SHORT REPORT
Total antioxidant capacity of plasma in asymptomatic carrier state of *Neisseria meningitidis*

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SUMMARY

Reduction of the antioxidant capacity of plasma has been linked with the impairment of an effective immune response and so we hypothesized that the carriage rate of *Neisseria meningitidis* in asymptomatic subjects might correlate with the levels of antioxidants in plasma. To this end we took pharyngeal swabs from 339 children in Marquesado Basic Health Zone, Granada, Spain and in addition determined the total antioxidant capacity (TAC) in plasma samples from these subjects. The overall prevalence of *N. meningitidis* carriage was 5·9% (mean age 7·1 years) with rates of 10·3% in children aged $\leqslant 3$ years, 3·9% between 4 and 7 years and 2·4% in older subjects. Plasma TAC for the $\leqslant 3$-year-olds was 0·13 for carriers and 1·10 for non-carrier controls ($P = 0.04$), 0·13 for carriers aged 4–7 years (controls 0·63) and 0·28 for carriers aged >7 years (controls 0·52). We analysed the association between TAC in plasma ($<0.37 - 2$ s.D.) and the carrier state of *N. meningitidis*. In the carrier state, the odds ratio for this association (TAC in plasma <0.25) was 8·44 (95% CI 1·5–48·9). These findings may suggest a reduced immune response in the host favourable to nasopharyngeal persistence of meningococci.

Research interest in asymptomatic carriers of *Neisseria meningitidis* has traditionally been based on the possibility that the host may carry and transmit potentially pathogenic strains. Indeed, asymptomatic carriers may be significantly more efficient transmitters of the organism than symptomatic individuals [1]. In such situations, after identifying the index case, chemoprophylaxis is carried out to eliminate the virulent strain in the population group [2, 3]. Also, strains of *N. meningitidis* considered to be non-virulent in the carriage state may become virulent when transplanted into communities different from those of their origin as a result of migratory movements [4, 5]. The virulence of the species is due, among other factors, to adaptive changes in the host environment and to the ease with which genetic information may be exchanged between bacteria [6]. The condition of the host together with the biodiversity of the carrier (facilitating the horizontal transmission of genetic material), are key elements in allowing the microorganism to avoid the host's immunological control mechanisms.

Moreover, the increasingly frequent and indiscriminate use of antibiotic treatments favours the periodic renewal of nasopharyngeal commensal flora and facilitates colonization by new bacteria, and thus genetic renewal. Prolonged contact between *N. meningitidis* and the host, under normal conditions, leads to the stimulation of antibodies to eliminate the bacteria from the nasopharynx. Various studies [7, 8] have reported a relation between low levels of immune response and oxidative stress particularly in individuals with tumours and rheumatic

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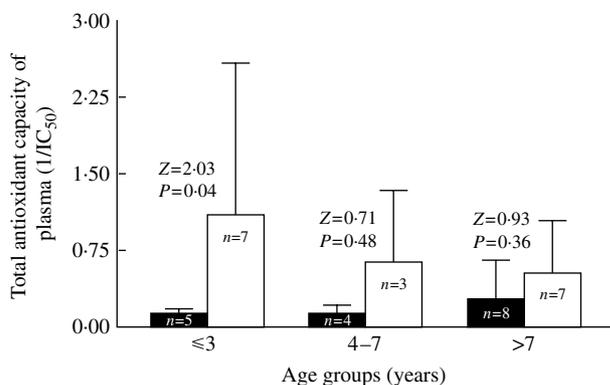(Email: joseuberos@telefonica.net)

Fig. Total antioxidant capacity in children carrying *N. meningitidis* (■) and controls (□) in the three age groups.

diseases. Furthermore immunological hyporesponsiveness can be reversed and normalized by antioxidant therapies [9].

In this study we sought to relate the prevalence of carriage of *N. meningitidis* with the total antioxidant capacity of plasma to determine whether low levels of antioxidants predispose individuals to carriage.

The study was carried out on the child population of the Marquesado Basic Health Zone (BHZ), in the province of Granada (Spain). Informed approval from parents or a guardian was received for all participating subjects (339 children) and the study was approved by the Committee of Ethics of the Hospital Clínico San Cecilio. Data were obtained concerning family history and consumption of antibiotics during the preceding 2 months.

Swabs were taken of the hypopharynx with the aid of a tongue depressor taking care to sample the tonsils, soft palate and pharynx without contact with the lips on withdrawal of the swab. The swab was maintained in Stuart's transport medium (Biomedics Ltd, Auckland, New Zealand) at 37 °C until being cultured on Müeller–Hinton agar which was incubated at 37 °C for 24 h. Gram-negative, oxidase-positive colonies were confirmed as *N. meningitidis* by their positive oxidation of glucose and maltose and negative reaction for lactose in Hugh and Leifson's medium.

Blood samples were taken from all subjects with EDTA-K as an anticoagulant. The plasma fractions were stored in aliquots at −40 °C until required. The determination of total antioxidant capacity (TAC) of the plasma is based on the fact that antioxidants inhibit the oxidization of crocin induced with the donor compound of free radicals 2,2′-azobis-2-methylpropionamidine (ABAP) and this is measured

by colorimetry [10, 11]. Briefly, 500 mg of saffron (Sigma-Aldrich, St Louis, MO, USA) were suspended in 20 ml diethylether (Sigma-Aldrich) and stirred for 2 min, after which the supernatant was discarded. This process was performed three times, and the residue was air-evaporated, resuspended in 15 ml methanol at 30 % (v/v) and stirred for 15 min at room temperature. The extract was passed through a 0·45 μm pore-diameter filter (Millipore Co., Billerica, MA, USA) and diluted to five times the original volume with 10 mM PBS (pH 7·4). The crocin concentration was adjusted to 25 μM by the addition of PBS. The sample was protected from light and stored at −40 °C in aliquots of 3 ml for a maximum of 2 months. The free radical donor used was ABAP, diluted (5 mg/ml) in PBS (10 mM, pH 7·4). The absorbance at 450 nm was measured twice with a blank of 400 μl crocin + 200 μl plasma + 400 μl PBS, and a sample mixture of 400 μl crocin + 200 μl plasma + 400 μl ABAP. In both cases, the mixtures were incubated for 20 min at 37 °C prior to measurements. The TAC of the plasma sample was calculated as TAC = 100 ($ABS_0 − ABS_1$)/ABS_0.

Statistical package SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used to calculate prevalence [95 % confidence interval (CI)] and association measures. The Mann–Whitney test was used to compare variables without normal distribution, and ANOVA of a factor and t test for the comparison of variables with normal distribution.

A cohort of 339 healthy children were recruited and grouped as cases (carriers of *N. meningitidis*) and controls (non-carriers). The mean age of cases was 6·2 years (95 % CI 4·3–8·1) and 7·1 years (95 % CI 6·7–7·6) for controls. The prevalence of carriers in the total study population was 5·90 % (95 % CI 3·64–8·96). In children aged ⩽3 years the carriage rate was 10·3 % (95 % CI 4·24–20·1), in those aged 4–7 years, 3·9 % (95 % CI 1·08–9·74), and for those aged >7 years, 2·4 % (95 % CI 0·64–5·94). There was no statistically significant difference between age groups in the prevalence of *N. meningitidis*.

As shown in the Figure, the TAC of plasma from children aged 1–3 years carrying *N. meningitidis* was 0·13 (95 % CI 0·07–0·19) and 1·10 (95 % CI −0·27 to 2·46) for controls ($P = 0·04$). For children aged 4–7 years, the TAC values were 0·13 (95 % CI 0·09–0·26) for carriers and 0·63 (95 % CI −1·10 to 2·39) in the controls ($P = 0·48$); in those aged >7 years the values were 0·28 (95 % CI −0·04 to 0·59) for carriers and 0·52 (95 % CI 0·06–0·99) for controls ($P = 0·36$).

According to the literature [10], the TAC of normal human plasma (expressed as the reciprocal of IC_{50}) is given as 0.37 ± 0.06. We analysed the association between TAC in plasma ($<0.37 - 2$ S.D.) and the carrier state of *N. meningitidis*. In the carrier state, the odds ratio (OR) for this association (TAC in plasma <0.25) was 8·44 (95 % CI 1·5–48·9) with a population attributable fraction of 77·8 % (95 % CI 47·2–108·4). We did not observe an association in our sample between consumption of antibiotics in the 2 months previous to the study and carriage of *N. meningitidis* (OR 1·35, 95 % CI 0·50–3·63).

Our results demonstrate that the prevalence of asymptomatic carriage of *N. meningitidis* is greater in children with diminished levels of plasma TAC; this difference being accentuated in the $\leqslant 3$ years age group. The oxidative balance in the plasma of carriers is biased towards the pro-oxidants, that is, there exists a relative deficit of antioxidants. The persistence of such a situation is known to impede an effective immune response [7, 12]. Indeed, a relative deficit of antioxidants has also been observed among tumour patients, for whom antioxidant treatment substantially improves the immune response. Thus, it is not surprising that a deficit of plasma antioxidants should impair the rapid, effective elimination of *N. meningitidis* from the nasopharynx. In line with our observations, other authors [13, 14], have demonstrated in animal models an increase of the phagocytic activity of leukocytes associated with a diet enriched in antioxidants. A positive association between irritants, such as tobacco smoke or repeated pharyngeal infections, and asymptomatic carriage of *N. meningitidis* has also been reported [15], but the reasons for this are unclear, but doubtless involve the effectiveness of mucociliary clearance mechanisms and the phagocytic capacity of polymorphonuclear cells or increase of oxidative local stress.

Our data show that 5·9 % of the children in the study group were asymptomatic carriers of *N. meningitidis*, a rate consistent with that reported for a population with a low incidence of endemic infection [16, 17]. However, the finding of a prevalence rate exceeding 10 % in children aged $\leqslant 3$ years is considerably higher than that reported by other authors [16, 18].

The assay of antioxidant activity by means of colorimetric techniques with crocin was developed by Lussignoli *et al.* [10] and subsequently automated by others [11]. The main advantage of this technique is that it enables the quantitation of antioxidant activity of plasma irrespective of whether or not the antioxidants involved are identified, with a precision of 4·8 %. Moreover, plasma samples can be frozen until required for measurement without affecting the results of the assay.

In summary our results show that asymptomatic carriers of *N. meningitidis* have consistently lower levels of antioxidants in plasma than do control subjects. We believe this situation may explain the altered immune response in the host, which enables the nasopharyngeal persistence of meningococcal bacteria.

DECLARATION OF INTEREST

None.

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