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Differential effect of appendectomy and tonsillectomy on anti-*Kudoa* sp. antibodies in patients with MALTectomy

J.C. Andreu-Ballester [a], F. Ballester [b], J. Pérez-Griera [c], V. Amigo [d], C. Peñarroja-Otero [e], E. Colomer-Rubio [a], I. Ortiz-Tarín [f], V. Pelayo [g], P. García-Hernández [g], M. Rodero [g], C. Cuéllar [g,*]

[a] *Research Unit, Emergency Department, Arnau de Vilanova Hospital, 46015 Valencia, Spain*
[b] *Unit of Epidemiology and Statistics, Valencian School of Studies for Health, 46017 Valencia, Spain*
[c] *Biopathology Department, Clinic University Hospital, 46010 Valencia, Spain*
[d] *Hematology Department, Arnau de Vilanova Hospital, 46015 Valencia, Spain*
[e] *Internal Medicine Department, Casa de Salud Hospital, 46021 Valencia, Spain*
[f] *Department of Surgery, Arnau de Vilanova Hospital, 46015 Valencia, Spain*
[g] *Department of Parasitology, Faculty of Pharmacy, Complutense University, 28040 Madrid, Spain*

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ABSTRACT

We found an association between tonsillectomized patients and subsequent appendicitis. We also observed that MALTectomy significantly decreased secretory IgA levels in serum of patients, being this decrease more pronounced when both operations (tonsillectomy and appendectomy) had been performed. The elevated humoral responses detected previously by us in BALB/c mice immunized with *Kudoa* sp. pseudocyst extracts and the high IgG1 and IgE levels induced by the oral administration of *Kudoa* sp. pseudocysts to BALB/c mice showed the possible immunopathological effects in man from the ingestion of *Kudoa* sp. infected fish. We use the ELISA method to investigate the possible relationship between MALTectomy (tonsillectomy and appendectomy) and specific antibody levels to *Kudoa* sp. Both anti-*Kudoa* sp. specific antibody levels and the number of patients that recognized *Kudoa* sp. antigens were greater in tonsillectomy patients when compared to the control and the other studied groups (appendectomized and appendectomized + tonsillectomies patients). Tonsillectomy was associated to a switch in the class of immunoglobulins involved in these responses and these responses may be abrogated by appendectomy. Tonsils and appendix may respond in different ways to *Kudoa* sp. antigens and these different reactions may be involved in some immunopathological reactions.

1. Introduction

The MALT system (Mucosa Associated Lymphoid Tissue), in the digestive tract GALT (Gut Associated Lymphoid Tissue), is an important part of secondary lymphoid tissues and is comprised of Peyer's patches of terminal ileum, and the appendix. In the nasopharyngeal area it is denominated NALT system (Nasopharyngeal Associated Lymphoid Tissues) composed of tonsils palatines, adenoids and tongue [1–3]. In adult human beings, 80–85% of immune cells are located in the digestive tract mucosa, hence the importance of the MALT in human immunology [4]. The appendix is highly vascular, contains a high concentration of lymphoid tissue, and produces immune system cells normally involved with the MALT [5–7].

In previous studies, we found an association between tonsillectomized patients and subsequent appendicitis [8,9]. Likewise [10], we studied the influence of the surgical removal of two important parts of the MALT (tonsils and appendix) on immune parameters. MALTectomy significantly decreased secretory IgA levels in serum of patients, being this decrease more pronounced when both operations (tonsillectomy and appendectomy) had been performed. Recently, we found that appendectomy significantly diminished specific immunoglobulin levels in serum against two food borne parasite antigens (*Anisakis* and *Kudoa* sp.) [11]. This decrease was detectable from three months to three years post-appendectomy. These facts prompted us to study the influence of the surgical removal of other important parts of the MALT on these anti-parasite humoral immune responses.

The majority of *Kudoa* species infect somatic muscle of fish, establishing cysts which contain many spores. There is currently no effective method to detect infected fish without destroying them, for this reason parasitized fish reach the consumer. Although there is no information about the prevalence of *Kudoa* sp. in fish markets in the

Abbreviations: MALT system, Mucosa Associated Lymphoid Tissue; GALT, Gut Associated Lymphoid Tissue; NALT system, Nasopharyngeal Associated Lymphoid Tissues; AP, Appendectomy; T, Tonsillectomy; T + AP, Appendectomy and tonsillectomy; C, Control group without either operation; Ig's, Total immunoglobulins; HRP, Horse radish peroxidase; O.D., Optical density; OR, Odds Ratio; CI 95%, Confidence Interval of 95%.

* Corresponding author. Tel.: +34 913941818; fax: +34 913941815.
E-mail address: cuellarh@farm.ucm.es (C. Cuéllar).

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studied region, multivalvulid myxozoan parasites, including *Kudoa* spp. have been described from fish from the Mediterranean coastal waters of Spain [12]. Likewise, in Spain, *Kudoa*-infected fish have lately been detected in both fresh and frozen imported Chilean hake (*Merluccius gayi gayi*) destined for human consumption. The elevated humoral responses detected previously by us in BALB/c mice immunized with *Kudoa* sp. pseudocyst extracts and the high IgG1 and IgE levels induced by the oral administration of *Kudoa* sp. pseudocysts to BALB/c mice showed the possible immunopathological effects in man from the ingestion of *Kudoa* sp. infected fish [13]. Previously [14] we investigated the seroprevalence of anti-*Kudoa* sp. antibodies in a Spanish healthy population and the possible association between the manifestation of allergic reactions after fish consumption and the humoral responses to *Kudoa* sp. antigens. Likewise, we determined specific anti-*Kudoa* sp. specific antibodies in sera of patients diagnosed with several digestive pathologies. Positive reactions to *Kudoa* sp. were observed in patients by means of the skin prick test [15]. For these reasons, this report aims to investigate the possible relationship between MALTectomy (tonsillectomy and appendectomy) and the levels of specific antibodies to *Kudoa* sp.

2. Materials and methods

2.1. Patients and serum samples

A population of adult patients was randomly selected from the database of appendectomized (AP) patients, treated over the last three years in our hospital (Arnau de Vilanova Hospital, Valencia, Spain). Patients who had been operated on in the last three months were excluded. By means of a telephone interview (in chronological order of intervention, starting with the most recently operated patient), information concerning a possible previous tonsillectomy (T) was obtained. So, patients were split into a group of 40 patients who had had both operations (T + AP), and another group of patients who had only undergone appendectomy. These groups were sorted according to sex, age (±2 years) and date of operation (the patient nearest on the list). Further, another two groups were also randomly selected from patients presenting to the emergency service: one group of patients who had had a tonsillectomy but not appendectomy; and the other group made up of controls that had not had either operation. These groups of patients were paired by sex and age with the two former groups. We excluded patients with any acute infections or inflammatory diseases, as well as those who had received immunosuppressive treatment or any kind of vaccine during the previous year. The age and the date when they had been operated on were also noted. The anatomopathological report on the appendicitis (type and size of appendices) was included. The tonsillectomy was confirmed by otorrinolaringological exploration. We examined a total of 160 patients. In this way, our study population was made up of four groups of 40 patients per group: Group 1 [patients with AP + T operations]; Group 2 [patients with only appendectomy (AP)]; Group 3 [patients with only tonsillectomy (T)]; Group 4 [control group without either operation (C)]. Each participant in the study gave their consent and the study was approved by the Ethics and Investigation Committee of our hospital.

Total immunoglobulins (Ig's), IgG, IgM, IgA and IgE against *Kudoa* sp. antigen were determined in the sera of the selected patients.

2.2. Antigens

"White" *Kudoa* sp. pseudocysts were manually obtained from the skeletal musculature of Chilean hake (*M. gayi gayi*) from local fish markets and destined for human consumption. Pseudocysts were separated from any associated fish tissue and then homogenised. In order to release the spore contents, glass beads were added and "white" *Kudoa* sp. pseudocysts were shaken and homogenates were extracted in PBS [13].

2.3. Specific antibody levels

Each well of 96 well microtitre plates (Costar, Corning, NY, USA) was coated by the addition of 10 μg/ml of *Kudoa* sp. antigen. Duplicate dilutions of sera at 1/100 in PBS-Tween, containing 0.1% BSA were added and incubated. Horse radish peroxidase (HRP) conjugate goat anti-human Ig's, IgM, IgG or IgA (Biosource International, Camarillo, CA, USA) was used [16,17].

For the IgE determination, test sera were added in duplicate at a 1/2 dilution. A murine monoclonal antibody against an epsilon human IgE chain (IgG1κ, E21A11, INGENASA, Madrid, Spain) was added and incubated, followed by a goat anti-mouse IgG1 (gamma) HRP conjugate (CALTAG Laboratories, Burlingame, California) [13,17]. Anti-*Kudoa* sp. positive and negative sera from mice were used as ELISA controls [13].

Values higher than the mean of the optical density (O.D.) of 160 subjects obtained for each immunoglobulin and antigen plus their respective standard deviation were considered as positive.

2.4. Determination of biological indicators of immunological status

Cells were stained for the surface markers CD8, CD4, CD3, CD19, CD2, CD8 and CD56. The data were collected and analyzed by flow cytometry (Coulter Epics XL-MCL; Beckman-Coulter). Nephelometry (BN-II Analyzer, Behring Diagnostics GmbH, Marburg, Germany) was employed for serum IgG and IgM assay. Total IgA, IgA1, IgA2 and secretory IgA were determined by ELISA using monoclonal mouse anti-human antibodies (HyTest Ltd., Turku, Finland) [10].

2.5. Statistical analysis

Statistical analysis was performed using the SPSS statistical package. Descriptive characteristics of the patients as well as variables of the immunoglobulin levels in each group were obtained. Contingency tables were made for the categorical variables. Their frequencies were compared by means of Pearson's Chi-Square test. To compare the mean values of these variables, a repeated measure ANOVA with contrast analysis of types of differences was used. Normality in distribution of the variables of immunoglobulin levels was confirmed by the Kolmogorov–Smirnov test. Sphericity was verified by the Mauchly test. In the case of non-sphericity, a low limit correction was obtained. Friedman's ANOVA non-parametric test was used to study the variables that did not have a normal distribution, and the exact or asymptotic test was chosen depending on the case. p values <0.05 were considered to be statistically significant. The Pearson's test was used to study the correlations.

3. Results

We examined a total of 160 subjects [40 appendectomy (AP), 40 tonsillectomy (T), 40 AP + T patients and 40 controls (C)], 76 were females (47.5%) and 84 were males (52.5%). The mean age (±SD) was 39.2 ± 14 years (CI 95%: 37.0–41.4) range 20–75. The four groups were matched for sex and age. In each of the four groups there were 21 men and 19 women. The mean age at which the tonsillectomy was performed was 6.87 years (CI 95%: 5.14–8.61). The mean age at which the appendectomy was performed was 36.33 years (CI 95%: 31.83–40.82). The selected population in our study was appendectomized between 3 months and 3 years before blood testing, and tonsillectomized 20 years before our investigation.

Anti-*Kudoa* sp. immunoglobulin levels are shown in the Fig. 1. Total antibody prevalences for Kudoa sp. Ig's, IgG, IgM, IgA and IgE