Introduction: The eosinophil is a multifunctional resident of the gastrointestinal tract that displays extensive regulatory ability to promote and balance mucosal inflammation. In irritable bowel syndrome (IBS), the prototype of gastrointestinal functional disorders, mucosal immune activation associated with altered barrier function and psychological stress has been identified. However, the role of specific leukocytes such as the eosinophil remains unknown. Here we used qualitative and quantitative approaches combining gene expression with light and electron microscopy techniques (structure, ultrastructure and immunolocalization) in order to study the role of eosinophil in IBS etiology.

Methods: Healthy (H) subjects (n=12) and age-matched, naïve participants fulfilling diarrhea-prone IBS (IBS-D) Rome III criteria (n=17) were included. Jejunal biopsies were obtained by Watson's capsule in all participants. RNA was isolated for the study of gene expression by microarray and identification of differential expression associated with biological functions by Ingenuity Pathway Analysis. Mucosal eosinophil counts were evaluated by immunohistochemistry for major basic protein (MBP) and its ultrastructure was analyzed by transmission electron microscopy as well as the quantification of corticotropin-releasing factor (CRF) by immunolocalization.

Results: The analysis of gene expression revealed functions related to eosinophil activity (P<0.0001) and stimulation (P<0.001) as differentially expressed between IBS-D and H. The number of MBP+cells was similar in both groups (H:44±7; IBS-D:83±19 cells/mm2), however the ultrastructure showed fragmentation of cytoplasmic granules and the presence of tubular structures and sombrero-like vesicles, indicative of secretory activity in the IBS-D group (Figure 1). In the intestinal mucosa, labelling of CRF was only present in the granules of eosinophils, and its content was higher in the group of patients (IBS-D:5.8±2.4, H:2.6±0.9 particles/granule; P<0.05).

Conclusions: Mucosal eosinophil activation in IBS-D was identified by gene expression and further confirmed by ultrastructure analysis. Additionally, although there are no differences in eosinophils density, the increased CRF granular content suggest the existence of alternative pathways of eosinophil activation involving the stress response.
Fig. 1: Representative images of degranulation of eosinophils (black arrows) from healthy (H) and diarrhea-prone IBS (IBS-D) subjects. Labelling of CRF localized in eosinophil granules (white arrows).