Hearing loss associated to ulcerative colitis

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Abstract: Objective: To value the eventual immunomediation in sensorineural hearing loss (SHL) on patients bearing of ulcerative colitis (UC). Material and methods: In a group of forty-nine cases with a mean age of 41.6 ± 9.3 years old we studied the hearing loss level, the disease activity index, the peripheral blood inflammation markers and the anticochlear antibodies by mean of western-blot technique (WB). Results: The 26.5% knew about their deafness, although SHL was detected in 59.1% of cases. The mean age of onset was 40.3 ± 9.8 years. 48.9% showed a positive WB, always in 68-70 kDa molecular weight blots. Moreover, patients with positive WB showed more severe deafness, higher disease activity and more altered parameters, specially erythrosedimentation rate. Conclusions: Audiologic and peripheral blood findings observed allow us to establish a reasonable suspicion of an autoimmune or immunomediated pathway of hearing loss on UC.

Key words: Deafness. Ulcerative colitis. Western-blot.

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory process of the digestive mucosa of the large intestine. Its cause is unknown and its evolution is recurrent, with outbursts and remissions which may derive from local complications and extra-digestive manifestations. The set of symptoms called inflammatory intestinal disease matches that of Crohn's disease (CD), but UC can be differentiated from the former by its mono-segmentary affectation in the colon and/or rectum and the limitation of its histopathological affectation in the form of granulations, erosion or ulcers at the mucosal surface.

With UC in particular, the deterioration of systemic immunity is significantly greater and is proportional to an increase in permeability of the colic wall. Because of this, it is not unusual to find diarrhea and abdominal pain associated with the intestinal symptoms of bleeding stools, symptoms more typical of distance immunomediated mechanisms, such as reactive arthritis and dermatitis, uveitis and vasculitis.

The association of progressive neurosensorial hearing loss (NHL) with UC was known of even before McCabe's description of autoimmune inner ear disease (AIED). In 1973, Levitan observed its presence in 46% of the study group as an effect of the enteropathy. In the following decade, numerous cases of the disease with concomitant deafness were reported: cases receiving steroid treatment, even on top of sulfasalazine - the specific treatment for inflammatory disease.

The immunomediation pathogen in hearing disorders in UC patients is strongly suspected. The appearance of laboratory tools that classify this disimmunity can help explain the generator mechanism of an occurrence which, in neurosensorial quality, is often irreversible and that nowadays is potentially diagnosable, predictable and even treatable.

Our objective is to assess the incidence of NHL in UC patients, studying the possible immunopathological pathways that could offer an explanation for the appearance of deafness in these cases, and then to discuss the possible consideration that NHL is an extra-intestinal manifestation of the disease.

PATIENTS AND METHODS

49 patients diagnosed with ulcerative colitis were studied using rectosigmoidscopy and opaque enema radiological studies and/or computed tomography. The degree of activity was evaluated in accordance with the Truelove and Witts index which awards between 0 and 5 points depending on whether the following signs are present or not: more than 6 watery-bloody stools per day, temperature higher than 37.5°C, tachycardia over 120ppm, anemia with a hemoglobin below 10g/dl and ESR over 30 mm in the first hour. The absence of histopathological confirmation of the disease, previous
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intestinal resections, and concomitant administration of sulfasalazine or 5-aminosalicylic acid enemas did not signify exclusion from the study.

One of the criteria for not being included in the study was the administration of immunomodulated treatments such as glucocorticoids, azathioprine, methotrexate or cyclosporine in the 15 days prior to the audiological evaluation and analysis. Patients were also excluded if another disorder or circumstance susceptible to generating hearing loss had or had possibly been detected. These circumstances were: exposure to working environments with acoustic levels greater than 85dB (n=2); previous administration of ototoxic agents (n=2), or ear surgery (n=1).

The audiological evaluation of the patients included in the study was done by means of pure-tone threshold audiometry, the percentage of monaural and binaural hearing loss of each individual being measured in accordance with the specifications of the Royal Decree 1971/1999, of December 23 for the Procedure, Declaration and Qualification of the Degree of Handicap (Reconocimiento, Declaración y Calificación del Grado de Minusvalía, BOE of January 26, 2000), in Annex 1A, Chapter 13.

The peripheral blood evaluation classified an unspecific analytical profile that included 16 parameters susceptible to modification in disimmunity circumstances for all the patients. This profile included total levels of immunoglobulin G (IgG), M (IgM) and A (IgA), fibrogen, C-reactive protein (CRP), IgG, IgM and IgA rheumatoid factor fractions, anti-streptolysin O (ASLO) titles, complement factors C3, C4 and activity CH50, ESR, absolute recount of leukocytes and lymphocytes and T4/T8 ratio. All the measurements were obtained from the serum from blood which was not anticoagulated, and was centrifuged within 2 hours following extraction, except the fibrinogen and the ESR which were obtained from blood plasma treated with potassium citrate and the cellular recounts from the blood anticoagulated with EDTA-K. The serum and plasma samples are stable for 48 hours at 4°C and for up to 4 weeks at -70°C. The whole blood measurements were stable for 24 hours at room temperature.

Serum was also obtained for the classification of anti-cochlear autoantibodies using western-blot (WB) towards bovine cochlear antigen extract diluted in sodium dodecyl sulfate migrated in polyacrylamide gels and transferred to nitrocellular membranes in a minitransforetic unit as described by Barnette. Following the incubation of the membranes in Tween-TRIS buffer solution, they were added to 1:10 dilutions of the serum to be tested for 8 hours at 4°C. The product of the incubation was subsequently added to an excess of antiserum with anti-heavy chains immunoglobulins and combined with an alkaline phosphatase, activator of a phosphate chromogen associated with nitroblue tetrazolium. The results of the WB were qualitative and were labeled “positive” or “negative” according to the presence or absence of anti-cochlear antibodies.

The audiometric threshold results and the percentages were obtained from the 49 patients that showed positive WB values. The percentages of hearing loss of the patients with UC were also compared depending on whether their WB results came back positive or negative. The volume of parameters belonging to the unspecific tests that were significantly modified and that bore some correlation to a positive WB were also noted. To evaluate the possible relationship between actual hearing loss and the patient’s subjective sensation of loss, straight line equations were calculated between ages and percentage of hearing loss, obtaining the linear correlation co-efficient R2. The comparisons between the volume of cases with a positive and negative WB established by studying the average and standard deviations were analyzed statistically using Student’s t-Test and the comparisons established between proportions were analyzed by means of χ², all of which was included in the IT packages provided by SPSS and Statgraphics.

RESULTS

The 49 cases of the study group included 23 men and 26 women (M/F=0.88), with an average age of 41.6±9.3 years (ranging from 22 to 62 years). According to the Truelove and Witt index, the distribution was bimodal with two groups making up 77.5% of the study: those with 0 points (n=12) and those with 3 points (n=16). The most common clinical finding among the patients was anemia, followed by an increase in ESR. In accordance with the previously defined criteria for hearing loss stipulated by Royal Decree, some degree of hearing loss was detected in 29 cases (59.1% of the study group), even though only 13 patients (26.5%), had a subjective sensation of uni- or bilateral hearing loss. Of these 13 individuals, this feeling of deafness had been easily identifiable for at least 5 years in 11 of them. The average age at the onset of subjective deafness was 40.3±9.8 years, ranging from 22 to 58. In figure 1, the distribution of the study group is shown in intervals according to the percentage of hearing loss.

In the study of the 16 unspecific parameters, in peripheral blood, but with the potential capacity to detect immunomodulated disorders, an average of 2.3±1.8 alterations per patient was observed (range: 0 to 6). A total of 114 alterations (above or below values) in the 304 measurements taken (14.5% of them) were detected. In 11 of the patients studied, no alteration was detected. The most common analytical modification was the increase in ESR (n=28), followed by increases in CRP (n=19) and the total levels of IgG (n=12).

Carrying out the WB with anti-cochlear bovine antigen allowed us to observe the appearance of bands in
Figure 1. Distribution of the sample volume (n=49) by intensity of hearing loss detected, calculated as a percentages (abscise). Up to 28 cases suffered less than 10% hearing loss. Shading indicates the individuals that reported a subjective sensation of hearing loss.

24 patients with UC (48.9% of the total). Of the 13 individuals with a subjective feeling of deafness, 12 had a positive WB. The band detected always migrated in the molecular weight of 68-70KD, although in two patients a second band also existed between 60 and 63KD.

The study of hearing loss bore no correlation whatsoever to the patient's age, as is reflected in figure 2, any possible relationship between hearing disorder and sense of loss felt by the older patients can therefore be ruled out. The average hearing loss recorded in the pure-tone threshold audiometry is reflected in figure 3, in which a progressive tendency can be observed for the threshold to increase by air conduction in accordance with the pitch of the tone being examined.

Furthermore, dividing the study group into two subgroups depending on whether the patient expressed anti-cochlear antibodies in the WB bands (n=48 ears) or not (n=50 ears) confirmed a greater degree of hearing loss in the former group compared to the latter. This difference was observed both in terms of the percentage of monaural hearing loss and the hearing threshold by air conduction for all the frequencies studied, as reflected in table 1. On the other hand, the patients with positive WB results had a greater number of altered parameters belonging to the unspecific tests and a clinical index of significantly higher UC activity.

Finally, the correlation study between the monaural hearing loss quantitative variables and the number of altered parameters of the unspecific tests and the UC clinical activity index allowed us to obtain the straight line equations presented in figure 4. These show a tendency towards hearing loss in patients with UC and a greater volume of altered analytical or clinical markers, even though this tendency did not prove to be statistically significant.

**DISCUSSION**

The concomitance of ulcerative colitis and NHL is not a new finding. Even though it was documented in isolated cases during the 1980s and 1990s, it was Kumar who detected it in the year 2000 when comparing control subjects and 20 patients with UC perceptive deafness of varying degrees, labeling hearing disorder as an extra-intestinal manifestation of the inflammatory intestinal process, whose activity is well correlated with the intensity of neurosensorial loss.

The clinical characteristics of NHL, described by Kumar and other authors, coincide with those we have observed. Diverse publications emphasize the
Table 1: Comparative and statistical analysis of hearing loss, analytical findings and degree of activity of the disease according to whether the patients had a negative or positive WB result for anticochlear antibodies.

<table>
<thead>
<tr>
<th>+WB (n=24 patients 48 ears)</th>
<th>-WB (n=25 patients 50 ears)</th>
<th>t</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42.7±10.1 years</td>
<td>40.5±8.34 years</td>
<td>1.173</td>
</tr>
<tr>
<td>↓250Hz</td>
<td>26.7±11.1 dB HL</td>
<td>20.6±7.9 dB HL</td>
<td>3.123</td>
</tr>
<tr>
<td>↓500Hz</td>
<td>32.5±14.6 dB HL</td>
<td>22.1±8.8 dB HL</td>
<td>4.25</td>
</tr>
<tr>
<td>↓1000Hz</td>
<td>37.3±16.9 dB HL</td>
<td>22.2±9.3 dB HL</td>
<td>5.449</td>
</tr>
<tr>
<td>↓2000Hz</td>
<td>41.0±19.9 dB HL</td>
<td>24.6±9.9 dB HL</td>
<td>5.133</td>
</tr>
<tr>
<td>↓4000Hz</td>
<td>46.9±22.6 dB HL</td>
<td>27.1±3.6 dB HL</td>
<td>5.228</td>
</tr>
<tr>
<td>↓8000Hz</td>
<td>55.3±24.5 dB HL</td>
<td>38.8±18.4 dB HL</td>
<td>3.759</td>
</tr>
<tr>
<td>Σ↓(500-4000Hz)</td>
<td>157.8±69.6 dB HL</td>
<td>96.0±36.5 dB HL</td>
<td>5.474</td>
</tr>
<tr>
<td>↓Monaural hearing</td>
<td>24.3±22.6%</td>
<td>4.8±7.4%</td>
<td>12.68</td>
</tr>
<tr>
<td>Altered tests</td>
<td>3.75±1.06</td>
<td>0.96±1.12</td>
<td>5.692</td>
</tr>
<tr>
<td>Degree of UC activity</td>
<td>2.2±1.3</td>
<td>0.8±0.9</td>
<td>13.22</td>
</tr>
</tbody>
</table>

peculiarities of this type of deafness which are very suggestive of its proving to be pathogenically immunomediated: its sensorineural character and rapid or even sudden evolution\(^8_9\); its clinical response to corticoids and other immunosuppressors\(^3,5,8,9\) and its association with other entities with potential autoimmune pathogenicity such as gangrenous pyoderma, iritis or vasculitis\(^10,12\). The unspecific inflammatory profile we used, and especially the development of WB with cochlear antigen, enabled us to document the degree of unusual immunomediation in the patient that approximates this model of detected hearing loss even more than that described by McCabe in defining labyrinthine autoimmunity. Hoistad reinforces this hypothesis documenting UC and NHL histopathological findings - very similar to those found in previous experimental studies on immunized guinea pigs - in the postmortem examination of a leukemia patient: atrophy of Corti’s organ, reduction in the cellularity of the spiral ganglion, spiral endolymphatic hydrops, utricle and saccule, and osteoneogenesis of the tympanic slope of the spira basa with lymphocytosis and fibrosis of the endolymphatic sac\(^13\).

The immunological response in UC has shown an increase in B cells: producers of immunoglobulins - mainly IgG. In the active disease this increase is even greater and the most classic theory attributes these intensifications to the cytotoxicity measured by lymphocytes and induced by immunoglobulins\(^14\). However, the exaggerated production of immunocomplexes is equally documented with local generation of an Arthus-type reaction and a serum response at the systemic level, similar to that which took place in the serum disease\(^15\). If the immunocomplexes are voluminous – formed in equivalence and in excess of the antibody – they rapidly disappear from circulation and are phagocytosed by cells of the reticular-endothelial system, favoring the development of local granulomatous disease.

On the other hand, the deposit of these aforementioned complexes in the walls of the vessels would explain the vasculitis and other extra-intestinal manifestations, at the same time conditioning an almost autolitic inflammatory response that raises the concentration of acute phase reactants, promotes
leukocyte infiltration and consumes complement products. This proves particularly justifiable when the complexes are small and prepared in excess of the antigen\(^16\). This necessitates an over-expression of antigenic epitopes that are not initially present in the intestinal lumen or in the colonic mucosa cells.

The following findings are frequently reported in UC patients: antibodies against anaerobic bacteria in their mucosa; colon antimucosal antibodies in their serum; antiproteins in their feces; and anti*Escherichia coli*\(^17-19\). Perhaps such low titles reflect their union with tissue antigens, or the constitution of immunocomplexes with the carcinoembryonate antigen. However, the correlation between the concentration of circulating immuno-complexes and the severity of the colitis and of some other symptoms has been proven. Regarding hearing, this implication of clinical intensity is still disputed today. Our results do identify greater depth of hearing loss when the disease shows aggressiveness indicators clinically, but it is not a very close relationship and it is not a result found by other authors.

The serum of UC patients can induce colon-cytotoxicity in normal lymphocytes if it contains anticolon antibodies or anticomplexes with these. It has been proven that these antibodies exist, that they can react against fetal colon mucosa and also against certain antigens present in many intestinal bacteria. The harmful reactivity must derive from the bacteria's structural similarity to epitopes and to other structures of the body.

Heat shock proteins (HSP) seem to be excellent candidates for the unleashing hearing damage antigen of the disease. These are constitutive polypeptide families with important immunogenic properties, intracellular molecules that are extremely structurally well-conserved over time and extraordinarily ubiquitous, but they can also be induced in the intestinal mucosa in the presence of diverse forms of cellular damage: hyperthermia, anoxia, ethanol, heavy metals or infection. These would initially be destined for the function of modular aggression which the living being may experience in hostile environments, encouraging the protein synthesis of useful substrates and the degradation and elimination of deteriorated structural material (receptors, transporters, membranes and even genome segments).

A strong staining tendency for the constitutive and inducible forms of HSP70 in mucosa specimens with UC, Crohn's disease, or intestinal ischemia has been demonstrated with immunohistochemistry, but only detected in mononuclear cells of patients with UC\(^20-22\). On the other hand, the same technique does not detect this over-expression in malignant intestinal tissue or intestinal tissue affected by polyposis of the colon\(^23\). The HSP70 family finds itself more and more involved as a triggering agent of immunomediaction that promotes labyrinthine damage resulting in hearing loss and/or rapid development vestibular episodes\(^24,25\). In UC, the circulation of anti-HSP70 antibodies has already been proven by Reumaux in the context of a hyper-expression of IgG polyclonal antihistones H2A, antipolymerases and antiactins\(^26\). Venkatraman has shown that, in these patients, the inhibition of the expression of colonic HSP70 with butyrate enemas reduces intestinal permeability, slows down the polymorphonuclear infiltration and protects the patient from the extra-intestinal manifestations of the disease\(^27\).

The implication of HSPs in the mediation of other damage in UC appears to be a fact. Experimental colitis by *Escherichia coli* generates histopathological damage similar to that of UC correlated with the hyper-expression of HSP60 and what is more, these lesions are reproducible when HSP60 itself, originating from *Yersinia enterocolitica*\(^28,29\), is inoculated in a rat's intestine. Different authors have shown the explosive presence of antiHSP60 and antiHSP65 in the serum of patients with UC in contact with *Escherichia*, but also with *Mycobacterium sp*\(^30-31\).

It is therefore reasonable to link the deterioration in hearing observed in some UC patients with an extra-intestinal manifestation of UC. UC is a disease that expresses immunological markers of a recognized harmful effect on the inner ear and which is an immunomeditated disease susceptible to being effectively treated in this regard with measures already contrasted for AIED. It has been documented that the same sequence of events does not generate in Crohn's disease, in itself a more extensive and deeper disorder of the intestinal wall, the same aggression on the inner ear\(^32,33\). The reasons for this immunitary discrimination, and above all the reasons why not all patients affected by UC develop the same labyrinthine damage, have still to be investigated.

References
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