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Short (GT)n Microsatellite Repeats in the Heme Oxygenase-1 Gene Promoter Are Associated with Antioxidant and Anti-Inflammatory Status in Mexican Pediatric Patients with Sepsis

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An adequate immune and antioxidant response is a key to the resolution of sepsis. Heme oxygenase-1 (HMOX1) is a stress protein with a polymorphic (GT)n repeat in its gene promoter that regulates its expression in response to oxidative injury, such as that present in sepsis. HMOX1 is the rate-limiting enzyme of heme degradation, and the heme breakdown products, CO, Fe, and bilirubin, are considered to be biologically active metabolites with direct or indirect antioxidant and anti-inflammatory properties. In this study, we investigated the inflammatory and antioxidant response and the relationship with the HMOX1 levels and *HMOX1* polymorphism in Mexican septic pediatric patients. In a case-control pilot study, we enrolled 64 septic patients and 72 hospitalized control patients without a diagnosis of sepsis. DNA extracted from buffy coat was genotyped for *HMOX1* (GT)n polymorphism by PCR and markers of antioxidant and inflammatory status were quantified in plasma by analysis of the oxygen radical absorbance capacity (ORAC), protein carbonyl (PC), interleukin (IL) 6, IL10, and HMOX1 levels. In septic children, oxidative and inflammatory markers were elevated, and HMOX1 levels were positively correlated with IL10 levels. Genotypic and allelic distribution of *HMOX1* polymorphism showed no difference between groups. *HMOX1* short-allele septic carriers (< 25 GT repeats) presented favorable ORAC, PC and IL10 levels. This study confirms that an active response against pediatric sepsis involves the expression of HMOX1 and IL10, suggesting that the high antioxidant status associated with *HMOX1* short-allele septic carriers might provide a beneficial environment for sepsis resolution.

Keywords: genetic polymorphism; heme oxygenase; inflammatory cytokines; oxidative stress; pediatric sepsis
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Introduction

Sepsis is a leading cause of death in pediatric patients despite widespread advances in diagnosis and treatment. Sepsis is a clinical syndrome defined by physiological changes indicative of systemic inflammation, which are likely attributable to documented or suspected infection (Standage and Wong 2011). Complex host and microbial factors are involved in the physiological response to sepsis. The interaction between these factors may finally result in

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the complete resolution of the condition or on the other side, the manifestation of septic shock, multiorgan dysfunction syndrome, and finally death. The septic response involves inflammatory and anti-inflammatory components, as well as oxidative, humoral, and cellular reactions (Hotchkiss and Karl 2003; Grimble 2008). A set of proinflammatory cytokines, including interleukin (IL) 1, IL6, and TNF-α, are produced to combat the infection as the first response to sepsis. An increased amount of antioxidant (*e.g.*, thioredoxin, glutathione, superoxide dismutase) and anti-inflammatory (*e.g.*, IL10, IL4) molecules are generated along with the inflammatory process to protect from tissue damage and deactivate the inflammatory response as the body becomes repaired (Grimble 2008). Additionally, synthesis of stress proteins is induced as a cell protecting mechanism against the "collateral damage" generated by the inflammatory septic response (Low-Friedrich et al. 1992).

A stress-responding molecule that plays a critical role in defending the body against oxidant-induced injury during inflammatory processes is heme oxygenase-1 (HMOX1), where elevated plasma concentrations of HMOX1 have been described in septic patients and experimental models of sepsis syndrome (Fujii et al. 2003; Takaki et al. 2010). HMOX1 is a cytoprotective enzyme that has anti-inflammatory, antioxidant, antiapoptotic, and antiproliferative effects. HMOX1 catalyzes the breakdown of heme to generate carbon monoxide, iron, and biliverdin. Each of these products plays an important function in the cellular protection against stress, presenting direct (carbon monoxide) or indirect (biliverdin through bilirubin, and iron through ferritin induction) antioxidant and anti-inflammatory actions (Morse and Choi 2005). Induction of HMOX1 is generated by exposure to various oxidative stress-related agents, such as heme, hyperoxia, hypoxia, heat shock, endotoxins, hydrogen peroxide, heavy metals, and nitric oxide, which has led to inference of the importance of HMOX1 expression in maintaining homeostasis of organ functions (Hayashi et al. 1999; Morse and Choi 2002). The human *HMOX1* gene is primarily regulated at the transcription level; this gene has a polymorphic (GT)n repeat in the 5′-flanking region that modulates the quantitative level of HMOX1 activity in response to a given stimulus and thus confers a protective factor against various stress-related illnesses. The identification of this polymorphic genetic marker has allowed the study of the involvement of HMOX1 in an extensive number of human diseases (Kimpara et al. 1997). Several studies have demonstrated that in individuals with short (GT)n repeats (where $n < 25$ or $n < 27$, according to the cutoff of different studies), higher expression levels of HMOX1 are induced and have a decreased susceptibility to oxidant-related diseases than in individuals with long (GT)n repeats (where $n \geq 25$) (Yamada et al. 2000; Exner et al. 2004; Wagener et al. 2008). Although most of the evidence indicates higher protection for subjects carrying the short (S)-allele for several oxidative and inflammatory diseases, some studies have found no relationship with the S-allele or the long (L)-allele on the *HMOX1* (GT)n promoter variant with a given disease or surgical interventional outcome (Kanai et al. 2003; Hausmann et al. 2008); and some other studies have established that the S-variant is a risk factor (Takeda et al. 2005; Okamoto et al. 2006; Bozkaya et al. 2010; Cordova et al. 2012).

Thus, in order to determine whether the S-allele HMOX1 promoter and higher HMOX1 expression might act as beneficial factors in protecting against sepsis among Mexican pediatric patients, we investigated the association of the HMOX1 (GT)n promoter polymorphism and HMOX1 expression with oxidative and inflammatory status by evaluating total antioxidant capacity, carbonyl proteins, IL6, and IL10 plasmatic levels.

Materials and Methods

Study design and participants

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee of the Universidad de Guadalajara and the Hospital Civil de Guadalajara, México. Informed consent was obtained from the legal guardians of each pediatric patient prior to inclusion in the study. A total of 136 patients between the ages of 2 months to 18 years were recruited from October 2010 to March 2011. Of these patients, 64 children developed sepsis, and 72 children were non-septic controls who did not show suspicious symptoms or develop signs of infection during their hospitalized stay in the Intensive care unit or the Infectology pediatric unit of the Hospital Civil de Guadalajara. Furthermore, 101 blood bank donors from the general population, who had previously provided informed consent for genetic studies, were included as healthy controls for the *HMOX1* (GT)n genotyping association analysis. All subjects were ethnically representative of the population in the occidental region of México.

Diagnosis of sepsis was made in accordance with the modified criteria from the 2005 International Pediatric Sepsis Consensus conference (Goldstein et al. 2005). Sepsis was defined as the presence of systemic inflammatory response syndrome and an infection proven by a positive blood culture or bacterial culture from a normally sterile site, or with high suspicion of infection by clinical or radiological signs. Although a bacterial infection may often be confirmed by culture or other methods, other pathogens like virus, fungi, and rickettsiae may not be positively confirmed. In general, to be diagnosed with sepsis, children must have a probable or confirmed infection and exhibit at least two of the following symptoms: 1) core temperature above 38.5°C or below 35°C; 2) tachycardia, defined as a heart rate higher than 2 standard deviation above normal for age in absence of external stimulus, or bradychardia, defined as a mean heart rate below 10th percentile for age in the absence of external vagal stimulus; 3) mean respiratory rate higher than 2 standard deviation above normal for age; and 4) leukocyte count elevated or depressed for age or > 10% immature neutrophils (Goldstein et al. 2005).

DNA extraction and HMOX1 genotyping

Anticoagulated blood samples were collected within the first 24 h after an overnight fasting period, ensuring that the criteria for diagnosing or excluding sepsis among controls were met. Samples were

processed within 2 h to obtain plasma and buffy coat by density gradient centrifugation at 200 × *g* for 10 min, and these fractions were stored at −80°C until assayed. Genomic DNA was extracted from the buffy coat using a standard phenol-chloroform method (Sambrook and Rusell 2001). The 5′-flanking region of the *HMOX1* gene containing the (GT)n dinucleotide repeat was amplified by end-point PCR on DNA extracted from buffy coats using a sense primer 5′-AGAGCCTGCAGCTTCTCAGA-3′, and an antisense primer 5′-ACAAAGTCTGGCCATAGGAC-3′, as previously published (Denschlag et al. 2004). Briefly, a 10-min initial denaturation step at 95°C was followed by 35 cycles of 30 s at 95°C, 30 s at 60°C, and 2 min at 72°C; a final extension step at 72°C for 15 min completed the reaction. The PCR products were separated by size using 12% polyacrylamide gel electrophoresis and analyzed after silver staining. A size marker of 10 pb (Invitrogen, Carlsbad, CA) and *Φ*X174 DNA/*Hinf*I (Promega, Madison, WI) was used in every electrophoresis run for correct allele assessment. Genotypes were classified as S-allele to those with < 25 GT repeats (PCR fragments less than 117 bp, according to *HMOX1* GenBank sequence X14782), and L-alleles with ≥ 25 dinucleotide repeats, as reported elsewhere (Yamada et al. 2000; Chen et al. 2002; Wagener et al. 2008).

Determination of total antioxidant capacity

The plasmatic oxygen radical absorbance capacity (ORAC) assay was carried out as previously described (Cao et al. 1993). Briefly, deproteinized plasma, or vitamin E analogue Trolox calibration standard, were incubated with 14 mM fluorescein for 30 min at 37°C in a 96-well microplate. Then, 153 mM AAPH (2,2′-azobis (2-amidino-propane) dihydrochloride) was added to start the peroxyl reaction. The fluorescence (Ex, 485 nm; Em, 520 nm) was monitored at 1-min intervals for 1 h by a microplate reader (BioTek Instruments; Winooski, VT). Results are given as μmol of Trolox equivalents/L (μmol TE/L).

Determination of protein carbonyls

This assay was performed as described by Levine (Levine et al. 1994). Briefly, an aliquot of plasma was diluted with 2% DNPH (2,4-dinitrophenylhydrazine) in 2.5 M HCl, and another aliquot was diluted with a 2.5 M HCl solution as blank. After 30 min of incubation in the dark, samples were protein-precipitated with 50% trichloroacetic acid (TCA), centrifuged, and pellets were washed with ethanol-ethyl acetate (1:1) and solubilized with 6 M guanidine-HCl. Absorbance was measured (370 nm), and carbonyl concentration was calculated using the extinction coefficient of DNPH, $e = 2.2 \times 10^4$ M^{-1} cm^{-1}. Total protein content was determined using a bovine serum albumin standard curve. Results are expressed as nmol protein carbonyls/mg total protein.

Determination of HMOX1, IL6, and IL10

HMOX1, IL6, and IL10 were measured on plasma aliquots by commercial human solid phase sandwich enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) operating strictly according to the manufacturer instructions. Endpoint reading was determined by measuring the absorbance at 450 nm on a Synergy H4 microplate reader using Gen5 software (Biotek, Winooski, VT).

Statistics

The sample size of 51 subjects required to achieve 80% statistical power given an alpha of 0.05 and a one-sided comparison was calculated from data of Lublinghoff et al. (2009). All data were assessed using the Kolmogorov-Smirnov test. Parametric data were compared by Student's *t* test, and the U-Mann-Whitney test was used for non-parametric data. Chi-square and Fisher's exact test were used for categorical variables. Bivariate correlations were assessed by Spearman rank correlation. A probability value of $P < 0.05$ was considered significant. Statistical calculations were performed with SPSS 17.0 software (SPSS, Richmond, WA).

Results

Population characteristics

The demographic and clinical features of the pediatric patients with sepsis and the control patients who did not develop sepsis are summarized in Table 1. Both groups were similar in demographic background and underlying disease, with the main difference being the presence or absence of a proven infection. The microbiological and infection characteristics on septic diagnosis are shown in Table 2.

Oxidative status

The oxidative status of patients was assessed by measuring the ORAC potential and the PC content of plasma of both cases and control patients. Plasmatic ORAC levels were significantly lower in septic patients when compared to control subjects [566.0 μmol TE/L (46.7-1,404) vs. 724.2 μmol TE/L (74.2-1,436); $P = 0.027$] [median (interquartile range)] (Fig. 1A). In addition, higher carbonyl levels were observed in patients with sepsis as compared to control non-septic patients [0.875 nmol/mg (0.25-4.67) vs. 0.855 nmol/mg (0.08-1.92); $P = 0.045$] (Fig. 1B).

HMOX1 and cytokine biomarkers

Patients with sepsis showed a marked response to the infectious condition. In septic patients versus control patients, the antioxidant/anti-inflammatory HMOX1 plasmatic protein was increased [4.44 ng/mL (3.87-5.26) vs. 3.50 ng/mL (2.71-4.69)] [median (interquartile range)] (Fig. 2A). Also, IL10 [15.3 pg/mL (8.42-62.2) vs. 4.72 pg/mL (1.70-13.3)] (Fig. 2B), and IL6 [24.0 pg/mL (7.62-43.6) vs. 16.4 pg/mL (6.47-24.7)] (Fig. 2C) cytokines were significantly elevated in those patients with sepsis.

HMOX1 genotype and allelic frequencies

The genotyping of the (GT)n promoter polymorphism in *HMOX1* was carried out by electrophoresis of PCR (GT)n repeats on the basis of differential mobility of amplicons with different sizes. The alleles were classified into two subgroups: the short "S" class with (GT) repeat numbers < 25, and the long "L" class with (GT) repeat numbers ≥ 25. Subjects were then classified as having an SS, SL, or LL genotype according to each of their *HMOX1* alleles. The genotype distribution of the (GT)n microsatellite polymorphism in the *HMOX1* promoter region among the general population did not deviate from the Hardy-Weinberg equilibrium ($P > 0.05$). Table 3 describes the genotype and

Table 1. Demographic characteristics of patients with sepsis and control patients without sepsis.

Variable	Sepsis (n = 64)	Control (n = 72)
Gender, n (%)		
Male	29 (45)	47 (65)
Female	35 (55)	25 (35)
Weight, mean ± sd		
Kg	25.2 ± 19.7	39.4 ± 23.4
Height, mean ± sd		
cm	119 ± 36.3	145 ± 29.6
BMI, mean ± sd		
Kg/m^2	15.7 ± 4.9	18.2 ± 6.9
Age range, n (%)		
1 mo - 1 yr	12 (19)	4 (6)
> 1 yr - 5 yr	14 (22)	2 (3)
> 5 yr - 12 yr	25 (39)	37 (51)
> 12 yr - < 18 yr	13 (20)	29 (40)
Underlying disease, n (%)		
Neurologic	19 (30)	17 (24)
Abdominal	16 (25)	13 (18)
Pulmonar	9 (14)	2 (3)
Metabolic	3 (5)	0 (0)
Genetic	1 (2)	1 (1)
Cardiac	1 (2)	6 (8)
Oncological	1 (2)	2 (3)
None	0 (0)	4 (6)
Other	14 (22)	27 (38)

Table 2. Microbiological and infection characteristics of the septic patients.

Sepsis classification, n (%)		Culture, n (%)		Assessment of infection, n (%)			
Sepsis	60 (94)	Positive	22 (34)	**Gram + bacteria**	**9** (41)	**Gram − bacteria**	**10** (46)
Severe sepsis	1 (1)	Negative	42 (66)	*S. aureus*	3 (14)	*E. coli*	5 (23)
Septic shock	3 (5)			*Staph. coag (−)*	2 (9)	*K. pneumoniae*	2 (9)
				S. pyogenes	1 (4)	*P. aeruginosa*	2 (9)
				Other	3 (14)	*E. cloacae*	1 (9)
				Candida spp	**2** (9)	**Mix**	**1** (4)

allele frequencies of the general population, septic patients, and control patients without sepsis. The SS genotype was present in 2% of the septic patients, and it was not detected in the control group. No significant difference in genotypic or allelic frequencies between the two groups of patients versus the general population was observed ($P > 0.05$).

Association of HMOX1 genotype with oxidative and inflammatory biomarkers

Carriers of an S-allele (heterozygotes and homozygotes) were compared to assess statistical power with L-homozygotes as the S-allele being associated with higher rates of *HMOX1* transcription (Baan et al. 2004). As shown in Table 4, septic patients with an S-allele had higher ORAC potential and lower protein carbonyls levels when compared with those with the LL genotype ($P = 0.011$ and $P = 0.013$, respectively). The *HMOX1* S-allele carrier state was associated with a significantly higher HMOX1 protein levels in the control group ($P = 0.044$). No significant differences in the IL6 or IL10 cytokines were demonstrable among the *HMOX1* genotypes either for the case or control groups.

Spearman correlations

A positive discrete correlation was observed between HMOX1 and IL10 levels ($\rho = 0.271$) and the levels of IL10

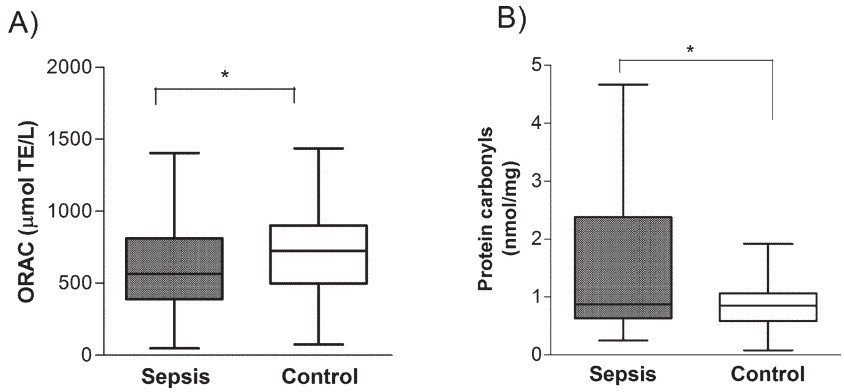

Fig. 1. Plasmatic antioxidant status of patients with sepsis and control patients without sepsis.
A) Total antioxidant capacity measured by the ORAC assay from septic (n = 58, *gray bars*) and control (n = 64, *white bars*) patients. B) Protein carbonyls from septic (n = 62, *gray bars*) and control (n = 62, *white bars*) patients. Data are presented as box plots with median and interquartile range ± maximum and minimum values. *P < 0.05, significantly different by Mann-Whitney-U test.

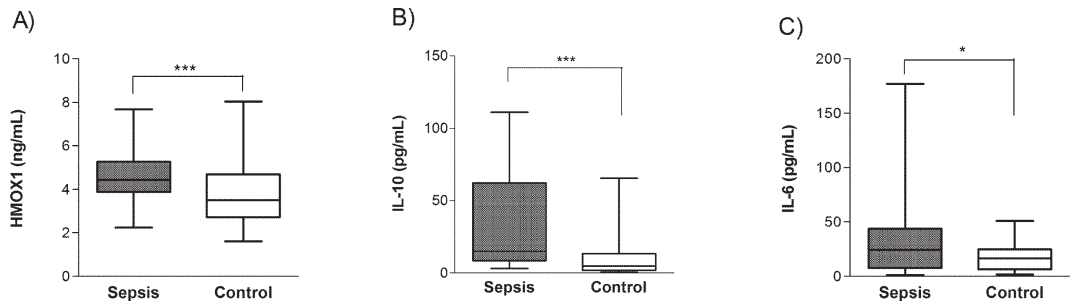

Fig. 2. Plasmatic HMOX1 and cytokine levels of patients with sepsis and control patients without sepsis as determined by enzyme-linked immunosorbent assay.
A) HMOX1 protein levels from septic (n = 62, *gray bars*) and control (n = 66, *white bars*) patients, ***P = 0.0003. B) IL10 from septic (n = 38) and control (n = 38) patients, ***P = 0.0001. C) IL6 from septic (n = 38) and control (n = 38) patients, *P = 0.038. Data are presented as box plots with median and interquartile range ± maximum and minimum values. All statistical analysis was calculated from Mann-Whitney-U test.

Table 3. Allele and genotype frequencies for dinucleotide (GT)n *HMOX1* polymorphism between general population, septic patients and control patients without sepsis.

	General population n = 101 n (%)	Sepsis n = 56 n (%)	P †	Control n = 62 n (%)	P ‡	P ¥
Genotype						
LL	56 (55)	34 (61)		42 (68)		
SL	39 (39)	21 (37)	0.454	20 (32)	0.079	0.457
SS	6 (6)	1 (2)		0 (0)		
Allele						
L	151 (75)	89 (80)	0.422	104 (84)	0.072	0.480
S	51 (25)	23 (20)		20 (16)		

Chi-square test. †General population vs. septic patients; ‡General population vs. control patients without sepsis; ¥ Septic patients vs. control patients.

and protein carbonyls (ρ = 0.275) among septic patients, irrespective of the *HMOX1* genotype (P = 0.045). For control patients without sepsis, there was a negative discrete correlation between ORAC and protein carbonyl levels (ρ = −0.223; P = 0.037). When the *HMOX1* genotype was considered in the Spearman correlation analysis, carriers of the SL+SS *HMOX1* genotype among control patients showed a moderate positive correlation between HMOX1 and ORAC levels (ρ = 0.549; P = 0.011) and a negative association between IL10 and IL6 levels (ρ = −0.486; P = 0.039),

Table 4. Association of dinucleotide (GT)n *HMOX1* promoter polymorphism with plasmatic antioxidant and inflammatory markers in septic patients and control patients without sepsis.

Variable		HMOX1 genotype		*P*
		LL	SL+SS	
ORAC [a] (mEq Trolox/L)	Sepsis	606.2 ± 40.4 (*n* = 33)	861.0 ± 123.0 (*n* = 20)	**0.011***
	Control	742.0 ± 41.5 (*n* = 38)	691.3 ± 85.9 (*n* = 18)	0.275
Protein carbonyls [b] (nmol/mg)	Sepsis	0.88 (0.66-1.60) (*n* = 32)	0.65 (0.38-0.91) (*n* = 20)	**0.013***
	Control	0.80 (0.50-1.15) (*n* = 36)	0.97 (0.8-1.19) (n = 16)	0.06
HMOX1 [a] (ng/mL)	Sepsis	4.31 ± 0.16 (*n* = 32)	4.55 ± 0.27 (*n* = 22)	0.21
	Control	3.54 ± 0.20 (*n* = 39)	4.23 ± 0.40 (*n* = 18)	**0.044***
IL-6 [b] (pg/mL)	Sepsis	27.41 (7.55-42.72) (*n* = 23)	15.07 (5.72-34.09) (*n* = 13)	0.224
	Control	16.36 (6.47-24.66) (*n* = 28)	17.66 (6.91-24.63) (*n* = 12)	0.389
IL-10 [b] (pg/mL)	Sepsis	14.9 (8.10-62.51) (*n* = 25)	15.35 (8.84-64.33) (*n* = 14)	0.482
	Control	4.54 (1.33-14.1) (*n* = 26)	3.66 (1.85-6.93) (*n* = 10)	0.382

Values are: [a]mean ± SEM analyzed by non paired *t*-student test; [b]median (interquartile range) analyzed by Mann-Whitney-*U* test; **P* < 0.05, significantly different.

whereas carriers of the LL *HMOX1* genotype among control patients showed a moderate negative correlation between IL10 and ORAC ($\rho = -0.410$; $P = 0.036$). No statistically significant correlations in oxidative and inflammatory markers were observed for septic patients when considering their *HMOX1* genotype.

Discussion

Sepsis syndrome is the result of a systemic inflammatory response to infection. The individual ability to resist infection is determined by many factors including the virulence of the organism, the size of the inoculums, the coexisting conditions of the patient, and the genetic inheritance of critical polymorphic inflammatory genes (Villar et al. 2004). The important antioxidant and anti-inflammatory properties of HMOX1 have prompted the analysis of the influence of this protein on the course of sepsis. In the present exploratory study, we evaluated the antioxidant and inflammatory conditions on pediatric septic patients in the western region of México and analyzed the relationship between their *HMOX1* polymorphisms and the selected oxidative and inflammatory markers.

In our study, septic patients showed an increase in oxidative stress when compared to control patients without sepsis, as observed by a decrease in the plasmatic ORAC potential and an increase in protein carbonyl plasmatic levels. Oxidative stress has been documented in association with sepsis syndrome. Several authors have reported the implication of oxidative damage on the pathophysiology of sepsis in critically ill adults and children (Macdonald et al. 2003; Cancelier et al. 2009); however oxidative injury may have more serious consequences in pediatric patients than in older people because of the lower functional reserve and due to the need for subsequent tissue growth to match somatic and normal development (Tsukahara 2007; von Dessauer et al. 2011). In the children septic patients in our study we observed a mixed antioxidant, anti-inflammatory, and inflammatory response as described for adult septic patients. Pediatric septic patients significantly augmented the plasmatic levels of HMOX1, IL10, and IL6 as compared to control patients. In a multivariate analysis to evaluate for potential associations of oxidant or inflammatory

markers with demographic or infection characteristics of septic patients, no significant differences were detected. The increase in HMOX1, IL10, and IL6 levels has been previously described by several authors in neonate, pediatric, and adult septic patients as part of the complex response of the organism against sepsis (Wu et al. 2009; Briassoulis et al. 2010). Our results corroborate the association of HMOX1 and IL10 as a dual modulator of the anti-inflammatory response against sepsis, and importantly verify the dynamic nature of response in the pediatric septic patients under study. Remarkably, the significant increase of plasmatic IL10 levels when sepsis occurs (3.3 times), after the slight but significant increment on plasmatic HMOX1 expression (1.3 times) suggests an important signal amplification by HMOX to immunomodulate the response to pediatric sepsis, as observed by the HMOX induction of IL10 expression in murine models of sepsis (Tamion et al. 2006; Piantadosi et al. 2011), and the narrow threshold of HMOX1 expression required to afford protective effects in organ failure (Bauer et al. 2008).

Several genetic variants of critical immune and antioxidant genes have been evaluated for their association with sepsis morbidity and mortality. The present work is the first report on Mexican *HMOX1* promoter polymorphisms associated with pediatric sepsis. The distribution of the *HMOX1* genetic variance among control patients, septic patients, and the general population of western México noted in the present study showed no statistical difference for the S-allele frequency (16%, 20%, and 25%, respectively). A recent study on the association between the *HMOX1* promoter polymorphism and rheumatoid arthritis and lupus erythematosus in a Mexican population showed a similar S-allele frequency as was observed in the present study (14% for control subjects and rheumatoid arthritis patients, and 19% for patients with systemic lupus) (Cordova et al. 2012). The allele frequency observed in our work was closely approximated to those reported among controls in a Hispanic population in the United States (15%) (Islam et al. 2008), and individuals of Caucasian origin (20-31%) (Islam et al. 2008; Wagener et al. 2008; Lublinghoff et al. 2009), but it was largely different from those described among a Hispanic population in Chile (45%) (Arredondo et al. 2007), and from Asian subjects (43-56%) (Song et al. 2009; Kuesap et al. 2010) showing the ethnically based heterogeneity for this promoter sequence. A focused study with a larger number of subjects and analysis of ancestry informative markers in the admixed Mexican individuals would be required to address the corresponding prevalence of the promoter *HMOX1* genotype on the stratified Mexican population.

Taking into account the small sample size and genetic power of this preliminary study, the statistical analyses did not reveal a significant association of the *HMOX1* promoter S- or L-allele with the occurrence of pediatric sepsis in our group of study; however, the association between an S-allele among septic patients with higher antioxidant response (*i.e.*, increase in plasmatic ORAC values and a decrease in protein carbonyl levels) supports the idea that shorter variants of the *HMOX1* (GT)n polymorphism might play an important role in improving outcomes in sepsis severity. Similarly, carriers of the *HMOX1* SL+SS genotype in control patients showed a positive correlation between HMOX1 and ORAC levels and a negative correlation between IL10 and IL6 protein which reflects the significant influence of the S-allele towards a balanced antioxidant and anti-inflammatory profile, and suggests that when homeostasis is changed by septic disturbance, a new set of inflammatory cytokine and stress response genes interact as a response to the infection.

Evidence obtained under the conditions of this pilot study corroborates the oxidative insult and the antioxidant and anti-inflammatory response of pediatric patients under sepsis, and describes for the first time in a Mexican pediatric population the association of the S-allele on the *HMOX1* genetic polymorphism with favorable antioxidant conditions in septic and control patients. Findings from our work need to be followed in future studies among larger sample sizes to assess the impact of gene interactions with particular environmental or pathogen elements while grouping critical inflammatory and antioxidant genes to identify an haplotype prognostic factor.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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