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Human enteroviral infection in fibromyalgia: a case-control blinded study

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SUMMARY

OBJECTIVE: This study aimed to test the hypothesis that fibromyalgia is associated with a human enteroviral infection.

METHODS: Venous peripheral blood samples from 27 patients fulfilling the American College of Rheumatology revised diagnostic criteria for fibromyalgia and from 26 age- and sex-matched controls, who underwent immunofluorescence assays for coxsackievirus A7 IgG, coxsackievirus B1 IgG, coxsackievirus A7 IgA, coxsackievirus B1 IgA, echovirus IgG, and echovirus IgA. These immunological tests were performed blind to group status.

RESULTS: There were no significant differences between the patient and control groups in respect of positive results for coxsackievirus A7 IgG (p=0.467), coxsackievirus B1 IgG (p=0.491), coxsackievirus A7 IgA (p=0.586), coxsackievirus B1 IgA (p=0.467), echovirus IgG (p=0.236), and echovirus IgA (p=1).

CONCLUSIONS: The results of this systematic study do not support the hypothesis that fibromyalgia is associated with infection by a human enterovirus.

KEYWORDS: Coxsackievirus. Echovirus. Fibromyalgia. Human enterovirus. Immunofluorescence.

INTRODUCTION

Fibromyalgia is a relatively common cause of chronic widespread musculoskeletal pain and tenderness; however, its etiology remains unknown[1]. Since the past century, it has been suggested that infections may trigger this condition[2]. In particular, the possibility has been raised that human enteroviruses may have an etiological role.

The human enteroviruses coxsackie A virus, coxsackie B virus, and echovirus belong to the genus *Enterovirus*, which, in turn, is part of the Picornaviridae family of positive-sense ssRNA viruses[3]. Infection with coxsackieviruses and echoviruses may be associated with myalgia, myocarditis, and central nervous system disorders, possibly including self-limiting meningitis in adults[4-7]. In 1989, Nash and colleagues described the case of a patient with symptoms consistent with primary fibromyalgia who showed evidence of chronic coxsackie B virus infection[8]. Subsequently, Wittrup and colleagues assayed immunoglobulin M (IgM) antibodies to hepatitis C, hepatitis B, cytomegalovirus (CMV), human herpesvirus-6 (HHV-6), rubella, parvovirus B19, and enterovirus in 19 acute-onset fibromyalgia patients compared with 19 healthy controls; IgM antibodies were only found against HHV-6 and enterovirus in the patient group and against CMV, HHV-6, and enterovirus in the control group[9]. A third comparative group, of nonacute-onset patients, also had IgM antibodies against enterovirus. However, no operational criteria were given for classifying patients as being acute onset versus nonacute onset; there was no further classification of the enterovirus; and no IgG or IgA antibody assay results were reported for either the patients or the control subjects[9]. Finally, in their muscle biopsy study, Douche-Aourik and colleagues reported that 4 out of 30 patients diagnosed with either fibromyalgia or chronic fatigue syndrome showed evidence of enteroviral RNA compared with none of 29 controls[10].

We hypothesized that fibromyalgia is associated with infection with one or more of the relatively common human enteroviruses coxsackievirus A7, coxsackievirus B1, and echovirus. To test for evidence of infection by these viruses and the formation of neutralizing antibodies and given the importance of the feco-oral route in the spread of these viruses, we decided to test our hypothesis by assessing the levels of IgG and IgA against each of these viruses in a cohort of well-characterized fibromyalgia patients.

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METHODS

Experimental design and participants

The study was carried out using a cross-sectional, blinded case-control experimental design. Patients were eligible to enter the study if they were aged between 18 and 80 years and fulfilled the revised diagnostic criteria of the American College of Rheumatology, with the diagnostic cutoff threshold employed of ≥13 from the composite painful sites number and symptom severity scale[11]. Healthy volunteers were eligible to enter the study if they were aged between 18 and 80 years and had no history of suffering from a rheumatological disorder (including fibromyalgia) or neurological disorder. Exclusion criteria for the patient group included treatment with antimicrobial/antiviral therapy or corticosteroids; exclusion criteria for the control group included the diagnosis of fibromyalgia in any first-degree relative. This study received ethical approval from a Research Ethics Committee and was carried out according to the Declaration of Helsinki. All participating patients and control subjects gave written informed consent.

Immunofluorescence assays

Notably, 8.5-mL venous peripheral blood was collected from each subject in a labeled sterile serum SST™ II Advance Tube consisting of a plastic (PET) tube with a Hemogard closure incorporating an inert stable gel to aid separation of serum from the blood clot. The labeling consisted of an alphanumeric code which fully anonymized the subsequent laboratory analysis of the serum; no record appeared on each tube of the subject's name, date of birth, sex, or group allocation (patient or control). Following venipuncture, each filled tube was immediately inverted 10 times and then placed vertically at room temperature for 30 min. The tubes were then packed upright and protected from light during transportation, at ambient temperature, to the laboratory where human enterovirus immunofluorescence assays were carried out, blinded to group status, with the following thresholds for positive results: coxsackievirus A7 IgG 1:100, coxsackievirus B1 IgG 1:1000, coxsackievirus A7 IgA 1:10, coxsackievirus B1 IgA 1:10, echovirus IgG 1:1000, and echovirus IgA 1:10.

Statistical analyses

Comparison of the mean age of the two groups was carried out using the Student's t-test, after confirming that there was no significant violation of either normality, using the Shapiro-Wilk test, or equality of variances, using Levene's test. Analysis of two-by-two contingency tables was carried out using the Fisher's exact probability test. All statistical tests were two-tailed. The statistical programs used were R v. 4.1.1 and JASP 0.15[12,13].

RESULTS

There were 27 subjects in the fibromyalgia group (26 females; mean age 49.6 years, standard error [SE] 2.1 years) and 26 subjects in the control group (25 females; mean [SE] age 48.2 [2.4] years). The two groups did not differ significantly in respect of age (p=0.655) or sex (p=1).

The immunofluorescence assay results are given in Table 1. There were no significant differences between the patient and control groups in respect of positive results for coxsackievirus A7 IgG, coxsackievirus B1 IgG, coxsackievirus A7 IgA, coxsackievirus B1 IgA, echovirus IgG, or echovirus IgA.

DISCUSSION

The results of this study did not provide any evidence in favor of the hypothesis.

These results are at variance with expectations, given the muscle biopsy findings by Douche-Aourik and colleagues mentioned above[10]. However, closer examination of those positive muscle biopsy findings reveals that the patient and control groups were not matched for either age or sex. Furthermore, it is not clear how many of the four positive biopsy results were in patients with fibromyalgia and how many in those with a diagnosis of chronic fatigue syndrome; the report simply states that 22 of the patient group were diagnosed with fibromyalgia, and the remainder with chronic fatigue syndrome[10].

As seen in Table 1, relatively high levels of positive results were found in the control group, as follows: coxsackievirus

Table 1. Immunofluorescence assay results.

Immunofluorescence assay	Number positive in fibromyalgia group (n=27)	Number positive in control group (n=26)	p
Coxsackievirus A7 IgG	21	23	0.4672
Coxsackievirus B1 IgG	25	26	0.4906
Coxsackievirus A7 IgA	13	15	0.5857
Coxsackievirus B1 IgA	21	23	0.4672
Echovirus IgG	24	26	0.2358
Echovirus IgA	19	19	1

A7 IgG in 88%, coxsackievirus B1 IgG in 100%, coxsackievirus A7 IgA in 58%, coxsackievirus B1 IgA in 88%, echovirus IgG in 100%, and echovirus IgA in 73%. It is noteworthy that coxsackievirus B1 infection has been found to be associated with a wide range of diseases[14-16]. Coxsackievirus A7 is associated with neurological diseases and can cause paralytic poliomyelitis[17]. In a recently published analysis of 153 worldwide epidemiological studies, the weighted median prevalence in Europe of all enteroviruses examined, which included, but was not limited to, coxsackievirus A7, coxsackievirus B1, and echovirus, was well under 10%, as was the median prevalence of enteroviruses in those at least 18 years of age[18]. The much higher prevalence figures in our cohort of healthy adult volunteers are difficult to explain. All members of the control group were white Caucasian British subjects who lived in the United Kingdom (as indeed were all members of the patient group). It would be interesting to carry out a larger study of the prevalence of enteroviruses in healthy subjects residing in the part of the United Kingdom from which the subjects of this study were recruited.

CONCLUSIONS

The results of this systematic study do not support the hypothesis that fibromyalgia is associated with infection by a human enterovirus.

AUTHORS' CONTRIBUTIONS

BKP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **GSL:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing – review & editing. **AS:** Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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