and 13 aged 3–10 years) and 20 healthy black adults (aged 20–40 years) were also included as controls.

2.3. Methods

Standard hematological parameters were determined in each patient. For parasitemia quantification blood smears, stained by standard May Grunwald Giemsa, were observed, whereas tick-smear technique was preliminarily used for the diagnosis of malaria. The blood samples were collected in sterile tubes containing EDTA. All the samples were centrifuged and the plasma were frozen at −40 °C and then sent to the Department of Clinical Biochemistry, Catholic University of Rome, Italy, for the determination of plasma Hcy, cysteine, cysteinylglycine and glutathione. HPLC method separates in the same run all thioles [6], giving simultaneous information on Hcy metabolism and trans-sulphuration route.

2.4. Statistics

Data were presented as mean ± standard deviation. Statistical comparison of Hcy and other thiol concentrations among samples of all groups were performed using paired and unpaired Student's *t*-test or Mann–Whitney test when appropriate, considering statistically significance at $P < 0.05$. All computations were made using the SPSS-10 program for Windows. Sensitivity, specificity, positive predictive value and negative predictive value of thioles as parameters to predict disease severity and outcome were calculated. A multivariate analysis was made considering severe and mild malaria or Hb as dependent variables. Age, parasitemia and thioles were considered as independent variables.

3. Results

Table 1 reports the clinical and hematological parameters of studied subjects affected by acute *P. falciparum* malaria and relative controls. Fifteen children (group 1) showed a hemoglobin level below 6 g/dl, an elevated degree of parasitemia (365,000/μl, range $2.5–5.0 \times 10^5$) and are considered affected by *severe malaria*. Other 15 children (group 2) did not show severe alteration in the haematological parameters and the disease was considered uncomplicated. The levels of Hb in this second group of children were medially comparable with those of patients belonging to the groups 3 and 4 and the parasitemia degree was below 2.5×10^5/μl in all patients. The levels of plasma Hcy were higher in all subjects (children and adults) with *P. falciparum* malaria, especially in 5–24 month old patients (group 1). These values correlate positively with the severity of disease, number of parasites and correlate negatively with the Hb levels and the ages of the subjects. There was a significant difference in the Hcy level between children with *severe* and *mild* malaria. Cysteine was higher in all patients, whereas cysteinylglycine was lower in 19–35 year old patients, compared with the data of the control groups (Table 1). Glutathione level was found significantly lower in all subjects affected by acute malaria. The correlation between Hcy and cysteine was not significant ($r^2 = 0.06$), suggesting that the trans-sulphuration is variably influenced by the presence of the parasite. The correlation between plasma Hcy level and severity of malaria was found significant at $P < 0.001$ in the 5–24 months group of patients (n. 30). However, the correlation between Hcy and severity of malaria was not calculated in the 4–10 years (n. 5) and 19–35 years (n. 5) groups because of the reduced number of patients. The correlation between Hcy and Hb was significant ($r^2 = 0.35$,

Table 1
Values of Hb, parasitemia, homocysteine and other thioles in 30 children, 5 boys and 5 adults with *P. falciparum* malaria and in relative controls according to the age

Group	Patients (*N*)	Age, median (range)	Hb (g/dl)	Parasitemia (μl)	Homocysteine (μmol/l)	Cysteine (μmol/l)	Cysteinylglycine (μmol/l)	Glutathione (μmol/l)
1	15	9.7 (5–24)^ months	4.9 ± 1.2*£	345,000* (250–500,000)	18.3 ± 8.3&*£	123.5 ± 25.9£	25.3 ± 13.5	1.5 ± 1.3&£
2	15	14.8 (6–24) months	7.4 ± 1.1&£	165,000 (100–250,000)	16.4 ± 7.3&£	132.3 ± 23.7£	26.4 ± 14.6	2.5 ± 1.2£
3	5	8.4 (4–10) years	9.5 ± 1.9£	144,000 (90–200,000)	12.3 ± 8.8$	135.6 ± 22.0£	31.9 ± 20.3	2.3 ± 1.1£
4	5	27.2 (19–35) years	11.1 ± 3.5$	176,000 (120–245,000)	16.3 ± 9.4£	122.1 ± 23.0%	21.5 ± 8.1§	1.5 ± 1.5£
5	Controls, 15	12.4 (6–24) months	12.2 ± 2.3	–	5.9 ± 1.9	95.0 ± 14.4	34.3 ± 12.9	4.2 ± 0.95
6	Controls, 13	7.0 (3–10) years	12.2 ± 2.3	–	6.5 ± 1.7	95.7 ± 12.6	35.5 ± 11.8	4.2 ± 0.79
7	Controls, 20	29.7 (20–40) years	14.2 ± 2.5	–	6.9 ± 1.3	96.3 ± 13.0	37.1 ± 10.0	4.3 ± 0.75

1 versus 2: ^$P=0.027$; *$P<0.001$; &$P=0.037$; patients versus healthy controls: £$P<0.001$; %$P=0.003$; §$P=0.004$; $$P=0.03$.

$P<0.001$) in 5–24 months children (Fig. 2). The correlation between Hcy and age and between Hcy and parasitemia was also significant ($P<0.001$) in the 5–24 months children. Notably, the value of hyperhomocysteinemia as a parameter to predict disease severity gave a sensibility of 100%, a specificity of 97%, a positive predictive value of 97%, a negative predictive value of 100%, when 5–24 months children were considered. Including all the patients in the same analysis, the sensibility decreased to 95%, the specificity to 92%, and the positive and negative predictive values to 91% and 96%, respectively. According to the multivariate analysis considering severity of malaria or Hb as dependent variable, the most sensitive parameter ($P<0.001$) to predict malaria severity was plasma Hcy.

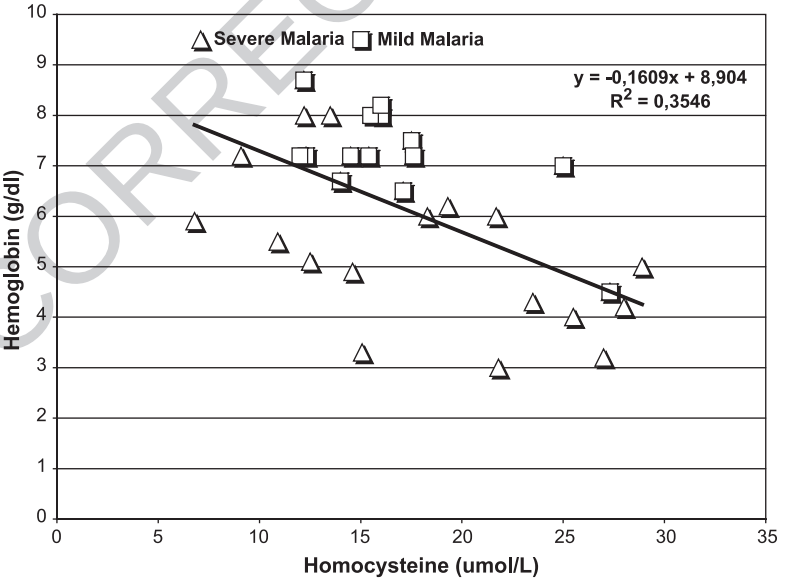

Fig. 2. Correlation between homocysteine and hemoglobin in 30 children with severe and mild malaria.

4. Discussion

The occurrence of high levels of polyamines in the *P. falciparum* or in other *Plasmodium*-infected cells is well documented in the literature [7]. In fact, HPLC analysis of the polyamines in *Plasmodium knowlesi*-infected erythrocytes versus normal erythrocytes showed a stage-dependent increment in the level of putrescine, spermidine and spermine. A significant increment of putrescine influx rate has been also documented during the trophozoite stage of malaria infection. This increment of putrescine stimulates the activity of erythrocyte AdoMetDC, activating polyamine biosynthesis [8]. Since in the presence of *P. falciparum* malaria SAM is greatly utilised in the polyamine shunt, one should expect a decrement in the Hcy level, but our results show instead increased level of Hcy. This increase could be due to the oxidative stress exerted by the *P. falciparum* infection. The inactivation of methionine synthase, as a consequence of the oxidation susceptibility of its cofactor Vit B_{12} [9] and the decreased level of NADPH, deactivate the folate cycle and then give rise to high level of Hcy (Fig. 1). The increment of Hcy, because of the reversible activity of *S*-adenosylhomocysteine hydrolase (SAHH), will result in an increase of SAH [3], which is both a product and an inhibitor of SAM-dependent transmethylations, determining a decrease of adenosine. SAM, in turn, will recycle methionine by means of the polyamine shunt, via 5′-deoxy-5′-(methylthio)adenosine (Fig. 1). On the other hand, the increase of SAM in malaria patients determines an increase of plasma cysteine, activating the trans-sulphuration pathway, mediated by CBS [10].

Although the glutathione synthesis should be increased by a higher level of Hcy, our data show a very low glutathione concentration (Table 1). On the other hand, the oxidative stress due to *P. falciparum* parasites determines a higher consumption of glutathione [5–11]. Clinical observations and experimental evidences reported in the literature show that glutathione plays a critical role for the defence of *P. falciparum* and its host cell against oxidative stress [12,13]. Other parasites such as *Giardia*, *Toxoplasma*, *Trichomonas*, etc., to protect themselves from oxidation stress, utilize, in addition to glutathione, the trypanothione, N^1,N^8-bis(glutathionyl) spermidine, produced through the polyamine route [14]. Also, *P. falciparum* could synthesize a trypanothione-like compound, to compensate the reduced level of glutathione. In agreement with this hypothesis, Luersen et al. [5] demonstrated with HPLC analysis of thioles isolated from a culture of *P. falciparum*, in addition to the cysteine and glutathione peaks, a peak of an unidentified compound, which could be a trypanothione analogue. In accord with the increased consumption of glutathione, the plasma level of cysteinylglycine, a catabolic product of glutathione, was found lowered in our patients with acute malaria.

Since the folate pathway is determinant in Hcy remethylation (Fig. 1), an inhibition of the folate cycle, caused by the mutant allele C677T of MTHFR, could always be associated with an increment of Hcy.

This mutated allele is differently distributed in various geographic areas. In black African people living in the sub-Saharan regions, the frequency of C677T polymorphism is estimated to about 6–7% [15,16]. In other parts of the world, this percentage ranges from 35% to 40% [17,18] and the difference between the population of developed and underdeveloped countries has been attributed to the folate deficiency [19]. In fact, the homozygous condition in underdeveloped countries would be associated with elevated frequency of miscarriage in the first month of pregnancy, which are responsible for the disappearance of C677T mutation. On the contrary, in developed countries the homozygous condition is associated only to defects of neural tube [20,21] and idiopathic deep venous thrombosis [22].

The interdependence between the *P. falciparum* malaria and the Hcy metabolism seems to be clearly demonstrated in this study by the elevated level of Hcy, suggesting another mechanism for the selection of C677T mutant allele. In fact, the subjects who are heterozygote for the C677T, in presence of *P. falciparum*, could show a further increase of Hcy. If we consider that the parasite replicates in the red cell full of Hcy, this increase of Hcy could interfere positively with parasite metabolism. In fact, in such condition the reverse reaction of *P. falciparum* SAHH does not determine an increase of SAH such to damage the parasite metabolism, because the activity of *P. falciparum* SAHH is more than 21-fold smaller in parasite than in human cells [23].

Therefore the heterozygote of the C677T mutant with higher Hcy level should be more disadvantaged

by the presence of *P. falciparum* and should die prematurely, while on the contrary the wild-type MTHFR allele maintains a protective role against malaria infection. This genetic characteristic (high frequency of wild-type MTHFR in African population) is sustained by the finding that the black population living in Burkina Faso shows lower levels of Hcy [24], as a consequence of selective advantage of the wild-type MTHFR gene.

This condition could result in a new balanced polymorphism, as was demonstrated for G6PDH deficiency, Hb S and Hb C, beta and alpha thalassemia in the same areas at high malaria endemia. If this is true, the study of C677T MTHFR polymorphism in different geographic areas could allow the construction of a gene frequency map of the effect that the presence of *P. falciparum* or of the folate deficiency has left in the genetic structure of underdeveloped population.

According to this model, some inhibitors of enzymes of polyamine biosynthesis, such as ODC, AdoMetDC and 5′-deoxy-5′-(methylthio)adenosine phosphorylase [25], or inhibitors of *S*-adenosylhomocysteine hydrolase [23], γ-glutamylcysteine synthase [5] and trypanothione reductase [26] would represent new therapeutic strategies against malaria infection.

In conclusion, our results suggest that in course of *P. falciparum* malaria infection, the dosage of plasma Hcy level could be introduced as a predictive parameter of severity, as well as of treatment efficacy. However, this strategy for the treatment of malaria appears suitable only in developed countries. In fact, it is difficult to introduce Hcy measurement as a marker of prognostic value in malaria in the so-called *third world countries*, which do not have the possibility of Hcy measurements. In these countries, the parasitemia and Hb measurement remain much more easy to determine, less expensive and more rapidly available.

Acknowledgements

The authors gratefully acknowledge the Department of Chemical Sciences, University of Catania, for hospitality in the late activities of the present study. Prof. Sebastiano Sciuto is similarly acknowledged for his precious suggestions and constant help in the preparation of manuscript and Dr. Michael Whalen for editing help.

References

[1] Scriver CR, Beaudet AL, Sly WS, Valle D. The Metabolic and Molecular Bases of Inherited Disease. 7th ed. New York: Mc Graw Hill International Book; 1995. p. 1279–327.

[2] Dudman NP, Guo XW, Gordon RB, Dawson PA, Wilcken DE. Human homocysteine catabolism: three major pathways and their relevance to development of arterial occlusive disease. J Nutr 1996;126(4 Suppl.):1295S–300S.

[3] Reed MC, Nijhout HF, Sparks R, Ulrich CM. A mathematical model of the methionine cycle. J Theor Biol 2004; 226(1):33–43.

[4] Sing S, Tekwani BL, Puri SK, Dutta GP, Pandey VC. Studies on polyamine metabolism during erythrocytic schizogony of *Plasmodium knowlesi*: a simian malaria. Indian J Parasitol 1991;15(2):147–51.

[5] Luersen K, Walter RD, Muller S. *Plasmodium falciparum*-infected red blood cells depend on a functional glutathione de novo synthesis attributable to an enhanced loss of glutathione. Biochem J 2000;346:545–52.

[6] Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987;422: 43–52.

[7] Krause T, Luersen K, Wrenger C, Gilberger TW, Muller S, Walter R. The ornithine decarboxylase domain of the bifunctional ornithine decarboxylase/*S*-adenosylmethionine decarboxylase of *Plasmodium falciparum*: recombinant expression and catalytic properties of two different constructs. Biochem J 2000;352(2):287–92.

[8] Sing S, Puri SK, Sing S, Srivastava R, Gupta R, Pandey VC. Characterization of simian malarial parasite (*Plasmodium knowlesi*)-induced putrescine trasport in rhesus monkey erythrocytes. A novel putrescine conjugate arrests in vitro growth of simian malarial parasite (*Plasmodium knowlesi*) and cures multidrug resistant murine malaria (*Plasmodium yoelii*) infection in vivo. J Biol Chem 1997;272(21):13506–11.

[9] Christensen B, Ueland PM. Methionine synthase inactivation by nitrous oxide during methionine loading of normal human fibroblasts. Homocysteine remethylation as determinant of enzyme inactivation and homocysteine export. J Pharmacol Exp Ther 1993;267(3):1298–303.

[10] Banerjee R, Evande R, Kabil O, Ojha S, Taoka S. Reaction mechanism and regulation of cystathionine beta-synthase. Biochim Biophys Acta 2003;1647(1–2):30–5.

[11] Becker K, Rahlfs S, Nickel C, Schirmer RH. Glutathione-functions and metabolism in the malarial parasite *Plasmodium falciparum*. Biol Chem 2003;384(4):551–66.

[12] Ginsburg H, Atamna H. Redox status of malaria-infected erythrocytes: an overview with an emphasis on unresolved problems. Parasite 1994;1:5–13.

[13] Mishra NC, Kabilan L, Sharma A. Oxidative stress and

malaria-infected erythrocytes. Indian J Malariol 1994;31(2): 77–87.
[14] Flohe L, Hecht HJ, Steinert P. Glutathione and trypanothione in parasitic hydroperoxide metabolism. Free Radic Biol Med 1999;27(9–10):966–84.
[15] Sadewa AH, Sunarti, Sutomo R, Hayashi C, Lee MJ, Ayaki H, et al. The C677T mutation in the methylenetetrahydrofolate reductase gene among the Indonesian Javanese population. Kobe J Med Sci 2002;48(5–6):137–44.
[16] Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol 2000;151(9):862–77.
[17] Shaw GM, Rosen R, Finnel RH, Wasserman CR, Lammer EJ. Maternal vitamin use, genetic variation of infant methylenetetrahydrofolate reductase and risk for spina bifida. Am J Epidemiol 1998;148:30–7.
[18] Pallaud C, Stranieri C, Sass C, Siest G, Pignatti F, Visvikis S. Candidate gene polymorphisms in cardiovascular disease: a comparative study of frequencies between a French and an Italian population. Clin Chem Lab Med 2001;39(2):146–54.
[19] Esfahani ST, Cogger EA, Caudill MA. Heterogeneity in the prevalence of methylenetetrahydrofolate reductase gene polymorphisms in women of different ethnic groups. J Am Diet Assoc 2003;103(2):200–7.
[20] Rosenberg N, Murata M, Ikeda Y, Opare-Sem O, Zivelin A, Geffen E, et al. The frequent 5,10-methylenetetrahydrofolate reductase C677T polymorphism is associated with a common haplotype in whites, Japanese, and Africans. Am J Hum Genet 2002;70(3):758–62.
[21] Van der Put NMJ, Steegers-Theunissen RP, Frosst P, Trijbels FJ, Eskes TK, van den Heuvel LP, et al. Mutated methylenetedrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995;346:1070–1.
[22] Berrut G, Ghali A, Quere I, Ternisien C, Gallois I, Roy PM, et al. A common mutation C677T in the 5,10-methyltetrahydrofolate reductase gene is associated to idiopathic deep venous thrombosis. Rev Med Interne 2003;24(9):569–76.
[23] Nakanishi M, Iwata A, Yatome C, Kitade Y. Purification and properties of recombinant *Plasmodium falciparum S*-adenosyl-L-homocysteine hydrolase. J Biochem (Tokyo) 2001; 129(1):101–5.
[24] Simpore J, Pignatelli S, Barlati S, Malaguarnera M, Musumeci S. Plasma homocysteine concentrations in a healthy population living in Burkina Faso. Curr Ther Res 2000;61(9): 659–68.
[25] Sufrin JR, Meshnick SR, Spiess AJ, Garofalo-Hannan J, Pan XQ, Bacchi CJ. Methionine recycling pathways and antimalarial drug design. Antimicrob Agents Chemother 1995; 39(11):2511–5.
[26] Li Z, Fennie MW, Ganem B, Hancock MT, Kobaslija M, Rattendi D, et al. Polyamines with *N*-(3-phenylpropyl) substituents are effective competitive inhibitors of trypanothione reductase and trypanocidal agents. Bioorg Med Chem Lett 2001;11:251–4.