



### **Entrega de l'Abstract**

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Títol de la recerca:

DETERMINATION WHETHER DIARRHOEA-PREDOMINANT IBS PATIENTS PRODUCE HIGHER LEVELS OF IMMUNOGLOBULIN A THAN THE CONTROL GROUP (HEALTHY VOLUNTEERS) IN THE JEJUNUM

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Abstract (màxim 500 paraules):

#### OBJECTIVES

The aim of this study is to quantify the reaction of the ADAPTIVE immune response in patients with IBS and in a CONTROL group (healthy volunteers), in order to determine whether IBS patients, whose intestinal barrier is known to be dysregulated, produce higher levels of immunoglobulin A

#### METHODOLOGY

For the assay performed, IBS patients with prevalence of diarrhoea, and healthy volunteers are required.

Once the IBS-D patients are diagnosed, 2 visits with the doctor before the intervention are required. In the first one, the following



information is obtained: Clinical data, questionnaires to know symptoms, personal information, toxic habits, psychological stress and depression, a prick test, an allergy questionnaire, and a blood test.

Before the 2<sup>nd</sup> visit, the doctor excludes following exclusion criteria, for example: food allergy, other gastrointestinal diseases...

Both, healthy and IBS patients, are subjected to the same procedures during the 2<sup>nd</sup> visit.. Samples will be obtained: a blood extraction for laboratory test, the jejunal content (aspirate) and a jejunal mucosal biopsy (with the *Watson capsule* and controlled by X-rays).

Then, 1  $\mu$ l/ml of protease inhibitor is added to the aspirate, and the tube is centrifuged at 2000 rpm during 10 min. After that, the supernatant is aliquoted in different eppendorfs with a final volume of 500 $\mu$ L. The ones assigned to determinate total protein content and IgA must be sonicated, subjected to 2 cycles of 10 seconds at ultrasounds with the Ultrasonic Homogenisor (waiting 10 seconds between both cycles). Next, 50  $\mu$ L are addressed for the determination of proteins, and the rest 450  $\mu$ L, will be aliquoted for the determination of IgA.

Then, the ELISA-Sandwich technique is used for the detection of IgA in serum.

The colorimetric reaction catalyzed by the peroxidise labeled conjugate, produces a blue product, which turns yellow when the reaction is terminated by addition of dilute sulphuric acid. The absorbance of the yellow product at 450 nm is proportional to the amount of IgA analyte present in the sample and a four-parameter standard curve can be generated.



After factoring sample dilutions the IgA concentration in the original sample is finally calculated.

## RESULTS

The concentration values of IgA are expressed in ng of IgA for each ml of intestinal fluid, and in ng of IgA each ng of proteins quantified in the same aspirate.

In order to compare the results obtained between healthy and IBS, different statistical parameters are calculated using the Statistical Package for the Social Sciences (SPSS).

## CONCLUSIONS

\*When the samples were subjected to ultrasound the signal was higher.

\*As this study was performed at the beginning, the little amount of data used could be the cause of obtaining no significant differences between Healthy volunteers and IBS patients, as it is shown with a normality test.

\*It would be necessary to repeat the statistical analysis after increasing the data.

## PERSONAL CONTRIBUTION

Only the doctor could perform the biological sample collection procedure, but under the supervision of one of the members of the research group I performed the following steps related with the processing of the samples, ELISAs, and the statistical analysis.