Identification of Mycoplasma in the Cerebro-Spinal Fluid (CSF) Sample from a Patient with Multiple Sclerosis (MS)

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Abstract

Background and aim: Multiple sclerosis (MS) is a complex neurological condition, characterized by demyelination and axonal loss. MS is the most common human demyelinating disease. The etiology of MS is unknown, but a lot of factors are considered. There are many studies on viral, bacterial and other factors one of which is Mycoplasma infections. In this study, we evaluated the mycoplasma pneumonia in cerebro-spinal fluid (CSF) of MS patients in the attack course. Methods: In this descriptive study, with the purposive sampling of CSF from 60 patients diagnosed with MS to diagnosis by the physician) by PCR identification method. Results: PCR identification of Mycoplasma species in a total of 42 enriched samples successful in 1 strain and showed specific amplicon at 163 bp. Conclusion: Mycoplasma pneumonia may be one of the causes or trigger

Keywords: mycoplasma pneumonia, Multiple sclerosis, MS, CSF, PCR

Introduction

age adults. MS is estimated to affect 400,000 persons in the United States and 2 million people worldwide. Women are affected twice as frequently as men, between the ages of 20 and 40, and whites are especially those of northern European extraction.

Generally accepted hypothesis is that MS is secondary to an autoimmune response to self-antigens in a genetically susceptible host. It should be pointed out that the inflammation found in the CNS of patients with MS is thought to represent an autoimmune response (7). There were thought to possibilities regarding the initiating event in MS. The first is that the CNS white matter is structurally normal,

Multiple sclerosis (MS) is an inflammatory disease limited to the central nervous system (CNS) white matter. MS is a chronic demyelinating disease of the central nervous system. Studies have shown that MS is a result of immune reaction that happens in a susceptible genetic condition (1-2). The inflammation varies among different patients, and consists of variable degrees of T lymphocytes, macrophages, B lymphocytes, and antibodies at the leading edge of the white matter destruction (3-4). MS is the most common inflammatory-demyelinating disease of the CNS and the most frequent cause of non-traumatic neurologic disability in young and middle-

arthritides (8-12-18). Patients with chronic fatigue syndrome, as a major sign, often have different clinical diagnoses, but display overlapping signs/symptoms similar to many of those found in CSF, FMS, and MS There is some preliminary evidence that mycoplasmal infections are associated with various autoimmune diseases. In some mycoplasma-positive GWI cases signs and symptoms of MS, Amyotrophic Lateral Sclerosis (ALS), Lupus, Graves's disease, and other complex autoimmune disease have been seen. Such usually rare autoimmune responses are consistent with certain chronic infections, such as mycoplasma infections, that penetrate into nerve cells synovial cells, and other cell types. The autoimmune signs and symptoms could be the result of intercellular pathogens, such as mycoplasmas, escaping from cellular compartments and incorporating into their own structures pieces of host cell membranes that contain important host antigens that can trigger autoimmune responses. Alternatively, mycoplasma surface components, sometimes called super-antigens, may directly stimulate autoimmune responses. Perhaps the most important event, the molecular mimicry of host antigens by mycoplasma surface components, may explain, in part, their ability to stimulate autoimmune responses (19). We used PCR (polymerase chain reaction) to determine the presence of mycoplasma genus in CSF of patients in Kerman province, Iran.

and that an autoimmune response is initiated by auto-reactive T cells. The second possibility is that the inflammation is a result of some alteration of the CNS white matter, which could be the result of a microbial CNS infection.

The trigger of showing immunity responses is totally unknown, but some factors are considered as contributing, such as personal factors (age, sex hormones) and environmental factors, including vitamin D deficiency, smoking, previous infection by Epstein-Barr Virus (EBV), contact to metals living in high prevalence ecological regions and cold weather (8-9-10). The prevalence of MS in Kerman is nearly 60 cases Chemokine and interleukins play an important role in the autoimmune diseases (12-13-14-15). Mycoplasma may have a role in triggering Mycoplasmas (class Molicutes) lack a rigid cell wall and are bound by a single membrane, the plasma membrane. Wall-less bacteria were first described years ago, and now 190 species, widely distributed among humans, animals, insects and plants, are known (17). Mycoplasmas can cause acute disease at multiple sites with wide-ranging complications and have been implicated as cofactors in disease. Recently, Mycoplasmas have been linked as a cofactor to AIDS pathogenesis and to malignant transformation, chromosomal aberrations, the Golf War illness (GWI), and other unexplained and complex illnesses, including chronic fatigue syndrome, Crohn's disease, and various

Material and methods

system can support, supplement, or even replace some clinical criteria (22-23), as recently emphasized by the so-called McDonald Criteria of the International Panel on Diagnosis of MS (23-24). The McDonald Criteria have resulted in earlier diagnosis of MS with a high degree of both specificity and sensitivity (25-26),

MS Diagnosis: Diagnostic criteria for MS include clinical and paraclinical laboratory assessments (20-21), emphasizing the need to dissemination of lesions in space (DIS) and time (DIT) and to exclude alternative diagnoses. Although the diagnosis can be made on clinical grounds alone, magnetic resonance imaging of the central nervous

min. The tube and an equal volume of mixed phenol/cholorophorm (1:1) was added. After centrifuging at 13 000rpm for 20 min, the aqueous transferred to another tube to which an equal volume of pure cholorophorm was added, and then centrifuged at 13 000 rpm for 5 min. was transferred to a new tube, mixed with 1/10 volume of acetate sodium (3 M) and was precipitated in a -20øC refrigerator with a 2-fold cool and pure ethanol (20 min). Next, the tube was centrifuged at 13 000 rpm for 15 min. 200 æL of 70% ethanol was at 13 000 rpm for 5 min, and finally, the DNA was dried and re-suspended in DDW at 4øCto be used.

PCR: For mycoplasma genus, amplification of the 16S rRNA gene was performed with a pair of primers complementary to the regions close to the 5' the 3' termini of the gene. DNA amplification was carried out in a total volume of 35.25æl, containing 17.5æl of DNA, 0.1æl of each primer, 0.5æl dNTP mix (10mM) {Roche}, 4æl Mgcl2 (25mM) {Roche}, 2.5æl PCR buffer (10x) {Roche}, and 0.25æl Tag DNA polymerase (5unit/æl) {Roche}. The reaction mixture was thermo-cycled. The thermal-cycling conditions for Mycoplasma strain were as follows: 95øC for 6 min, followed by 35 cycles of 94øC min, 55øC for 1 min, and 70øC, for one minute. The PCR products were stored at 4øC. Negative and positive controls were PPLO broth media and standard strain of M. Pneumonia were included in all tests. Each microliter aliquot of every PCR product was mixed with 2æl of the loading buffer (6x The PCR products and a 100bp DNA ladder were, then, separated by electrophoresis on 1% agarose gel and stained with 0.5æl/ml ethidium bromide volts for 1hr), following a UV transluminator From the work on mycoplasma genus amplification, two designed primers (forward and reverse), were used; a 163bp region of 16S rRNA gene was amplified

allowing for better counseling of patients and treatment.

Since the revision of the McDonald Criteria in 2005, new data and consensus have pointed to the need for their simplification to improve comprehension and utility and for evaluating their appropriateness in populations that differ from the largely Western Caucasian adult populations from which the Criteria were derived. In May 2010, in Dublin, Ireland, the International Panel on Diagnosis of MS (the Panel) met for a third time examine requirements for demonstrating DIS and DIT and to focus on the application of the McDonald Criteria in pediatric, Asian, and Latin American populations.

Mycoplasma isolation: In this descriptive study, the purposive CSF sampling from 42 patients diagnosed with MS (according to diagnosis by physician) was conducted in the first 6 months of 2013. Following the clinical examination, samples were collected from epidural space. were immediately placed in test tubes with transport Mycoplasma culture medium and were kept at 4øC until they have been transported to the respective department of Pasargad Microbiology Research Group's Microbiology Laboratory within 24 h. Transport media contain thallous acetate (250mg liter), that reduce bacterial contamination of the clinical sample (28). The collected samples in transport culture medium were, then, incubated PPLO broth in order for the primary Mycoplasma to be propagated and enriched in a humid air with 5% CO2 at 37øC overnight in the DNA extraction: Consequently, DNA was extracted according to the procedures of Kojima (29). 500 æL of the samples were placed in a 1x tube and micro centrifuged at 13 000rpm for 15 min. 100 æL of lyses buffer was added to 100 æL of precipitate, and, the tube was bath for 4 h. Then, 200 æL saturated phenol was added and the tube was centrifuged at 13 000 rpm for 20

For the M. hominis species, the primer sets RNAH1 and RNAH2 had already been designed (30) which amplified a 334bp region of lipoprotein gene. Sequences of the primers with their corresponding genes are shown in Table 1. DNA amplification was done in a total volume of 25 æL, DNA, 1 æL of each primer, 0.5 æL dNTP mix (200 mM) (Cinnagen Inc.), 2 æL Mgcl2 (50 mM) (Cinnagen Inc.), 2.5 æL æL Tag DNA polymerase (5 units/æL) (Cinagen Inc.). The reaction mixture was thermocycled 30 times, beginning with an initial denaturation step minimum 94øC. The temperature and time profile of each cycle was as follows: 94øC for 3min (Annealing) and 72øC for 1 min (Extension), while were finished with a final extension step at 72øC for 10 min. PCR products were stored at 4øC. PCRs were carried out using two programmable, cyclers (Primus and Master gradient). Positive and negative controls were included in all tests. Each æL aliquot of every PCR product was mixed with a 2 æL loading buffer (6X). The PCR products and 100bp DNA ladder were then separated by electrophoresis on 1% agarose gel and stained with ethidium bromide (100 volts for 1h), following a UV

Results

PCR identification of Mycoplasma species in a total of 42 enriched samples was successful in 1 strain and showed specific amplicon at shows the bands (163bp) obtained from Mycoplasma-positive male and female samples. All of the electrophoresed PCR products were run with positive and negative control

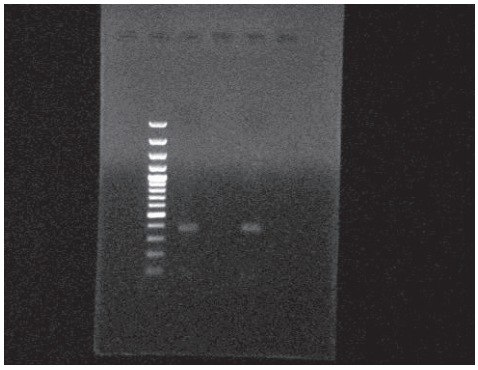

Figure 1: PCR products of Mycoplasma strain on Agarose gel. From left to right: lane +. Mycoplasma positive control. Lane control. Lane 1. Positive male

Discussion

In this study, we detected one case of mycoplasma in 42 known MS cases at acute attack. PCR identification of Mycoplasma species in a enriched samples was successful in 1 strain and showed specific amplicon at 163 bp. A similar study attempted to isolate M. pneumonia the organism by PCR from CSF of pediatric patients with Central nervous system manifestations. Of the 244 CSF samples, no M. pneumonia was Six (2.5%) of the CSF samples were positive by PCR amplification. More efforts are necessary to isolate the organism from CSF samples in order ascertain the role of M. pneumonia in causing neurological complications.(31) Another study developed a sensitive two-stage PCR method using nested or semi-nested primers specific for the bacterial 16S ribosomal DNA to test the presence of bacterial DNA in CSF. They

aseptic meningitis, while, the highest amount was observed in cases with Gillain-Barre syndrome and those with focal neurologic deficit (34). In another study conducted in Iran using ELISA method, IgM and IgG to M. pneumonia were determined in 130 patients with relapsing-remitting MS (85 Remitted phase and 45 Relapsed phase) and 50 sex-controls. The groups were compared with a significant level of $p < 0.05$. The median [interquartile range] titer of IgG in remitted MS RU/ml versus 64 [52.6-71.4] RU/ml in relapsed group and 57.5 [29.2-74.3] RU/ml in the control group ($p = 0.442$). There was difference between the groups based on median titer of IgM too ($P = 0.446$). The median [interquartile range] titer of M. pneumonia IgG in RU/ml in remitted patients, versus, 63.85 [52.45-71.25] RU/ml in the relapsed patients, and 55.2 [29.17-72.75] RU/ml in controls (p hoc analysis demonstrated significant difference between remitted patients and controls ($p = 0.002$). There was not any significant the men in the groups (p).

Conclusion

Consequently, we were unable to find evidence for direct CNS infection with M, pneumonia in MS

designed seven sets of primers to amplify DNA from spirochetes, Campylobacter, Mycoplasma, Chlamydia, Barton Ella, Mycobacteria, and Streptococcus, and tested CSF in patients with relapsing-remitting MS, primary progressive MS, transverse myelitis and controls. They did not detect DNA from any of the groups of bacteria in patients or controls (32). In a study, 30 samples of human brain and 57 pairs of serum and CSF were examined. No Mycoplasma-specific sequence was detected, and the consistent observation of an endogenous gene, human serum albumin (HSA), as a positive control, documented the adequacy of the method. Real-time PCR analysis of serum and CSF was also done, targeting using the Mycoplasma 16S rDNA gene, and this, also demonstrated the lack of Mycoplasma in these samples. The presence of Mycoplasma at extra-neural sites in MS patients is now being explored.(33) A study was carried out in Iran on 55 pediatric patients with central nervous system disorders. The CSF anti body level of M, pneumonia significant difference between the cases and the control group (0.08ñ0.26 versus 0.001ñ 0.001, p=0.02). It showed poor agreement between cases the control group (Kappa=0.27). The lowest amount was seen in cases with

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Investigating the Relationship between Nurses' Moral Sensitivity and Patients' Satisfaction with the Quality of Nursing Care

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Abstract

Background & Aims: Patient satisfaction is considered as an important indicator of the quality and effectiveness of health system. Moral sensitivity plays an important role in how professional responsibilities and moral decisions are made by nurses. This study aims to investigate the relationship between nurses' moral sensitivity and patients' satisfaction with quality of nursing care.

Materials & Methods: This study is descriptive and analytic. First, nurses in internal, surgical and special wards were selected through census method. Then, patients were selected using quota sampling to the ratio of nurses from each section. Data were collected through Demographic Questionnaires, Patient Satisfaction Instrument and Moral Sensitive Questions. Data were analyzed using SPSS 23.

Results: There was a significant positive correlation between nurses' moral sensitivity and patients' satisfaction with quality of nursing care ($P<0.05$). The majority of patients (70.5%) had moderate level of satisfaction with quality of nursing care. 93.5% of nurses had high moral sensitivity. There was no statistically significant relationship between nurses' moral sensitivity and variables of sex, location of work, marital status, type of responsibility and work shift. Relationship between patient satisfaction and type of admission ward was significant ($P = 0.03$).

Conclusion: Increased moral sensitivity in nurses is effective on improving patients' satisfaction with the quality of nursing care. It is suggested to conduct further research with larger sample size and investigate other factors affecting patient satisfaction in order to ensure the generalizability of research results.

Key Words: Nurses' Moral Sensitivity, Patients' Satisfaction, Quality of Nursing Care

Ethics are set of behavioral characteristics originated from individual's beliefs, values, commitments, inner faith and piety and play role in determination of attitudes and values by him (1). Moral sensitivity is the first component to observe ethics (2) and includes individual's skill and ability to interpret the reactions and feelings of others. This kind of sensitivity is effective on detection of moral conflicts, emotional and intellectual perception of vulnerable situations as well as increasing awareness of ethical consequences resulted from making decisions about others (3, 4). The attention paid to moral sensitivity is increasing especially in social fields and occupations (2). Nursing is a social profession (5). Due to the certain circumstances of their profession as well as providing acute and intensive care, nurses are regularly exposed to situations of conflict and tension caused by ethical confusions (6). Lack of attention to ethical issues by nurses may result in ignoring these