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Viral and Bacterial Coinfection in Patients with Neurological Disorders: An Analytical Cross-Sectional Study from Karachi

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Abstract

To study the existence of coinfection of the meninges caused by *Neisseria meningitidis*, Herpes simplex virus-1 (HSV-1) and Herpes simplex virus-2 (HSV-2) in the CSF of patients admitted with neurological disorders. 92 CSF samples collected from patients having neurological ailments were subjected to the microbiological analysis for the detection of possible bacterial etiology. In order to screen the viral coinfection, DNA was extracted from the samples found positive for bacterial presence. Extracted DNA was amplified for the presence of glycoprotein G gene of HSV-1 and HSV-2. Of the 92 samples, 20 samples (21.7%) found infected with *Neisseria meningitidis* (Nm). PCR results highlighted that of this 20, 8 samples (40%) were found coinfected with both HSV-1 and HSV-2 in addition to the Nm. Whereas, 12 samples (60%) were found negative for the viral etiology. Moreover, out of 8 samples showed viral and bacterial conifection, 5 (62.5%) were from female patients. Whereas 3 (37.5%) were from male patients. Findings from present study provide a considerable evidence of existence of bacterial and viral i.e. Nm and HSVs coinfection in CSF of the patients with neurological disorders. Nonetheless, use of microbiological and molecular testing methods in medical diagnostic laboratories and hospitals are pivotal to differentiate between bacterial and viral meningitis and even detect coinfection of both etiologies.

Keywords: Coinfection; Viral meningitis; Bacterial meningitis; *N. meningitidis*; Herpes simplex virus; Central nervous system; Cerebrospinal fluid; Diagnosis

Introduction

Meningitis is established to be the most severe and potentially fatal infection of central nervous system (CNS) involving the risk for permanent disability among survivors [1,2]. This infection is mainly attributed to different bacterial and viral etiologies thus, referred as bacterial meningitis (BM) and viral meningitis (VM) respectively [1, 3,4]. *Neisseria meningitidis* is commonly responsible for BM [5-9] whereas herpes simplex viruses (HSVs) are the main cause of VM [10-12].

Extensive literature review has suggested several studies reporting individual infections of both type of aforementioned meningitis [6-12]. Although, coifections of CNS with microorganisms of quite different texa are extremely rare [13] Nonetheless, evidences suggested that infections have been described with two bacterial species [14] Furthermore, simultaneous infection of CNS caused by bacterial i.e. *Streptococcus pneumoniae* and viral etiology i.e. HSVs has been reported as well [2,13].

CNS pathogen may adopt various mechanisms to cause dual infections. This may include a well-documented fact that viral infections of the CNS surpass bacterial infections [15,16]. Furthermore, HSVs becomes latent in dorsal root ganglia subsequent to the primary infection. Reactivation of these viruses may occur by several stimuli including febrile illnesses, stress and immunosupperation [13] and other diseases where these viruses might not constitute the aetiopathogenic agents [17] In addition, another important mechanism of viral and bacterial coinfection was studied by Wang et al. mentioning that significant damage to nasal mucosa caused by bacteria favours the establishment of viral infection [18]. Although clinical relevance of these coinfections may not be clear; however, it is tempting to speculate about the interplay between viral and bacterial CNS infections, for example by facilitating the entry of one or the other into the CNS compartment.

Since the detection of viral and bacterial coinfection of the meninges is not routine in many medical laboratories and hospitals in Karachi. Consequently, the diagnosis of this fatal infection with varied etiology may be missed. Therefore keeping in view, present study was aimed out to determine the existence of coinfection of the meninges caused by *Neisseria meningitidis*, Herpes simplex virus-1 (HSV-1) and Herpes simplex virus-2 (HSV-2) in the CSF of patients admitted with neurological disorders.

Materials and Methods

Chemicals

Unless otherwise stated, all the analytical grade chemicals and formulated bacteriological media were purchased from Merck, Frankfurter, Darmstadt, Germany. Polymerase chain reaction (PCR) reaction mix was purchased from Promega, Madison, Wisconsin, USA.

Study design

Present study is a sub-analysis of cerebrospinal fluid (CSF) samples collected randomly from the patients admitted with neurological ailments in the two major government hospitals of Karachi, Pakistan [9]. Sample collection was carried out subsequent to the consent and approval of lab authorities. All informed patients were given a questionnaire for their consent prior to inclusion in the study. All procedures performed in this study involving human participants

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were in accordance with the 1964 Helsinki declaration and its later amendments.

All the collected CSF samples were aliquotted and stored at -80°C immediately. CSF samples proven the presence of meningitis bacteria i.e. *Neisseria meningitidis* (Nm) subsequent to the microbiological processing as previously described [9] were included in the present study. An analytical cross-sectional study design was used to conduct the study reported herein.

Isolation of *Neisseria meningitidis*

Isolation and identification of bacterial etiological agent was carried out according to the previously described study conducted in our lab [9]. Briefly, different culture media i.e. nutrient agar, blood agar and chocolate agar were separately streaked with 10 µL of CSF samples. The experiment was run in duplicate and all plates were incubated overnight at 37°C. Bacteria were identified on the basis of visual colonial characteristics, cellular morphology, and standard biochemical reactions.

Total 20 Nm positive CSF samples were additionally checked for the presence of Herpes simplex virus 1 (HSV1) and Herpes simplex virus 2 (HSV2) and for the occurrence of their coinfection.

Total DNA extraction

For total cellular DNA extraction, 150 µL of CSF was mixed with 150 µL of cell lysis buffer (containing 2 mg/mL proteinase K in 1 M Tris-HCl (pH 8.0), 0.1 mM ethylenediamine tetraacetic acid, and 5% (w/v) sodium dodecyl phosphate) and incubated at 37°C for 1 hour. The lysed samples were extracted twice with equal volume of phenol and chloroform-isoamyl alcohol (24:1), both steps followed by a centrifugation step at 5000 rpm for 2 min. DNA was precipitated by adding 2.5 volumes of chilled absolute ethanol and placed overnight at -20°C. The DNA was centrifuged at 13,000 rpm for 20 minutes at 4°C. The DNA pellet was washed with 70% ethanol. After air-drying, the DNA was dissolved in 25 µL of sterile distilled water (D/W). Extracted DNA was quantified spectrophotometrically at 260 nm.

PCR reaction

PCR amplification was carried out using GoTaq® Green Master Mix (Promega). For each PCR reaction, the 25 µL reaction mixture contained 12.5 µL of Master Mix, 6.5 µL of the D/W, 2 µL of each forward and reverse primer and 1.7-194 µg of the template. Furthermore, two different sets of viral specific primers targeting glycoprotein G genes reported elsewhere [19] were selected to carry out PCR. The temperature profile used for the PCR is summarized in (Table 1). Amplified PCR products were detected by agarose gel (2%) electrophoresis and gels were visualuzed using GelDoc (BioRed, USA) after staining with ethidium bromide.

Results

Total ninety-two cerebrospinal fluid (CSF) samples were screened for the presence of bacterial neuropathogens, out of which, 20 samples (21.7%) found infected with *Neisseria meningitidis* (Nm). Cellular DNA quantification profile of these Nm positive CSF samples showed that 8 samples (40%) high yield of DNA i.e. 60.931 µg/mL, whereas the amount of quantified DNA in 12 samples (60%) was found to be of moderate concentration i.e. 24.18 µg/mL (Figure 1).

Furthermore, the results of PCR (Figure 2) highlighted that out of this 20 samples, 8 (40%) revealed the presence of viral coinfection of both HSV-1 and HSV-2 in addition to the Nm. Whereas, 12 samples (60%) were found to be infected with *Neisseria meningitidis* exclusively. In contrast, (Figure 3) describes the frequency of the coinfection in the CSF samples of patients.

Moreover, the results of the present study indicated out of 8 samples showed viral and bacterial conifection, 5 (62.5%) were from female patients. Whereas 3 (37.5%) were from male patients (Figure 4).

Disscussion

The study herein reported is the first study to provide a better overview of the bacterial and viral coinfection in the cerebrospinal fluid (CSF) of patients suspected different neurological disorders.

Existence of the coinfection was evaluated initially by screening of bacterial etiology. Therefore 92 CSF samples were analyzed for the presence of bacterial neuropathogen. The results of the present study indicated that 20 samples (21.7%) showed infection with *Neisseria meningitidis* (Nm) subsequent to the standard microbiological and biochemical examination. These results are in line with the findings of other authors [6-9] who reported Nm as the principle pathogen of bacterial meningitis (BM).

Since the detection of viral and bacterial coinfection of the meninges is pivotal from the standpoint of both diagnosis and to better manage the infection. Consequently, reflecting the local epidemiological trend of the microbial agents associated with neurological disorders. Therefore, Nm positive CSF samples were additionally screened for the presence of Herpes simplex virus-1 (HSV-1) and Herpes simplex virus-2 (HSV-2). Our results showed a considerable rate (40%) of coinfection of HSV-1 and HSV-2 in CSF samples that have already showed the infection of Nm (Figures 2 and 3). Since the lifelong latency of HSVs in neuronal cells is the characteristic feature of these viruses [17] therefore, the results of current study can be explained by the fact that the host complex immune response triggered by severe BM can have a phase of decreased immunity i.e. immune paralysis, [20] allowing latent HSVs to reactivate. Our findings can be further supported by the fact that many patients of HSV infection may shed

S. No	Virus	Primer name	Sequence	Tm	Reference
01	Herpes Simplex Virus- 1	HS1F3	GCCGTTGTTCCCATTATCCC	59°C	[19]
02		HS1B3	TACTTGGCATGGGGGGTG		
03		HS1LPF	TTGGTGGGAACCCCCGATAC	57°C	
04		HS1LPB	AACATGACCCAGACCGGCAC		
05	Herpes Simplex Virus- 2	HS2F3	GGCCTTGACCGAGGACAC	58°C	
06		HS2B3	CGACTCCACGGATGCAGT		
07		HS2LPF	GCCGACACAGGGAGGGGCGT	64°C	
08		HS2LPB	GATGGCCACACAAGCCGCAA		

Table 1: Primers used for Herpes Simplex Virus amplification.

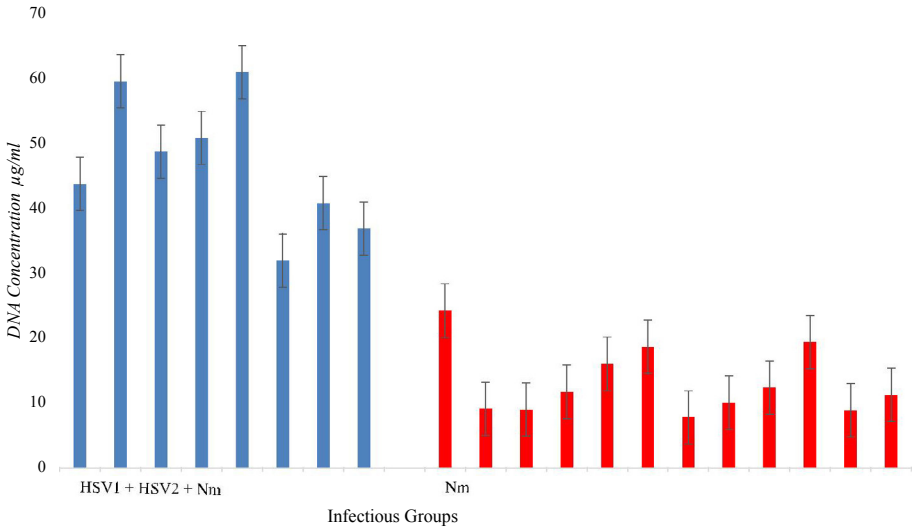

Figure 1: Cellular DNA quantification profile in CSF infected bacterial and viral etiological agents.

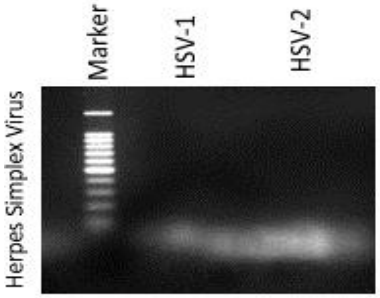

Figure 2: PCR results of HSV-1 and HSV-2.

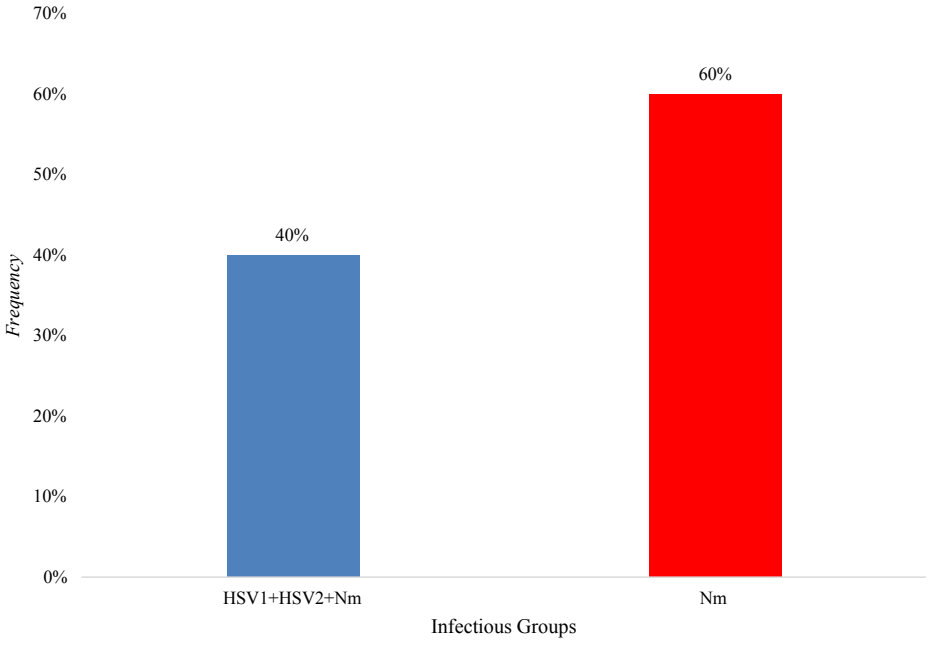

Figure 3: Frequency of bacterial and viral coinfection.

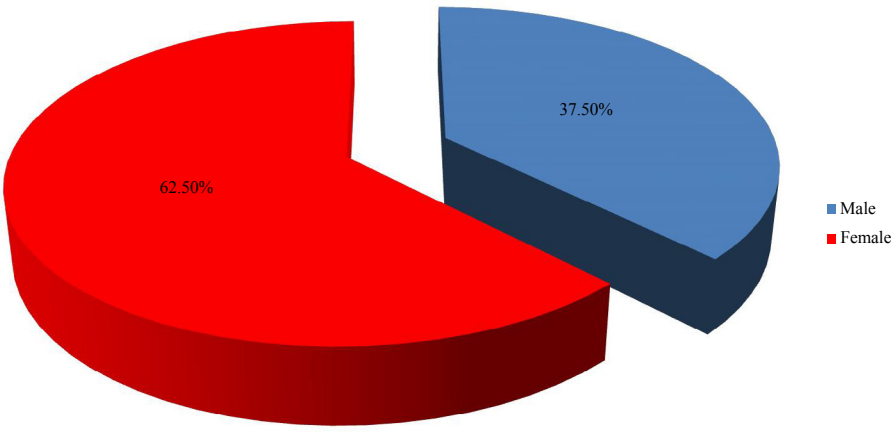

Figure 4: Gender distribution in individuals showed bacterial and viral coinfection.

low levels of viruses continuously without demonstrated reactivation [21] In current scenario it can be hypothesized that these viruses were present latently in the cells of CNS. They may get activated after the occurrence of meningococcal infection where this bacterium served as strong stimulus for the viral reactivation.

Interestingly, all the CSF samples showed bacterial and viral coinfection, were collected from the local government hospitals of Karachi. People living in shanty towns belonging to the low socioeconomic status were the common visitors of these hospitals. Therefore, despite of the aforementioned possible clinical causes of coinfection, stressful conditions due to compromised socioeconomic status may also be another cause of viral reactivation. The results of the present study are in accordance with the findings of Padgett et al. [22] who reported that physcological and social stress significantly contribute in the reactivation of the HSV-1.

DNA quantification profile of CSF samples revealed coinfection rendered the high yield of the DNA concentration i.e. 60.931 µg/mL (Figure 1). This result may be explained by the fact that HSVs may stimulate the apoptosis in the infected cells which may lead to either loss or dysfunctioning of the cells of CNS. Neuronal and/or glial cell breakdown during viral infection yields end products which seem to be deleterious for the healthy cells and may facilitate the secondary infection. Our results are consistent with the study of other researcher [23] who concluded that HSVs induced neural cell oxidative tissue damage and cytotoxicity by activating microglial cell reactive oxygen species through a toll like receptor-2 (TLR2) dependent mechanisms. This may contribute to the neurodegeneration both in peripheral and central nervous system that may leads to the permanent disabilities in the survivors of these infections.

Conclusion

Present study provides a considerable evidence of existence of bacterial and viral i.e. Nm and HSVs coinfection in CSF of the patients with neurological disorders. It is noteworthy to state hererein that coinfection of varied aetiologies greatly enhances the sensitivity of host immune, peripheral and central nervous system to subsequent exposure to microbial bioactive molecules. Therefore, patients suspected for BM, VM and even coinfection of both may be kept under strict observation even after convalescence. Furthermore, use of microbiological and molecular testing methods should be required in medical diagnostic laboratories since they can differentiate between bacterial and viral meningitis and even detect coinfection of both etiologies. This would be immensely helpful to shorten hospital stays for patients and avoid unnecessary use of antibiotics. Since antibiotics would not effectively cure the infection if the cause is of viral origin therefore correct diagnosis is pivotal. Nevertheless, present study constitutes a small number of CSF samples which was mainly due to difficulty of CSF sample acquisition from the aforementioned hospitals. This can be explained by the fact that very small number of CSF samples is being considered by the clinicians to be checked for the presence of BM. However, it is suggested that such study must be carried out continuously to establish the surveillance department in the hospitals to acquire the information about the modality of epidemic, pandemic, prevailing and existing disease programme.

Conflict of Interest

The authors of this manuscript declare that they have no conflict of interest.

Authors' Contributions

Conceived and designed the experiments: NJ. Collection of the samples: AT. Experimental work: AT. Analysis of the data and Conclusion: AT, NJ. Writing of the manuscript: AT. All authors read and approved the final manuscript.

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