Study of technical validation of molecular biomarkers for the development of a diagnostic tool for Fibromyalgia.

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Introduction

Fibromyalgia (FM) is a disease characterized by widespread pain and both physical and psychological symptoms. These influence the normal course of daily life on a personal and professional level, generating a considerable deterioration in the quality of life of patients[1].

The prevalence of FM is estimated to be between 2% and 8% of the world population, with women being the most affected. Currently, a large proportion of patients with FM are not properly diagnosed and face sub-optimal treatment, whose efficacy is variable among them[2]. While the molecular etiopathogenesis of FM remains unclear, several factors that could underlay it have been suggested in recent years, including oxidative stress, inflammation, mitochondrial dysfunction and disturbances in the gut microbiome[3,4].

Therefore, the aim of this study is to validate the use of various biomarkers for the molecular diagnosis of FM through the determination of mitochondrial balance, total antioxidant capacity (TAC) and intestinal status in patients in order to develop a diagnostic tool.

Materials and Methods

To carry out the study, FM patients and healthy controls were given an informed consent form which, once understood and signed, provided the use of information for the research. Prior to the technical validation, patients' data were collected through a series of questionnaires, such as the Fibromyalgia Impact Questionnaire (FIQ) and the Fibromyalgia-Endometriosis Prevalence, in order to characterize the profile of fibromyalgia patients. The samples used were:

- **Blood:** Total blood was extracted from all participants in 2 Heparin tubes. One of them was immediately centrifuged 10 minutes at 4^{o}C and 1300g for plasma collection, sample needed in TAC determination. The other tube was processed with Ammonium chloride (NH_4Cl) for PMBCs isolation, cells used in mitochondrial balance study throughout western blot.
- **Feces:** Each participant was provided with an specific fecal collection kit to be used in their own house. Intestinal markers were determined with Enzime-linked Immune Assays.

At the moment, the prevalence of Endometriosis has been ascertained, while the biological samples are being collected and the analytical processes regarding its molecular determinations have just started.

Results

We analyzed the data collected from the questionnaires with the aim of identifying the profile of FM patients, particularly those afflicted with another inflammatory disease such as endometriosis. Out of 41 patients who participated, only 8 of them had a confirmed diagnosis of endometriosis, however, those patients who reported having some type of infertility, heavy and/or painful periods and pelvic pain were considered patients with suspected undiagnosed endometriosis. As a result, 41% of the FM patients who participated could have undiagnosed endometriosis.

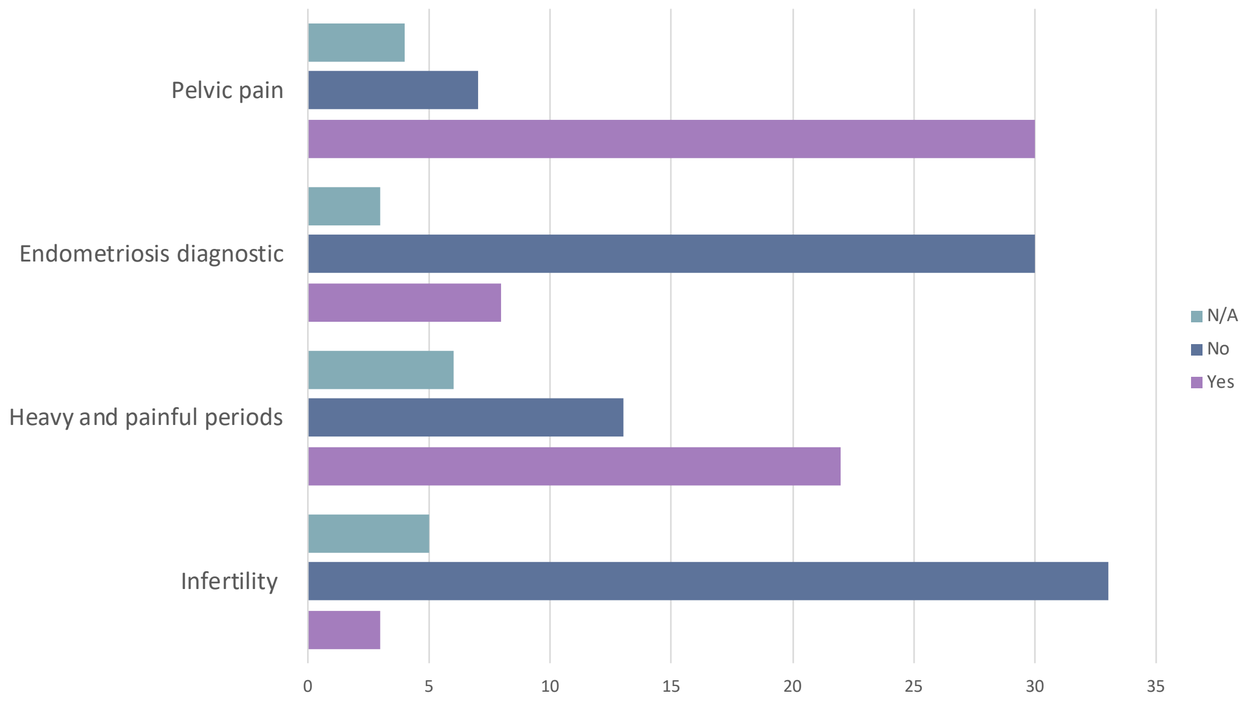

Figure 1. FM patients with diagnosis or symptoms of endometriosis.

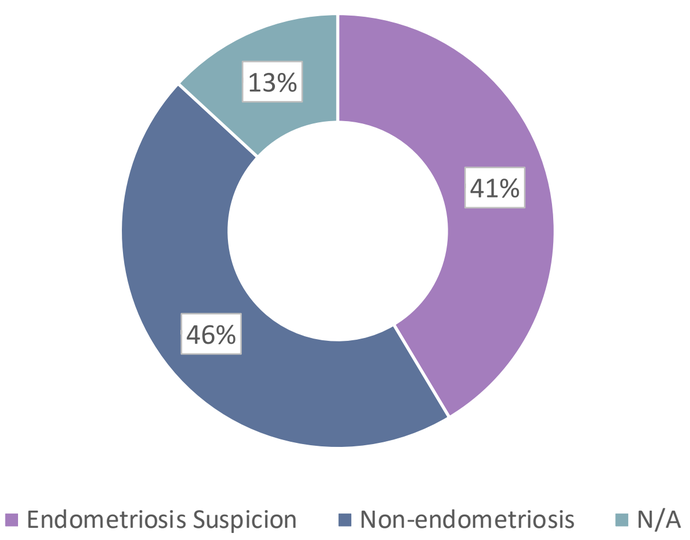

Figure 2. Proportion of FM patients with and without endometriosis suspicion.

Conclusions

- Interestingly, while the prevalence of endometriosis in the general population is approximately of 10%[5], we observed a prevalence of 19.5% of endometriosis in FM patients, which is almost twice as high. These results obtained so far suggest that **fibromyalgia has an inflammatory profile that can affect even the reproductive organs of the affected person**, thus reinforcing the need to use molecular biomarkers to differentiate between FM patients and both patients from different inflammatory conditions and healthy people.

- Further studies will be performed in an extended cohort of patients, to verify the results by a proteomic approach and sequencing of FM patients for the determination of the proteome and intestinal dysbiosis. **A complete characterization of these biomarkers will contribute for a more accurate diagnosis of the disease**, leading to a better course and allowing the monitorization of the treatment outcome.

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