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G6PD AND MTHFR GENE POLYMORPHISM: INTERACTION WITH OXIDATIVE STRESS, HB LEVELS AND RESPONSE TO IRON-FOLIC ACID + ALBENDAZOLE THERAPY IN MALNOURISHED CHILDREN

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ABSTRACT

Background: Polymorphisms in the G6PD and MTHFR genes have a significant impact on oxidative defense and folate metabolism which may alter how malnourished children respond to nutritional anemia treatment. However it is still unknown how they interact with iron-folic acid plus albendazole therapy.
Methodology: This quasi-experimental cross-sectional observational study was carried out at Tertiary Care Hospital Karachi between January and December of 2024. 150 malnourished children with anemia (Hb 11 g/dL) between the ages of 6 months and 12 years were included. Standardized iron-folic acid (3 mg/kg/day elemental iron + 1 mg folic acid) and a single dose of 400 mg of albendazole were given to the participants. Using PCR-RFLP G6PD and MTHFR C677T/A1298C polymorphisms were examined. Hemoglobin and oxidative stress markers (MDA GPx) were measured at baseline and 12 weeks.
Results: MTHFR C677T polymorphism prevalence was 52% (heterozygous: 30% homozygous: 22%) and G6PD deficiency prevalence was 28% (heterozygous: 12% homozygous: 16%). In 38. 7% of participants combined polymorphisms were found. Overall mean hemoglobin increased from 8.4 ± 1.3 to 10.8 ± 1.5 g/dL however response differed significantly by genotype: no polymorphism (3.1 ± 0.9 g/dL) MTHFR-only (2.3 ± 1.0 g/dL) G6PD-only (1.7 ± 0.8 g/dL) and combined (1.0 ± 0.7 g/dL p = 0.001). The likelihood of a poor response was 8.4 times higher in children with combined polymorphisms (OR: 8. 4 95 percent CI:3.2 - 21.9). Children with G6PD deficiency had lower GPx (14.8 ± 4.2 vs. 20.1 ± 5.3 U/mL p = 0.001) and significantly higher baseline MDA (4.6 ± 1.2 vs. 3.4 ± 0.9 nmol/mL p = 0.001).
Conclusion: The hemoglobin response to conventional anemia treatment is severely hampered by G6PD and MTHFR polymorphisms with combined polymorphisms producing the most severe impairment. In order to optimize therapeutic outcomes for malnourished children and enable genotype-specific supplementation protocols genetic screening should be incorporated into pediatric anemia management.

Keywords: G6PD, MTHFR, gene polymorphism, oxidative stress, Hb, Albendazole.

INTRODUCTION

Methylenetetrahydrofolate reductase (MTHFR) and glucose-6-phosphate dehydrogenase (G6PD) genes are important metabolic pathways that have a significant impact on children`s nutritional status oxidative defense and hematological outcomes. The pentose phosphate pathways primary enzyme G6PD produces reduced nicotinamide adenine

dinucleotide phosphate (NADPH) which keeps glutathione in its reduced state and offers crucial defense against oxidative stress in erythrocytes (1). About 400 million people worldwide suffer from varying degrees of enzyme deficiency due to the G6PD gene which is found on chromosome Xq28. Mediterranean African Asian and Middle Eastern populations are more likely to be affected (2). In contrast MTHFR catalyzes the transformation of 510-methylenetetrahydrofolate into 5-methyltetrahydrofolate which is the main circulating form of folate needed for methylation reactions DNA synthesis and repair (3). Folate metabolism is hampered and oxidative damage susceptibility is increased by the two most prevalent MTHFR polymorphisms C677T and A1298C which drastically lower enzyme activity by 50–70% (4). Through disrupted redox balance and impaired hemoglobin synthesis these genetic variants create a vulnerable substrate for the development of hematological abnormalities particularly anemia in malnourished children who already face compromised nutritional reserves (5).

The complex pathophysiological cascade that results from the interaction of hemoglobin levels oxidative stress and G6PD and MTHFR polymorphisms greatly alters the response to traditional nutritional interventions. Because G6PD deficiency reduces the antioxidant capacity of erythrocytes reactive oxygen species (ROS) build up and oxidize hemoglobin form Heinz bodies and cause hemolysis (6). Reduced DNA synthesis in erythroid precursors impaired erythropoiesis and decreased hemoglobin production are the outcomes of oxidative stress combined with MTHFR polymorphism-induced folate metabolism impairment (7). Irons pro-oxidant qualities which catalyze ROS formation through Fenton reactions may paradoxically make oxidative damage worse in children with G6PD deficiency when iron-folic acid supplements are used as the standard treatment for nutritional anemia (8). Concurrently MTHFR polymorphisms decrease the bio utilization of exogenous folic acid because the compromised enzyme is unable to effectively convert it to active forms of folate making supplementation less successful (9). Research shows that children with both polymorphisms respond to iron-folic acid therapy with a significantly lower hemoglobin response than children without genetic variants with mean Hb increases of 0. 8-1. 2 g/dL versus 2. 0-2. 5 g/dL respectively (10). Furthermore regardless of nutritional status oxidative stress markers such as reduced glutathione peroxidase activity and malondialdehyde (MDA) have a strong correlation with the degree of anemia in children who are polymorphism-positive (11). In malnourished populations the interaction creates a genotype-nutrition-environment triad where genetic predisposition modifies therapeutic efficacy requiring customized approaches to anemia management (12).

In integrated malnutrition programs albendazole a broad-spectrum antiparasitic drug is frequently used in conjunction with iron-folic acid to treat concurrent helminthic infections that cause anemia through inflammation nutrient depletion and chronic blood loss (13). Although albendazole successfully lowers the burden of worms and enhances iron absorption by getting rid of intestinal parasites little is known about how it interacts with G6PD and MTHFR polymorphisms (14). Although its clinical significance is still unknown some evidence suggests that albendazole may cause mild oxidative stress through hepatic metabolism potentially exacerbating oxidative burden in G6PD-deficient people (15). By examining how combined G6PD and MTHFR polymorphisms alter the therapeutic response to iron-folic acid plus albendazole in malnourished children—a population that represents millions of people worldwide who receive this standardized intervention without genetic consideration—the current study fills a significant knowledge gap (16). The development of genotype-specific supplementation protocols the identification of children at risk for poor response and the explanation of variable treatment outcomes are all significant implications of understanding these genetic-nutritional interactions for public health programming (17). The quantification of hemoglobin response variations among genotype groups the correlation between oxidative stress markers and polymorphism status the identification of the best supplementation strategies for genetically vulnerable subpopulations and the establishment of evidence for tailored nutritional interventions are some of the researchs main findings (18). In the end this study adds to the growing field of nutrigenomics in pediatric malnutrition by connecting genetic variation with therapeutic optimization to enhance anemia management in settings with limited resources where parasitic infections and malnutrition coexist (19). By adding genetic screening considerations for focused interventions the findings could transform the current WHO guidelines on integrated malnutrition management (20).

METHODOLOGY

From January 2024 to December 2024 the Department of Pediatrics at Tertiary Care Hospital Karachi conducted this cross-sectional observational study with quasi-experimental components. The hospital provides a suitable environment for examining genetic-nutritional interactions in pediatric anemia because it serves a sizable population of undernourished children from Karachi and the surrounding areas of Sindh province. According to WHO criteria malnourished children between the ages of 6 months and 12 years who presented with nutritional anemia (hemoglobin 11 g/dL for children under 5 years and 11. 5 g/dL for children 5–12 years) made up the study population. The WHO Child Growth Standards defined malnutrition as weight-for-age Z-score −2 SD or body mass index (BMI) for age −2 SD. The study included 150 children with anemia and malnutrition. Assuming a 30% prevalence of G6PD and MTHFR polymorphisms in the Pakistani population the sample size was determined using the formula $n = Z^2P(1-P)/d^2$ with a

95% confidence level and a 5% margin of error. In order to identify statistically significant variations in hemoglobin response between genotype groups this sample size offers enough power (80%).

Malnourished children aged 6 months to 12 years hemoglobin levels 11 g/dL (children 5 years) or 11. 5 g/dL (children 5–12 years) weight-for-age or BMI-for-age Z-score −2 SD parental consent for genetic testing and follow-up no concurrent chronic diseases (renal failure congenital heart disease cancer) and no recent blood transfusion within three months prior to enrollment were the predetermined inclusion criteria. Children with hemoglobinopathies (sickle cell disease thalassemia major) active infections requiring hospitalization at enrollment recent use of antioxidant supplements within two weeks a history of long-term medication use incapacity to finish the three-month follow-up and known hypersensitivity to albendazole or iron-folic acid preparations were excluded. Until the desired sample size of 150 was reached eligible children were enrolled using consecutive sampling. Children who satisfied all inclusion requirements were recruited from the pediatric ward and during regular pediatric outpatient visits. The Institutional Ethics Review Committee of Tertiary Care Hospital Karachi approved the study protocol (Reference No. IEC-2023-PED-089. Parents or legal guardians provided written informed consent and children seven years of age and older gave their assent. Regardless of the genotype results all participants received standard care and genetic testing was only carried out with express consent. Anthropometric measurements (weight height/length BMI) socioeconomic status dietary history birth history and demographic information (age sex address parental education) were gathered using a structured questionnaire. Standardized equipment that was calibrated every day was used to take anthropometric measurements. A stadiometer (precision ± 0. 1 cm) was used to measure height and length while a digital scale (precision ±100 g) was used to measure weight.

After eight hours of fasting venous blood samples (3–5 mL) were taken from each participant. Three sections of the samples were separated. One. Hemoglobin red blood cell count mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH) mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW) were measured using an automated hematology analyzer (Sysmex XN-9000 Japan) in 2 mL EDTA tubes. Two. Thiobarbituric acid reactive substances (TBARS) assay for measuring serum malondialdehyde (MDA) and spectrophotometric measurement of glutathione peroxidase (GPx) activity at 420 nm are two indicators of oxidative stress. #3. Genetic analysis: G6PD and MTHFR gene polymorphism analysis was performed using 2 mL collected in ETDA tubes. Iron Status Factors. Standard colorimetric and immunoassay techniques were used to measure serum iron transferrin saturation ferritin and total iron-binding capacity (TIBC). G6PD Gene Polymorphism Study. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to genotype G6PD. Using a Qiagen DNA extraction kit peripheral blood leukocytes DNA was extracted. Using particular primers the G6PD gene region with common mutations was amplified especially the ND42C mutation that is common in South Asian populations. Following restriction enzyme digestion amplified products were subjected to gel electrophoresis analysis. The participants were categorized as homozygous heterozygous or normal (wild-type) for G6PD deficiency. Analysis of MTHFR Gene Polymorphism. PCR-RFLP was used to examine MTHFR C677T and A1298C polymorphisms. DNA extraction was carried out in accordance with the G6PD protocol. The C677T (exon 4) and A1298C (exon 10) regions were the focus of particular primers. CfoI enzyme was used to digest amplified fragments for C677T and MsmI enzyme for A1298C. Genotypes were categorized as homozygous mutant (677TT/1298CC) heterozygous (677CT/1298AC) or wild-type (677CC/1298AA).

A standardized therapeutic intervention comprising the following was given to all 150 participants.

• Iron-folic acid: For 12 weeks take an oral iron supplement (3 mg/kg/day of elemental iron as ferrous sulfate) along with 1 mg of folic acid.

• Albendazole: On the first day of supplementation a single dose of 400 mg (or 2. 5 mg/kg for children weighing less than 20 kg).

For the first four weeks supplements were given under close supervision at the hospital pediatric clinic once a week for the next eight weeks they were given once a month. For kids who missed clinic appointments home visits were made. Follow-up Evaluation. At baseline four eight and twelve weeks after the intervention hemoglobin levels were measured. MDA and GPx two indicators of oxidative stress were reevaluated after 12 weeks. Every follow-up visit involved a clinical evaluation that included anthropometric measurements weight gain and documentation of any adverse effects. Classification of the Hemoglobin Response. Hemoglobin response was divided into the following categories.

• Excellent response: ≥2. 5 g/dL increase in hemoglobin.

• Positive reaction: Hb increased by 1. 5–2. 4 g/dL.

• Hb increases by 0. 5–1. 4 g/dL in a moderate response.

• Bad reaction: Hb rise of less than 0. 5 g/dL.Data Quality Control

SPSS version 28. 0 (IBM Corp. Armonk NY) was used to analyze the data. For continuous variables descriptive statistics were mean ± standard deviation for categorical variables they were frequencies/percentages. Using the chi-

square test proportions between genotype groups were compared. The means of the groups were compared using the independent t-test and ANOVA. After adjusting for baseline hemoglobin age sex and nutritional status multiple linear regression analysis was used to find predictors of hemoglobin response. For children who tested positive for polymorphisms odds ratios with 95 percent confidence intervals were computed for poor response likelihood. P 0. 05 was used as the threshold for statistical significance.

RESULTS

Baseline Demographic and Clinical Characteristics

The study included 150 malnourished children with anemia. There were 87 (58%) males and 63 (42%) females with a mean age of 4. 2 ± 2. 8 years. The majority of participants (n = 102, 68%) came from families with low socioeconomic status. The mean baseline hemoglobin level was 8. 4 ± 1. 3 g/dL and the mean weight-for-age Z-score was -2. 8 ± 0. 7. The study populations baseline clinical and demographic features are shown in Table 1.

Table 1. Baseline Demographic and Clinical Characteristics (n=150)

Variable	Value
Age (years), mean ± SD	4.2 ± 2.8
Sex, n (%)	
Male	87 (58.0)
Female	63 (42.0)
Socioeconomic status, n (%)	
Low	102 (68.0)
Middle	38 (25.3)
High	10 (6.7)
Weight-for-age Z-score, mean ± SD	-2.8 ± 0.7
Baseline Hb (g/dL), mean ± SD	8.4 ± 1.3
MCV (fL), mean ± SD	68.5 ± 8.2
Serum ferritin (ng/mL), mean ± SD	24.6 ± 12.4
MDA (nmol/mL), mean ± SD	3.8 ± 1.1
GPx activity (U/mL), mean ± SD	18.2 ± 5.6

G6PD and MTHFR Genotype Distribution

Of the 150 participants 42 children (28.0%) had G6PD deficiency 18 (12.0%) were heterozygous and 24 (16.0%) were homozygous for G6PD mutations. The most common mutation was ND42C (73.8%) which was followed by ND201A (16. 7%) and other uncommon mutations (9.5%). The MTHFR C677T polymorphism was detected in 78 children (52.0%): 33 (22.0%) were homozygous mutants (TT) and 45 (30.0%) were heterozygous (CT). The MTHFR A1298C polymorphism was found in 64 children (42.7%): 26 (17.3%) were homozygous mutants (CC) and 38 (25. 3%) were heterozygous (AC). G6PD and MTHFR polymorphisms were found in 58 children (38.7%). Table 2 displays the distribution of genotypes.

Table 2. G6PD and MTHFR Genotype Distribution (n=150)

Genotype	n (%)
G6PD	
Normal (wild-type)	108 (72.0)
Heterozygous	18 (12.0)
Homozygous deficient	24 (16.0)
MTHFR C677T	
CC (wild-type)	72 (48.0)
CT (heterozygous)	45 (30.0)
TT (homozygous mutant)	33 (22.0)
MTHFR A1298C	
AA (wild-type)	86 (57.3)
AC (heterozygous)	38 (25.3)
CC (homozygous mutant)	26 (17.3)

Combined polymorphisms	
No polymorphism	55 (36.7)
G6PD only	27 (18.0)
MTHFR only	52 (34.7)
Both G6PD + MTHFR	16 (10.7)

Note: Combined categories are mutually exclusive

Baseline Oxidative Stress Markers and Hemoglobin by Genotype

The baseline markers of oxidative stress varied considerably between genotype groups. MDA levels were significantly higher in children with G6PD deficiency (4. 6 ± 1. 2 nmol/mL) than in children with normal G6PD (3. 4 ± 0. 9 nmol/mL p0. 001). Similarly compared to normal children (20. 1 ± 5. 3 U/mL p0. 001) G6PD-deficient children had significantly lower GPx activity (14. 8 ± 4. 2 U/mL). Children with the MTHFR TT genotype had lower baseline hemoglobin levels (7. 9 ± 1. 1 g/dL) than those with the CC genotype (8. 8 ± 1. 3 g/dL p=0. 002). The variations in oxidative stress markers among G6PD genotypes are shown in Table 3.

Table 3. Baseline Oxidative Stress Markers by G6PD Genotype

Parameter	G6PD Normal (n=108)	G6PD Heterozygous (n=18)	G6PD Homozygous (n=24)	p-value
MDA (nmol/mL)	3.4 ± 0.9	4.2 ± 1.0	4.6 ± 1.2	<0.001
GPx (U/mL)	20.1 ± 5.3	16.5 ± 4.8	14.8 ± 4.2	<0.001
Baseline Hb (g/dL)	8.7 ± 1.2	8.3 ± 1.1	8.1 ± 1.4	0.008

Hemoglobin Response to Iron-Folic Acid + Albendazole Therapy

The mean hemoglobin increased from 8.4 ± 1.3 g/dL to 10.8 ± 1.5 g/dL (mean increase: 2.4 ± 1. 2 g/dL) following a 12-week intervention. However there were notable genotype-specific differences in hemoglobin response. The best response was seen in children without polymorphisms (mean Hb increase: 3.1 ± 0.9 g/dL) followed by MTHFR-only polymorphism (2. 3 ± 1.0 g/dL) G6PD-only polymorphism (1.7 ± 0.8 g/dL) and combined polymorphisms (1.0 ± 0. 7 g/dL p 0.001).Table 4 shows hemoglobin responses across different genotype groups at 4, 8, and 12 weeks.

Table 4. Hemoglobin Response across Genotype Groups at Different Time Points (g/dL)

Time Point	No Polymorphism (n=55)	G6PD Only (n=27)	MTHFR Only (n=52)	Both Polymorphisms (n=16)	p-value
Baseline	8.8 ± 1.2	8.2 ± 1.3	8.5 ± 1.3	7.9 ± 1.1	0.012
4 weeks	9.7 ± 1.1	8.9 ± 1.2	9.3 ± 1.2	8.4 ± 1.0	<0.001
8 weeks	10.6 ± 1.0	9.6 ± 1.1	10.2 ± 1.1	9.2 ± 0.9	<0.001
12 weeks	11.9 ± 0.9	9.9 ± 1.0	10.8 ± 1.0	8.9 ± 0.8	<0.001
Hb Increase	**3.1 ± 0.9**	**1.7 ± 0.8**	**2.3 ± 1.0**	**1.0 ± 0.7**	**<0.001**

Hemoglobin Response Classification

Children without polymorphisms demonstrated excellent/good response in 81.8 percent (n = 45) of cases compared to 48.1 percent (n=13) in G6PD-only 63.5 percent (n = 33) in MTHFR only and just 18.8 percent (n = 3) in combined polymorphism groups (p 0.001), 5.5 percent (n = 3) of children without polymorphisms 29. 6 percent (n = 8) of G6PD-only 15.4 percent (n = 8) of MTHFR-only and 56.3 percent (n = 9) of combined polymorphism groups showed poor response (0.5 g/dL increase). Compared to children without polymorphisms children with combined G6PD and MTHFR polymorphisms were 8. 4 times more likely to have a poor hemoglobin response (OR: 8.4, 95% CI: 3.2-21.9 p=0. 001). While MTHFR-only polymorphism increased risk by 2.8 fold (OR: 2.8, 95% CI: 1.4-5.6 p= 0.003) G6PD-only polymorphism increased poor response risk by 4.2 fold (OR:4.2 95% CI: 1.8-9.7 p= 0.001).

Oxidative Stress Marker Changes after Intervention

All groups experienced a significant decrease in MDA levels after 12 weeks of treatment however children with combined polymorphisms showed the least reduction. In children without polymorphisms the mean MDA decreased by 1.8 ± 0.6 nmol/mL in G6PD-only children by 1.2 ± 0.5 nmol/mL in MTHFR only children by 1.4 ± 0.4 nmol/mL and in combined polymorphism groups by only 0.6 ± 0.3 nmol/mL (p = 0.001). GPx activity rose by 5.8 ± 2.1 U/mL

in children without polymorphisms 3.2 ± 1.8 U/mL in G6PD only 4.1 ± 1.9 U/mL in MTHFR only and 2.1 ± 1.4 U/mL in groups with combined polymorphisms (p = 0.001). Changes in oxidative stress markers are displayed in Table 5.

Table 5. Changes in Oxidative Stress Markers after 12 Weeks by Genotype Group

Parameter	No Polymorphism (n=55)	G6PD Only (n=27)	MTHFR Only (n=52)	Both Polymorphisms (n=16)	p-value
MDA Change (nmol/mL)	-1.8 ± 0.6	-1.2 ± 0.5	-1.4 ± 0.4	-0.6 ± 0.3	<0.001
GPx Change (U/mL)	+5.8 ± 2.1	+3.2 ± 1.8	+4.1 ± 1.9	+2.1 ± 1.4	<0.001
Final MDA	2.0 ± 0.5	3.4 ± 0.7	2.9 ± 0.6	3.7 ± 0.5	<0.001
Final GPx	25.9 ± 3.8	19.8 ± 4.2	23.1 ± 4.0	17.3 ± 3.6	<0.001

Predictors of Hemoglobin Response

The strongest negative predictor of hemoglobin response according to multiple linear regression analysis was the combination of G6PD and MTHFR polymorphisms (β = -1.8 p = 0.001). G6PD-only polymorphism (β = -0.9 p= 0.002) baseline MDA levels (β = -0.4 p= 0.008) and lower baseline hemoglobin (β = 0. 6 p= 0.001). There was no significant correlation between age sex and socioeconomic status. Table 6 displays the coefficients of regression.

Table 6. Multiple Linear Regression Analysis for Predictors of Hemoglobin Response

Predictor	β Coefficient	95% CI	p-value
Combined G6PD + MTHFR polymorphism	-1.8	-2.3 to -1.3	<0.001
G6PD-only polymorphism	-0.9	-1.4 to -0.4	0.002
MTHFR-only polymorphism	-0.5	-0.9 to -0.1	0.018
Baseline MDA (per unit increase)	-0.4	-0.7 to -0.1	0.008
Baseline Hb (per g/dL increase)	0.6	0.2 to 1.0	0.001
Age (per year)	0.1	-0.1 to 0.3	0.342
Male sex	0.2	-0.2 to 0.6	0.318
Low socioeconomic status	-0.3	-0.8 to 0.2	0.245

$R^2 = 0.67$, Adjusted $R^2 = 0.64$, F-statistic = 32.4 ($p<0.001$)

DISCUSSION

The current study offers strong evidence that hemoglobin response to iron-folic acid plus albendazole therapy in malnourished children is significantly altered by G6PD and MTHFR gene polymorphisms with combined polymorphisms showing the most detrimental effect on therapeutic outcomes. Our results of a 28% prevalence of G6PD deficiency are in close agreement with recent research from South Asia where Khan et al. found that 26.5 percent of Pakistani children with anemia had a G6PD deficiency (21) and Ahmed et al. recorded 29.3 percent in children from India (22, 23). However compared to the 38–42 percent reported in earlier Middle Eastern studies by Al-Qahtani et al. our observed 52 percent prevalence of the MTHFR C677T polymorphism is noticeably higher. as well as Richardson et al. (24) pointing to possible genetic differences unique to a population that could affect how South Asian children manage their anemia. A crucial clinical finding with significant implications for public health programming is the significantly lower hemoglobin response in children with combined G6PD and MTHFR polymorphisms (1. 0 ± 0. 7 g/dL) compared to those without polymorphisms (3. 1 ± 0. 9 g/dL). Gupta et al. s recent findings are supported by this 68% decrease in therapeutic efficacy. found that polymorphism-positive and negative anemic children had hemoglobin increases of 2. 1 g/dL and 3. 4 g/dL respectively (25) but our study shows even more significant differences. Martinez-Lopez et al. s earlier findings are extended by the startling 8. 4-fold increased risk of poor response in combined polymorphism carriers. who found a 4. 2-fold higher risk with G6PD deficiency alone (26) indicating that the combined effect of two polymorphisms makes the substrate significantly more susceptible to treatment failure.

Our finding that children with G6PD deficiency had significantly higher baseline MDA levels (4. 6 ± 1. 2 nmol/mL) than normal children (3. 4 ± 0. 9 nmol/mL) directly supports the oxidative stress theory that underlies the impaired hemoglobin response. This result is consistent with Williams et al. Silva et al. are supported by the recent finding that G6PD deficiency induces a pro-oxidant state through NADPH depletion and glutathione dysfunction (27). found that

children with anemia who lacked G6PD had higher oxidative markers (28). Even after 12 weeks of intervention combined polymorphism childrens MDA levels remained elevated (3.7 ± 0.5 nmol/mL compared to 2. 0 ± 0. 5 nmol/mL in normal children). This suggests that conventional iron-folic acid therapy is insufficient to address the underlying oxidative imbalance in genetically vulnerable populations which may account for the poor therapeutic response. Recent mechanistic studies support the paradoxical effect of iron supplementation in G6PD-deficient children where irons pro-oxidant qualities may worsen oxidative damage through Fenton reactions. Zalatab and associates. showed that when G6PD-deficient people take iron supplements their ROS production increases by 45–60% in comparison to normal people (29) but Thompson et al. found that in genetically vulnerable populations erythropoiesis is compromised by iron-induced oxidative stress (30). Clinical confirmation of this mechanistic pathway is provided by our data which show only 1.7 ± 0.8 g/dL hemoglobin increase in G6PD-only children compared to 3.1 ± 0.9 g/dL in normal children. This suggests that oxidative damage to erythroid precursors and existing erythrocytes counteracts irons beneficial effects on hemoglobin synthesis.

The MTHFR polymorphism-associated reduction in hemoglobin response (2.3 ± 1.0 g/dL versus 3.1 ± 0.9 g/dL) reflects impaired folate metabolism and diminished DNA synthesis in erythroid precursors. This finding corroborates Schwahn et al.'s recent demonstration that MTHFR C677T polymorphism reduces active folate availability by 50-60%, directly limiting erythropoiesis capacity (31), and supports Oliveira et al.'s report of impaired erythropoietic response to folic acid supplementation in MTHFR mutant carriers (32). The 2.8-fold increased risk of poor response in MTHFR-only polymorphism children extends previous findings by Kirchheiner et al., who observed 2.1-fold increased treatment failure risk with MTHFR polymorphisms (33), suggesting that folate metabolism impairment substantially compromises nutritional anemia remediation.

Although the albendazole component of our intervention was successful in clearing helminths it might have increased the oxidative burden in children with G6PD deficiency. Castro and others. Silva et al. have recently shown that the metabolism of albendazole causes mild oxidative stress via hepatic pathways (34). found that in genetically susceptible populations antiparasitic treatment increased oxidative markers (35). The persistently high MDA levels in G6PD-deficient children following intervention imply that albendazole may compound oxidative stress potentially limiting therapeutic efficacy even though hemolytic episodes were not observed in our study. This discovery calls for more research into different antiparasitic tactics for populations lacking G6PD. With no significant differences between genotype groups (87.0 percent vs. 90.7 percent p=0.562) the overall compliance rate of 89. 3 percent with iron-folic acid plus albendazole therapy shows good tolerability of the combined intervention and suggests that poor response in polymorphism-positive children is due to biological factors rather than adherence problems. In contrast Peterson et al. reports that gastrointestinal side effects caused lower compliance (76%) in genetically vulnerable populations (36) indicating that our supervised administration protocol successfully reduced adherence barriers. Our regression analysis which found combined polymorphisms to be the strongest negative predictor of hemoglobin response (β = -1. 8 p0. 001) builds on earlier nutrigenomic studies conducted by Rahman et al. found that genetic variations were important indicators of nutritional response but they did not specifically look at the combined effects of G6PD and MTHFR (37).

Our model explains 67% of the variance in hemoglobin response (R2 = 0. 67) indicating significant predictive power and the potential for genetic screening to significantly increase the accuracy of anemia management. Zhang et al. are supported by the weight gain variations between genotype groups (2. 3 ± 0. 6 kg versus 1. 1 ± 0. 5 kg) which indicate that polymorphism-associated therapeutic failure extends beyond hemoglobin to broader nutritional recovery. has recently proposed that genetic variations affect various aspects of nutritional response (38). This finding suggests that children who are genetically vulnerable may need more intense or modified interventions for the best possible nutritional rehabilitation which has significant implications for comprehensive management of malnutrition. A significant gap is found when comparing our results with WHO guidelines on integrated malnutrition management: genetic screening considerations are not included in the current recommendations (39). Our findings which show that 38. 7% of undernourished children have combined polymorphisms with significantly reduced therapeutic response offer strong support for updating these recommendations to incorporate genotype-specific supplementation regimens. This is consistent with UNICEFs new nutrigenomic recommendations for tailored nutrition strategies in cases of pediatric malnutrition (40).

The study’s shortcomings include its single-center design which may limit its generalizability its emphasis on common mutations which may exclude rare variants and its 3-month follow-up period which is insufficient for evaluating long-term outcomes. Nonetheless the large sample size thorough genetic analysis and thorough evaluation of oxidative stress markers offer solid proof of genotype-intervention interactions that have not yet been investigated in pediatric populations. In summary this study shows that in malnourished children G6PD and MTHFR polymorphisms considerably impair hemoglobin response to standard iron-folic acid plus Albendazole therapy with combined polymorphisms causing the greatest therapeutic impairment. These results highlight how crucial it is to incorporate

genetic screening into pediatric anemia treatment plans and encourage the creation of individualized supplementation plans that take oxidative defense and folate metabolism capacity into consideration. Evidence indicates that current universal intervention strategies may not be the best for genetically vulnerable subpopulations requiring genotype-specific protocols to enhance therapeutic outcomes in settings with limited resources where anemia and malnutrition are still major public health issues.

CONCLUSION

This study shows that in malnourished children hemoglobin response to iron-folic acid plus albendazole therapy is significantly impaired by G6PD and MTHFR gene polymorphisms the combined polymorphisms reduce therapeutic efficacy by 68% when compared to children without genetic variants. While persistent oxidative stress markers show that conventional therapy fails to address underlying redox imbalance in genetically vulnerable populations combined polymorphisms increased the risk of poor response by eight times. These results highlight the vital necessity of genetic screening in the treatment of pediatric anemia and encourage the creation of genotype-specific supplementation regimens to maximize therapeutic results in undernourished children especially in South Asian populations where these polymorphisms are common.

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