Effect of Gum Arabic (Acacia Senegal)on C-reactive protein level among Sickle Cell Anemia patients

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Research note

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Abstract

Objectives: Inflammation is ongoing process among sickle cell anemia even during steady state. C reactive protein (CRP) is cardinal marker that utilized widely as inflammatory indicator. Gum Arabic (GA) is gummy exudates from Acacia Senegal tree. Fermentation by colonic bacteria increases serum butyrate concentrations, so considered as prebiotic agent. Gum Arabic (GA) has anti-inflammatory activity through butyrate. Earlier we proved that regular intake of GA increased fetal hemoglobin and anti-oxidant capacity most likely through raised level of butyrate, which would ameliorate symptoms of sickle cell anemia. Best of our knowledge this is the first study conducted to investigate GA intake on inflammatory markers among sickle patients.

Results : This was a retrospective study conducted on stored samples from trial of Gum Arabic and sickle cell anemia. Quantitative CRP was measured by Mindray BS 200 before and after Gum Arabic consumption for 12 weeks. Daily intake of GA significantly decreased C reactive protein level (P.V=001) (95% CI, 0.943 -3.098). No correlation between CRP and age, fetal hemoglobin, hemolysis markers and white blood cells. Our findings revealed novel effect of GA as anti-inflammatory agent can be consumed as natural dietary supplement to modulate disease severity and downregulate inflammatory process.

Trial registration: ClinicalTrials.gov Identifier: NCT02467257. Registered 3rd June 2015

Introduction:

Sickle cell Anemia (SCA) is an autosomal recessive genetic disease that results from solitary point mutation in position 6 of the β -globin chain, leading to production of hemoglobin S (HbS)(1). Africa is the main origin of the sickle (β S) mutations (2;3). Polymerization of deoxygenated sickle hemoglobin is the primary event in the molecular pathogenesis of sickle cell disease and is responsible for the vasoocclusive phenomena which is the hallmark of the disease(4). SCD has long been recognized as an inflammatory condition and oxidative stress plays important role in pathophysiology of SCA(5). SCA Patients have multiple indicators of an inflammatory response, including raised white cell counts, C-reactive protein (CRP) levels, cytokines, as well as activated monocytes, neutrophils, platelets, and endothelial cells(4). Liver produced CRP as part of the acute phase reaction, in response to a host of pro inflammatory cytokines (6;7). CRP has wide acceptance as reliable indicator of systematic inflammation and tissue damage (7;8). Elevated levels of CRP, as a general marker of inflammation, have been previously reported in patients with SCD(5) and in transgenic sickle cell mice(9). Elevated CRP in SS patients may be in response to endothelium damage due to vascular endothelium blockage by sickle erythrocytes(5). Further, it seem like CRP is elevated even during free crisis time i.e. among steady state condition(6;10).CRP elevation during steady state may be attributed to sub clinical vaso occlusive episodes, which raise covert inflammatory response (10). This response is mediated by cytokines primarily IL6 (10).

The US FDA recognized Gum Arabic (GA) as one of the safest dietary fibres (11;12). GA is indigestible for both human and animals; Its fermentation by colonic intestinal bacteria leads to formation of various degradation products, such as short-chain fatty acids(13). Gum Arabic ingestion increases serum short chain fatty acid concentration, mainly butyrate and propionate (11;14). Serum butyrate concentration increased following administration of GA in healthy subjects (11;15). Butyrate has a potent anti-inflammatory effect. It decreases the pro-inflammatory cytokine expression through inhibition of NFκB(16;17). Oral intake of GA has been shown to provide several health benefits(18), such as prebiotic effects(12). GA has immune-modulatory (13;19), anti-inflammatory(20), and antioxidant properties (11;12;21;22).

We hypothesized GA degradation delivers short chain fatty acids, which in turn have been shown to stimulate fetal hemoglobin expression in RBCs as studied previously(23). Also, serves as anti-inflammatory agent through SCFA production and provide some protection against damaging effects of inflammation and vaso-occlusive crisis. The present study tested whether Gum Arabic may influence the CRP level.

To the best of our knowledge, this is the first study conducted to investigate the effect of oral administration of GA on inflammatory markers in sickle cell anemia patients.

Methods:

This is retrospective study conducted on stored blood samples of GA and Sickle cell clinical trial. Entry criteria, clinical monitoring, and laboratory measurements have been described in detail previously(23). Patients were in steady state as define as crisis- free period for three weeks and three months and more after last blood transfusion(10). Blood samples were collected before administering GA and after 12 weeks,

Two ml in EDTA container, Three ml in plain container. The serum and plasma was separated by centrifugation and stored at -85°C. Blood samples were stored between twelve to eighteen months prior to analysis. Studies revealed that CRP can remains stable more than ten years when kept at/less than -80 °C (24, 25)

Gum Arabic administration:

GA dose and administration were described in details in the previous report(23). Properties and composition of GA are listed elsewhere (21).

Quantitative CRP was measured by Mindray BS 200 using turbidimetry method and expressed in mg/L. The principle of the test: Determination of the concentration of CRP through photometric measurement of immunocomplex between antibodies of CRP and CRP present in the sample, the absorbency increase is directly proportional to the concentration of CRP(26).

Data were analyzed using SPSS version 24. Paired samples T test was used to compare between pre and post intervention results. Person correlation was utilized to study correlation between contentious variables. P values equal or less than 0.05 was considered significant.

Results:

Thirty-four samples were available for CRP analysis before and after GA administration. Patients' background characteristics are presented in Table 1. All were Sudanese; 50% were males (age 5 to 42 years). Five patients were on a stable dose of hydroxyurea 500gram per day. Duration of treatment was for 12 weeks.

Daily oral intake of GA significantly decreased CRP level (Fig 1).

CRP showed no significant correlation with age, HbF level, TWBCs counts, Platelets count, and LDH level (Table 2).

Discussion:

Sickle Cell Disease is the most common hemoglobin defect around the globe, with a high incidence in sub-Saharan Africa(27). There is strong evidence generating a close connection between chronic inflammatory processes and sickle cell disease (28;29). Which it seems as inherent characteristic of sickle endothelia cells(29). Inflammation has fundamental role in many comorbidity and mortality associated with SCD like acute chest syndrome for example. CRP is the most commonly assessed marker for acute and chronic inflammation(28). In the current study, we revealed no significant relation between CRP level and Hb F (table 2). Our results are comparable to earlier study, who interpret their results to other external factors like inflammation and vaso-occlusion due to SCA(6). Monocytes, neutrophils, and platelets are also actively involved in the various adhesive interactions and clinical manifestations(30). Chronic hemolysis plays major role in inflammation, among steady-state HbSS patients likely through subclinical vascular endothelial injury and transient vasoocclusive events (9). Conversely, none of hemolytic markers (LDH, Reticulocyte counts, Hb concentration) correlated statistically with CRP level in this study (table 2). CRP level could be activated by other pro-inflammatory cytokines such as Tumor Necrosis Factor α and IL-1b(9), which found consistently elevated among SCA patients(31). These results were confirmed later by other investigators who found no significant difference between TNF α and other inflammatory cytokines level between SCA in steady state and in vaso-occlusive crisis(30).

GA significantly decreased CRP level (Figure 1). This novel effect of GA may be of great importance, since inflammation is a cardinal component of the pathophysiology of SCD (32). Earlier studies revealed GA anti-inflammatory effects as it decreased several inflammatory markers as TNF α , ESR and CRP (19;20). The reduction of CRP level could be accredited to GA prebiotics properties. Since several studies

proposed that alteration in gut microbiota can alleviate inflammation (33-35). SCFAs in particular butyrate have strong anti-inflammatory effect (16;17;36).

GA clinical trial among sickles induced HbF production(23)and this may have a role in reduction of ongoing inflammatory process and decreases CRP level. Nevertheless, we found no significant correlation between CRP and fetal hemoglobin (Table 2). On the other hand, there is strong relation concerning oxidative stress and inflammation and both are linked to SCD pathogenesis (5;32;37-39). Earlier we documented GA exhibited strong anti-oxidant properties among SCA patients(22). Therefore, reduction in CRP could be secondary to drop in oxidative stress markers. Numerous antioxidant therapies elicit anti-inflammatory responses (32).

In conclusion, our results reveled that inflammation among Sickle cell patients is ongoing process even during steady state period. GA significantly decreased CRP level, findings revealed an innovative effect of GA, which might be consumed as natural dietary fiber to attenuate inflammation in SCD patients and other pathogenesis linked with inflammatory process.

Limitations:

The short trial duration precludes us to confirm the clinical significance of our results in modulation of disease severity and related mortality. The study is single arm with no control group. The inference of our findings, that GA displays anti-inflammatory action among SCA patients. Longer and multi arm studies are recommended to validate our findings.

Abbreviations

CRP: C reactive protein GA: Gum Arabic, Hb: Hemoglobin, HbF: Fetal hemoglobin, IL6: Interleukin 6, LDH: Lactate dehydrogenase SCD: Sickle cell disease. SCA: Sickle cell anemia,

Declarations

Ethics approval and consent to participate:

The study was ethically approved from the Institutional Review Board at Alneelain University and from Research Ethics Committee- Sudan Academy of Sciences. Stored blood samples were coded, not identified by names. Data were kept anonymous. Informed consent is not applicable for this particular study.

Consent for publication:

Not Applicable

Availability of data and material:

The datasets used and/or analyzed during the current study are included in the main text. Further data can be obtained from the corresponding author on reasonable request.

Competing interests:

No conflicts of interest, financial or otherwise, are declared by the authors

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Supporting fund was obtained from Alneelain University Khartoum Sudan to principal Investigator LK. Funding bodies did not interfere in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contribution

LK, and AK participated in study design. LK and AK, were involved in all aspects of the study conduct. LK and AK analyzed data. AK performed laboratory studies. LK, and AK participated in the writing and review of the manuscript. All authors approved final version of manuscript.

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References

- (1) Fathallah H, Atweh GF. Induction of fetal hemoglobin in the treatment of sickle cell disease. Hematology Am Soc Hematol Educ Program 2006;58-62.
- (2) Diallo D, Tchernia G. Sickle cell disease in Africa. Current opinion in hematology 2002;9(2):111-6.
- (3) Makani J, Ofori-Acquah SF, Nnodu O, Wonkam A, Ohene-Frempong K. Sickle cell disease: new opportunities and challenges in Africa. ScientificWorldJournal 2013;2013:193252.
- (4) Belcher JD, Bryant CJ, Nguyen J, Bowlin PR, Kielbik MC, Bischof JC, et al. Transgenic sickle mice have vascular inflammation. Blood 2003 May 15;101(10):3953-9.
- (5) Bhagat S, Patra PK, Thakur AS. Association of Inflammatory Biomarker C-Reactive Protein, Lipid Peroxidation and Antioxidant Capacity Marker with HbF Level in Sickle Cell Disease Patients from Chattisgarh. Indian J Clin Biochem 2012 Oct;27(4):394-9.
- (6) Krishnan S, Setty Y, Betal SG, Vijender V, Rao K, Dampier C, et al. Increased levels of the inflammatory biomarker C-reactive protein at baseline are associated with childhood sickle cell

vasocclusive crises. Br J Haematol 2010 Mar;148(5):797-804.

(7) Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003 Jun;111(12):1805-12.

(8) Hirschfield GM, Pepys MB. C-reactive protein and cardiovascular disease: new insights from an old molecule. QJM 2003 Nov;96(11):793-807.

(9) Archer DR, Stiles JK, Newman GW, Quarshie A, Hsu LL, Sayavongsa P, et al. C-reactive protein and interleukin-6 are decreased in transgenic sickle cell mice fed a high protein diet. J Nutr 2008 Jun;138(6):1148-52.

(10) Bourantas KL, Dalekos GN, Makis A, Chaidos A, Tsiara S, Mavridis A. Acute phase proteins and interleukins in steady state sickle cell disease. Eur J Haematol 1998 Jul;61(1):49-54.

(11) Ali BH, Ziada A, Blunden G. Biological effects of gum arabic: a review of some recent research. Food Chem Toxicol 2009 Jan;47(1):1-8.

(12) Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM. Effects of Gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. Nutr J 2012;11:111.

(13) Ballal A, Bobbala D, Qadri SM, Foller M, Kempe D, Nasir O, et al. Anti-malarial effect of gum arabic. Malar J 2011;10:139.

(14) Tulung B, Remesy C, Demigne C. Specific effect of guar gum or gum arabic on adaptation of cecal digestion to high fiber diets in the rat. J Nutr 1987 Sep;117(9):1556-61.

(15) Matsumoto N, Riley S, Fraser D, Al-Assaf S, Ishimura E, Wolever T, et al. Butyrate modulates TGF-beta1 generation and function: potential renal benefit for Acacia(sen) SUPERGUM (gum arabic)? Kidney Int 2006 Jan;69(2):257-65.

(16) Luhrs H, Gerke T, Muller JG, Melcher R, Schaubert J, Boxberge F, et al. Butyrate inhibits NF-kappaB activation in lamina propria macrophages of patients with ulcerative colitis. Scand J Gastroenterol 2002 Apr;37(4):458-66.

(17) Luhrs H, Gerke T, Schaubert J, Dusel G, Melcher R, Scheppach W, et al. Cytokine-activated degradation of inhibitory kappaB protein alpha is inhibited by the short-chain fatty acid butyrate. Int J Colorectal Dis 2001 Aug;16(4):195-201.

(18) Nasir O. Renal and extrarenal effects of gum arabic (Acacia senegal)-what can be learned from animal experiments? Kidney Blood Press Res 2013;37(4-5):269-79.

- (19) Ali BH, Al-Husseni I, Beegam S, Al-Shukaili A, Nemmar A, Schierling S, et al. Effect of gum arabic on oxidative stress and inflammation in adenine-induced chronic renal failure in rats. *PLoS One* 2013;8(2):e55242.
- (20) Kamal E, Kaddam LA, Dahawi M, Osman M, Salih MA, Alagib A, et al. Gum Arabic Fibers Decreased Inflammatory Markers and Disease Severity Score among Rheumatoid Arthritis Patients, Phase II Trial. *Int J Rheumatol* 2018;2018:4197537.
- (21) Nasir O, Umbach AT, Rexhepaj R, Ackermann TF, Bhandaru M, Ebrahim A, et al. Effects of gum arabic (*Acacia senegal*) on renal function in diabetic mice. *Kidney Blood Press Res* 2012;35(5):365-72.
- (22) Kaddam L, Fadl-Elmula I, Eisawi OA, Abdelrazig HA, Salih MA, Lang F, et al. Gum Arabic as novel anti-oxidant agent in sickle cell anemia, phase II trial. *BMC Hematol* 2017;17:4.
- (23) Kaddam L, FdleAlmula I, Eisawi OA, Abdelrazig HA, Elnimeiri M, Lang F, et al. Gum Arabic as fetal hemoglobin inducing agent in sickle cell anemia; in vivo study. *BMC Hematol* 2015;15:19.
- (24) Doumatey AP, Zhou J, Adeyemo A, Rotimi C. High sensitivity C-reactive protein (Hs-CRP) remains highly stable in long-term archived human serum. *Clin Biochem* 2014 Mar;47(4-5):315-8.
- (25) Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. *Clin Diagn Lab Immunol* 2003 Jul;10(4):652-7.
- (26) Tugirimana PL, Holderbeke AL, Kint JA, Delanghe JR. A new turbidimetric method for assaying serum C-reactive protein based on phosphocholine interaction. *Clin Chem Lab Med* 2009;47(11):1417-22.
- (27) Diallo DA, Guindo A. Sickle cell disease in sub-Saharan Africa: stakes and strategies for control of the disease. *Curr Opin Hematol* 2014 May;21(3):210-4.
- (28) Damanhour GA, Jarullah J, Marouf S, Hindawi SI, Mushtaq G, Kamal MA. Clinical biomarkers in sickle cell disease. *Saudi J Biol Sci* 2015 Jan;22(1):24-31.
- (29) Sakamoto TM, Lanaro C, Ozelo MC, Garrido VT, Olalla-Saad ST, Conran N, et al. Increased adhesive and inflammatory properties in blood outgrowth endothelial cells from sickle cell anemia patients. *Microvasc Res* 2013 Nov;90:173-9.
- (30) Pathare A, Al KS, Alnaqdy AA, Daar S, Knox-Macaulay H, Dennison D. Cytokine profile of sickle cell disease in Oman. *Am J Hematol* 2004 Dec;77(4):323-8.
- (31) Francis RB, Jr., Haywood LJ. Elevated immunoreactive tumor necrosis factor and interleukin-1 in sickle cell disease. *J Natl Med Assoc* 1992 Jul;84(7):611-5.

(32) Owusu-Ansah A, Ihunnah CA, Walker AL, Ofori-Acquah SF. Inflammatory targets of therapy in sickle cell disease. *Transl Res* 2016 Jan;167(1):281-97.

(33) Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 2009 Aug;58(8):1091-103.

(34) Kang Y, Cai Y, Zhang X, Kong X, Su J. Altered gut microbiota in RA: implications for treatment. *Z Rheumatol* 2017 Jun;76(5):451-7.

(35) Khanna S, Jaiswal KS, Gupta B. Managing Rheumatoid Arthritis with Dietary Interventions. *Front Nutr* 2017;4:52.

(36) Cavaglieri CR, Nishiyama A, Fernandes LC, Curi R, Miles EA, Calder PC. Differential effects of short-chain fatty acids on proliferation and production of pro- and anti-inflammatory cytokines by cultured lymphocytes. *Life Sci* 2003 Aug 15;73(13):1683-90.

(37) Jison ML, Munson PJ, Barb JJ, Suffredini AF, Talwar S, Logun C, et al. Blood mononuclear cell gene expression profiles characterize the oxidant, hemolytic, and inflammatory stress of sickle cell disease. *Blood* 2004 Jul 1;104(1):270-80.

(38) Emokpae MA, Uadia PO, Gadzama AA. Correlation of oxidative stress and inflammatory markers with the severity of sickle cell nephropathy. *Ann Afr Med* 2010 Jul;9(3):141-6.

(39) Biswal S, Rizwan H, Pal S, Sabnam S, Parida P, Pal A. Oxidative stress, antioxidant capacity, biomolecule damage, and inflammation symptoms of sickle cell disease in children. *Hematology* 2018 Jul 16;1-9.

Tables

Table 1: Demographics and Baseline Characteristics

Characteristics	Mean	SD	Median	Minimum	Maximum
Age	15.65	8.9	14	5	42
Gender	17(50%) Male				
Base line weight (Kg)	35.34	1.68	35	13	63
Base line height (Cm)	147.1	23.21	154.5	107	190
Hb (g/dL)	7.28	1.105	7	11	5.5
Hb F (%)	7.83	6.53	6.2	.00	29.60
Hb S (%)	88.89	6.22	91	68.00	97.00
CRP (mg/L)	4.22	5.89	2.3	0.10	27.80
WBCs (10 ³ /uL)	14.51	4.45	13.8	7.60	26.00
LDH (U/L)	688.19	220.44	630	352.00	1250.00

Table 2: Correlation of different biomarkers with CRP level among SCA patients

Bio marker	Person Correlation	P value
Age	-.091	0.608
Hb F	-.133	0.452
Hemoglobin	.169	0.339
WBCs	.155	0.383
Platelets	-.124	0.486
Reticulocytes	.146	0.589
LDH	-.300	0.101
Urea	-.180	0.333
Creatinine	.070	0.710

Figures

Figure 1

Effect of GA intake on CRP (P. Value=0.001) * indicates significant difference from baseline.