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immune respRQ VWDWHG WR WHVW RI ÒDWRQ neutrophils, basophils, and monocytes produce cytotoxic proteins such as proteases, collagenases, and elastases as well as reactive oxygen radicals, which cause oxidative stress. In turn, this damage promotes the activation of endothelium and WKHKRQHOO@

,QKRORRSDWKVKV&DQWKODMD oxidative damage to erythroid cells plays a crucial role QRROWR HuHVH WHVKSHQWKRQ marrow and short survival of red blood cells in circulation WKWDOHSRWHWSWDWHQWKRZ OHMORKDQRDQWKSODMDRHRHMH WRRHRIRWDWHMRV may be triggered by several switches, including the interaction between sickled red blood cells and the endothelium, nitric RHQGDQWHSMQDWRQ

QWSOH DWRDW HQVH SHQ QHOOXDU RDHOOHVQDWRKDWHQHDWVW which provide protection from oxidative and chemical stress. @ROH HDP\VH SODHQRX WDHQHDWHG RX WDHQHDWHG RRHWDHQHDWHG RZHBSSHDWRHWKRWHOWHG XHHPQDMHSRORSK there has been considerable interest in determining the particular allelic variants and those associated with increased WDWHQHDW@RIWKV to investigate whether *GSTP1* and *TLR2* polymorphisms ÒHQ WSWHW\WRQHWRQHHOO SDWHQV ,WKRWHWRHWQHWSWHWQOHDWRDQvHRI metabolic pathways involved in antioxidant mechanisms or pathogen recognition increase the chances of infectious processes in SC$

Methodology

Casuistry

EDHERROGDERGEHGDPSOHRI SDWLHQWHIROORZHGDWWKH&HQWHU OHOHEHOODHPLDEHODHGRHHDJHRIDG IROORHGDHRGDRRIHPDRORJDGHPRHDS RIHDPEERZDHIIHLRDG ERROHRDIHELR

Ethical Aspects of Research

This study was performed after approval by the Research (WKLFV RDWHH RI (QUR DQ developed fully complying with the ethical principles set out HURWRQIWKDWRQOHDOWKRQWRQ

Hematologic and Clinical Analysis

DOIRHPDRORJLEDOGDDSHIRPHGLJ DHOHERLEEHOOERHROHRSRDRR HTDDEDRRIHDOHPRJOREDGDSHIRPHGEIHOLTGERPDRJDS were obtained by the analysis of medical records of patients.

Molecular Analysis

LHDEHGESHROEORRIRPHELTHD DPSOLHGEHDHLERIDJPHOHJ SROPRSLPSROPHDHEDLHDERIDJIRDG DGHHHSLPHIR*SROPRSLPDDOLI ***DG* ***HDOROPHRI HHDERIDHSDDPHHHGHHDLDO GHDDRIRPLHIROORHGEHEOH RIGHDDRIRPLHDHDOJDIR HERGHHRDIRHERGDGDDO HHRDIRPLHHSRGEHH treated with *AlwHLFLRHPHIRPLHD IHLHDPSOHHMHHGRHOHRSRHL RDJDRHJHODLHGLHLGLPRPLGHDG RIDGSHHLDOLHGERHSRGJRIOG SHJHRSHOHODGRISDGHDERG FRHSRGJRDGGSHJDGJRPJR DLDJHRSHDODOGDOGHHLDOLHGL HHHRJRDLDJHRSHOHDOHDGRI DGSHHRHHGRDSRODEODPLGHJHO* stained with ethidium bromide, where eight samples were DSSOHGHDWSHQWSHWKHKWHRIMDQV ,OHDODQRRJRMDQVDOO

In the DQOV RI SRORSLK WR HO HH MGWK SHVIRDG &DQ HMM @ K & RQQRKDOORKDOHQWRWQRU NHVROORZHHRKHQWRWQRU MRQDQWRHQWRWQ IRUQOHWHQWRWQ SRHWDOHWHDQOHGOHWRSKDDEM JHOKDQSRHUQWRWKHQWSH WKDQSRHUQWRHQWSHHOHDQWK DQSDQWRMDWHQWK HOHQWSHOWKDHWKQ transilluminator.

Statistical Analysis

H2GGDDRDEDOEODHGLJ*DSDGLP RIDHLDERFRGHEHLHDOHH DREDRERHHHHDDJHRSHRPRJR DGHHRJRDDIHERDOHPLDD evaluated.

Results and Discussion

GSTP1 polymorphism analysis

HDHDJHDJHRIHSDHDHD

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The distribution of frequency examined showed a higher percentage for the heterozygous variant genotype (Ile/Val) at 46.02%, followed by the wild genotype (Ile/Ile) at 36.23% and the lowest frequency of 17.75% for the homozygous variant (Val/Val). Such findings were similar to those found in two studies also conducted with the Brazilian population by Hatagima et al. [17] and Honma et al [18]. Kiss et al [19] and Ates et al [20] also obtained similar frequencies in studies carried out in Hungary and Turkey, respectively.

We did not find a statistically significant association between the variant genotypes (Ile / Val and Val / Val) and the risk of infection in patients with sickle cell anemia (Table 1). Silva et al found higher levels of glutathione and greater antioxidant capacity in patients with SCA who presented the Val / Val genotype. This was compared to those with the Ile / Ile genotype [21].

In the literature, it has been extensively studied the relationship between *GSTP1* Val genotypes and a variety of diseases. This includes determining the susceptibility to cancer, and responses to metabolism, efficacy, and toxicity of certain drugs [15, 19, 20, 22, 23]. The association not found in this study can be explained by the possibility that these individuals present antioxidant mechanisms to compensate for the deficiency in the activity of isoform GSTP1. It is imperative to note that GST enzymes are part of an integrated defense system, and the combined action of other enzymes such as γ-glutamylcysteine synthase (γGluCysS) and glutathione synthase guarantees the efficiency of this system. It provides glutathione, as well as carriers to facilitate the removal of GSH conjugates [24].

TLR2 polymorphism analysis

SNPs (Single nucleotide polymorphisms) in TLR2 have been associated with susceptibility to various infectious and inflammatory diseases such as leprosy [25], increased gram-negative sepsis risk [26], recurrent bacterial infections in children [27] and colorectal and cervical cancer [28, 29]. Also, a study linked variants in the non-coding region of the TLR2 gene with infections in pediatric sickle cell anemia [30].

The polymorphism corresponding to a deletion of 22 base pairs at position -196 to -174 of the TLR2 promoter region has been reported to alter the promoter activity, decreasing responsive promoters [31]. In this study, the wild homozygous genotype (Ins / Ins) was the most frequent in this population (58.27%), followed by the heterozygous variant genotype (Ins / Del, 40.65%) and finally the homozygous variant (Del / Del, 1.8%). The frequency of TLR2 polymorphism alleles investigated correlates well with previously published data on Brazilian, Japanese and German populations [32, 33, 34].

Individuals with the homozygous genotype for the deletion (Del / Del) showed a 95% increased chance of developing an infection compared to wild homozygotes (OR=1.95; CI=0.1 to 38.65) (Table 2). However, despite the central role of TLR2 in the recognition of pathogens and initiation of defense, this result was not statistically significant.

When pairing the analysis of polymorphisms of both genes (*GSTP1* Ile / Val and *TLR2* Ins / Del) for the development of infectious complications, we also did not find a statistically significant (OR=1.27, P= 0.61) (Table 3).

Woehrle et al. 2008 [35], studying 325 patients with septic shock, observed that in patients with sepsis caused by gram-positive bacteria, the variant profile TLR2 did not alter the pattern of cytokines produced, so, had no involvement in the immune response. TAHARA et al. 2008 [36] in Japan also studied polymorphisms -196 to -174 of the TLR2 gene, relating them to the risk of gastroduodenal disease induced by

Table 1: Association between GSTP1 variant genotype and the risk of infections in 278 patients with sickle cell disease

GSTP1 Genotype	*With infection*	*Without infection*	*Chi-square*	*Odds Ratio*	*CI* of 95%*	*P value*
Ile/Val	98	29	0.14	1.13	(0.60 - 2.08)	0.7
Val/Val	34	15	0.52	0.75	(0.35 - 1.16)	0.47
Ile/Val or Val/Val	132	44	0.01	1	(O.57 – 1.76)	1
Ile/Ile	75	25		1(reference)		

* ***Confidence interval***

Table 2: Association between TLR2 variant genotype and the risk of infection in 278 patients with sickle cell disease

Variant Genotype TLR2	*With infection*	*Without infection*	*Chi-square*	*Odds Ratio*	*CI*of 95%*	*P value*
Ins/Del	78	35	3.08	0.61	(0.35 – 1.06)	0.08
Del/Del	3	0	0.04	1.95	(0.1 – 38.65)	0.85
Ins/Del or Del/Del	81	35	2.63	0.63	(0.37 – 1.1)	0.1
Ins/Ins	127	35		1(reference)		

* ***Confidence interval***

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Table 3: Association between GSTP1 and TLR2 variant genotypes and the risk of infections in 278 patients with sickle cell disease

GSTP1 and TLR2 Genotypes	With infection	Without infection	Chi-square	Odds Ratio	CI* of 95%	P value
GSTP1(Ile / Val) and TLR2(Ins / Del)	43	17	0.95	0.67	(0.3 – 1.5)	0.33
GSTP1(Ile / Ile) and TLR2(Ins / Ins)	53	14		1(reference)		
GSTP1(Ile / Val) and TLR2(Ins / Del)	43	17	0.25	1.27	(0.5 – 3.16)	0.61
GSTP1(Ile / Ile) and TLR2(Ins / Ins)	22	11		1(reference)		
GSTP1(Ile / Val) and TLR2(Ins / Del)	43	17	1.71	0.57	(0.25 – 1.3)	0.19
GSTP1(Ile / Val) and TLR2(Ins / Ins)	53	12		1(reference)		
GSTP1(Val / Val) and TLR2(Ins / Del)	14	6	0.72	0.62	(0.2 – 1.89)	0.39
GSTP1(Ile / Ile) and TLR2(Ins / Ins)	53	14		1(reference)		
GSTP1(Val / Val) and TLR2(Ins / Del)	14	6	0.01	1.05	(0.3 – 3.6)	0.93
GSTP1(Val / Val) and TLR2(Ins / Ins)	20	9		1(reference)		
GSTP1(Val / Val) and TLR2(Ins / Del)	14	6	0.06	1.17	(0.35 – 3.9)	0.8
GSTP1(Ile / Ile) and TLR2(Ins / Del)	22	11		1(reference)		

* ***Confidence interval***

Helicobacter pylori in 309 patients, and no found association of this polymorphism with the risk of ulcer gastric, duodenal ulcers and gastritis.Taken together, our data suggest that deletion -196 to -174 of the promoter of the TLR2 gene region was not enough to establish a correlation of polymorphism as a risk factor for infections that affect individuals with sickle cell disease. Possibly it is explained by numerous genetic variations in the pathogens recognition system, responsible for specific differences [37], and also because of the wide range of functionally relevant genetic alterations of the innate immune system [38].

It is necessary to search simultaneously for other candidate genes such as those encoding receptors that interact directly in the formation of heterodimers with TLR2 (TLR1, TLR6, and CD14) and protein signaling pathways, to characterize the profile of severe infections. For example, the presence of CD14 enhances the efficiency of recognition of TLR2-specific ligands [39].

Conclusions

The genotypes Ile/Val and Ins/Ins related to GSTP1 and TLR2, respectively, were the most frequent in this study. No association was found between GSTP1 (Ile/Val) and TLR2 (Del/Del) variant genotypes and increased risk of infections in sickle cell anemia.

Declaration of interest

None

Author contributions

Géssyka Jerônimo Silva: Formal analysis, investigation; *Romério Alencar de Oliveira Filho*: Formal analysis, investigation; *Igor de Farias Domingos:* Formal analysis, investigation; *Rodrigo Marcionilo de Santana:* Writing - review & editing; *Thais Helena Chaves Batista*: Writing - review & editing; *Aderson da Silva Araújo:* Conceptualization, Funding acquisition; *Marcos André Cavalcanti Bezerra:* Methodology, Supervision; *José Luiz de Lima Filho*: Funding acquisition; *Danyelly Bruneska Gondim Martins*: Methodology, Supervision; *Rosângela Ferreira Frade de Araújo*: Methodology, Project administration, Roles/Writing - original draft.

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References

1. Lettre G, Uda M, Sanna S, Cao A, Schlessinger D, et al. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associated with fetal hemoglobin levels and pain crises in sickle cell disease. Proc Natl Acad Sci USA 105 (2008): 11869-74.
2. Piel F B, Steinberg M H, David C, Rees D C. Sickle Cell Disease. N Engl J Med 376 (2017): 1561-1573.
3. Lobo C L C, Nascimento E M, Jesus L G C, Freitas T G, Lugon J R, et al. Mortality in children, adolescents and adults with sickle cell anemia in Rio de Janeiro, Brazil. Hematol. Transfus. Cell Ther 40 (2018): 37-42.
4. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat immunol 11 (2010): 373-384.
5. Lorenz E, Mira J P, Cornish K L, Arbour N C, Schwartz D A. A novel polymorphism in the Toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect Immun 68 (2000): 6398-6401.
6. Booth C, Inusa B, Obaro SK. Infection in sickle cell disease: A review. Int J Infect Dis 14 (2010): 2-12.
7. Owusu-Poku A G, Gyamfi D, Togbe E, Opoku S, Ephraim R K D, et al. Interplay between foetal haemoglobin, micronutrients and oxidative stress biomarkers in sickle cell anemia children. Hum Nutr Metab 30 (2022).
8. Smith O S, Ajose O A, Adegoke S A, Adegoke O A, Adedeji T A, et al. Plasma level of antioxidants is related to frequency of vaso-occlusive crises in children with sickle cell anemia in steady state in Nigeria. Pediatr Hematol Oncol J 4 (2019): 17-22.
9. O'Brien C E, Ali S, Chevassut T. Sickle cell disease: an update. Clin Med (Lond) 22 (2022): 218-220.
10. Goto S, Kawakatsu M, Izumi S-I, Urata Y, Kageyama K, et al. Glutathione S-transferase π localizes in mitochondria and protects against oxidative stress. Free Radical Bio Med 46 (2009): 1392–1403.
11. Zhang W, Gao J, Lu L, Bold T, Li X, et al. Intracellular GSH/GST antioxidants system change as an earlier biomarker for toxicity evaluation of iron oxide nanoparticles. NanoImpact 23 (2021): 100338.
12. Gao Y, Pan X, Su T, Mo Z, Cao Y, et al. Glutathione S-transferase P1 Ile105Val polymorphism and colorectal cancer risk: A meta-analysis and HuGE review. Eur J Cancer 45 (2009): 3303 – 3314.
13. Wang J, Wu D, Sun A. Effects of GST null genotypes on individual susceptibility to leukemia: A meta-analysis. Exp Mol Pathol 108 (2019): 137-142.
14. Dutta T, Bhattacharjee S. Frequency of Glutathione S-Transferase M1, T1 and P1 genotypes and their combinations in northern West Bengal, India: implications for disease association. Gene Rep 25 (2021): 101313.
15. Sobti R C, Kaur S, Kaur P, Singh J, Gupta J, et al. Interaction of passive smoking with GST (GSTM1, GSTT1, and GSTP1) genotypes in the risk of cervical cancer in India. Cancer Genet. Cytogen 166 (2006): 117–123.
16. Tahara T, Arisawa T, Wang F, Shibata T, Nakamura M, et al. Toll-like receptor 2 −196 to 174del polymorphism influences the susceptibility of Japanese people to gastric Cancer. Cancer Sci 98 (2007): 1790–1794.
17. Hatagima A, Marques C F S, Koifman R J, Boffetta P, Koifman S, et al. Glutathione S-transferase polymorphisms and oral cancer: a case-control study in Rio de Janeiro, Brazil. Oral Oncol 44 (2008): 200-207.
18. Honma H N, Capitani E M, Perroud M W, Barbeiro A S, Toro I F C, et al. Influence of p53 codon 72 exon 4, GSTM1, GSTT1 and GSTP1*B polymorphisms in lung cancer risk in a Brazilian population. Lung Cancer. 61 (2008): 152-162.
19. Kiss I, Németh A, Bogner B, Pajkos G, Orsós Z, et al. Polymorphisms of glutathione-S-transferase and arylamine N-acetyltransferase enzymes and susceptibility to colorectal cancer. Anticancer Res 24 (2004): 3965–3970.
20. Ateş N A, Tamer L, Ateş C, Ercan B, Elipek T, et al. Glutathione S-transferase M1, T1, P1 genotypes and risk for development of colorectal cancer. Biochem Genet 43 (2005): 149–163.
21. Silva D G H, Junior E B, Torres L C, Júnior O R, Lobo C C, et al. Relationship between oxidative stress, glutathione S-transferase polymorphisms and hydroxyurea treatment in sickle cell anemia. Blood Cells Mol Dis 47 (2011): 23–28.
22. Bid H K, Konwar R, Saxena M, Chaudhari P, Agrawal C G, et al. Association of glutathione S-tranferase (GSTM1, T1 and P1) gene polymorphisms with type 2 diabetes mellitus in north Indian population. J Postgrad Med 56 (2010): 176-181.
23. Koch F P, Kämmerer P W, Kämmerer P, Al-Nawas B, Brieger J. Influence of Class M1 Glutathione S-transferase

(GSTM1) Polymorphism on GSTM1 gene expression level and tumor size in oral squamous cell carcinoma. Oral Oncol 46 (2010): 128-133.

24. Janowiak B E, Hayward M A, Peterson F C, Volkman B F, Griffith O W. Gamma-glutamylcysteine Synthetase-Glutathione Synthetase: Domain Structure and Identification of Residues Important in Substrate and Glutathione Binding. Biochemistry 45 (2006): 10461-73.

25. Kang TJ, Chae GT. Detection of toll like receptor 2 (TLR2) mutation in the leprosy patients. Fems Immunol Med Microbial 31 (2001): 53-58.

26. Schröder N W, Morath S, Alexander C, Hamann L, Hartung T, et al. Lipoteichoic acid (LTA) of Streptococcus pneumoniae and Staphylococcus aureus activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. J Biol Chem 18 (2003): 15587-15594.

27. Kutukculer N, Yeniay B S, Aksu G, Berdeli A. Arg753Gln polymorphism of the human toll-like receptor- 2 gene in children with recurrent febrile infections. Biochem Genet 45 (2007): 507–514.

28. Boraska Jelavić T, Barisić M, Hofman I D, Boraska V, Vrdoljak E, et al. Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. Clin Genet 70 (2006): 156–160.

29. Pandey S, Mittal R D, Srivastava M, Srivastava K, Singh S, et al. Impact of Toll-like receptors [TLR] 2 (-196 to -174 del) and TLR 4 (Asp299Gly, Thr399Ile) in cervical cancer susceptibility in North Indian women. Gynecol Oncol 114 (2009): 501-505.

30. David S, Aguiar P, Antunes L, Dias A, Morais A, et al. Variants in the non-coding region of the TLR2 gene associated with infectious subphenotypes in pediatric sickle cell anemia. Immunogenetics 70 (2018): 37-51.

31. Noguchi E, Nishimura F, Fukai H, Kim J, Ichikawa K, et al. An association study of asthma and total serum immunoglobulin E levels for Toll-like receptor polymorphisms in a Japanese population. Clin Exp Allergy 34 (2004): 177-183.

32. Oliveira J G, Silva A E. Polymorphisms of the TLR2 and TLR4 Genes Are Associated with Risk of Gastric Cancer in a Brazilian Population. World J Gastroenterol 18 (2012): 1235–1242.

33. Wang F, Tahara T, Arisawa T, Shibata T, Nakamura M, et al. Genetic polymorphisms of CD14 and Toll-like receptor-2 (TLR2) in patients with ulcerative colitis. J Gastroenterol Hepatol 22 (2007): 925-929.

34. Nischalke H D, Coenen M, Berger C, Aldenhoff K, Müller T, et al. The toll-like receptor 2 (TLR2) -196 to -174 del/ins polymorphism affects viral loads and susceptibility to hepatocellular carcinoma in chronic hepatitis C. Int J Cancer 130 (2012): 1470–1475.

35. Woehrle T, Du W, Goetz A, Hsu H-Y, Joos T O, et al. Pathogen specific cytokine release reveals an effect of TLR2 Arg753Gln during Candida sepsis in humans. Cytokine 41 (2008): 322–329.

36. Tahara T, Arisawa T, Wang F, Shibata T, Nakamura M, et al. Toll-like receptor 2 (TLR) -196 to -174 del Polymorphism in gastroduodenal diseases in the Japanese population. Dig Dis Sci 53 (2008): 919-924.

37. Liu F, Lu W, Qian Q, Qi W, Hu J, et al. Frequency of TLR 2, 4, and 9 Gene Polymorphisms in Chinese Population and Their Susceptibility to Type 2 Diabetes and Coronary Artery Disease. J Biomed Biotechnol 2012 (2012): 373945.

38. Frodsham AJ, Hill AV S. Genetics of infectious diseases. Hum Mol Genet 13 (2004): 187-194.

39. Compton T, Belko J, Latz E, Golenbock D T, Finberg R W, et al. Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-like receptor 2. J Virol 77 (2003): 4588-4596.

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