An-Najah National University
Faculty of Graduate Studies

THE PREVALENCE OF *ENTEROVIRUSES* ASSOCIATED WITH ASEPTIC MENINGITIS IN NORTH REGIONS OF WEST BANK, PALESTINE IN 2022-2023

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Dedication

To my lovely Family, who always told me that I can achieve anything I put my mind to. Also to my beloved husband, and darling daughter, who I am forever grateful for their endless love, support and encouragement. I dedicate this work to them.

Acknowledgments

All thanks and appreciation to my God Almighty who has helped me a lot

Thanks to all my rudder and teachers who have helped me during my university trip

Thanks and deep respect to Dr. Walid Bash for his help

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

THE PREVALENCE OF *ENTEROVIRUSES* ASSOCIATED WITH ASEPTIC MENINGITIS AMONG NEONATE IN NORTH REGIONS OF WEST BANK, PALESTINE IN 2022-2023

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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Date: 19. 9. 2024

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Abstract

Background: *Enteroviruses* (EV) is a significant cause of aseptic meningitis worldwide. The prevalence of EV in neonates in the West Bank, Palestine has not been previously studied**.**

Objectives: This study aimed to determine the prevalence of EV in neonates diagnosed with aseptic meningitis at three major governmental hospitals in northern regions of the West Bank, Palestine, examining associations with various demographic, clinical, and laboratory findings**.**

Methodology: A total of 150 cerebrospinal fluid (CSF) samples were collected from neonates younger than 8 weeks with aseptic meningitis from the three hospitals between June 2022 and May 2023. EV RNA in CSF was detected by reverse transcription-PCR and the association with demographic data, clinical symptoms, and laboratory findings were evaluated. Chi-Square and Fisher Exact tests were used for statistical analysis**.**

Results: Out of the 150 samples, 23 (15.3%) neonates were found to have tested positive for EV. The study found no significant association between EV positivity and gender, location, seasonality, or most clinical symptoms and laboratory findings. However, significant differences were observed with vomiting, diarrhea, and photophobia. The distribution of aseptic meningitis cases varied across cities and seasons**.**

Conclusion: EV was present in a minority of aseptic meningitis cases in neonates in the North regions of the West Bank. The study highlights the importance of routine testing for EV in neonates with aseptic meningitis, especially in cases presenting with specific

symptoms like vomiting, diarrhea, and photophobia**.** Further studies are needed to investigate the molecular epidemiology of EV among this age group.

Keywords: Aseptic meningitis, Cerebrospinal fluid, *Enteroviruses*, Neonates, Palestine.

Chapter One

Introduction and Theoretical Background

1.1 Background

Meninges serves as an anatomical nomenclature denoting the tripartite membranous layers that encapsulate the cerebral and spinal structures.(1) Its etymological origin can be traced back to the Greek word "meninx", signifying membrane. These membranous structures fulfill dual pivotal roles in safeguarding neurological tissues (2). Primarily, they offer direct protection and structural support to the cerebral and spinal tissues. Moreover, they indirectly shield these vital structures by means of their attachment to the cranial and vertebral bones. Notably, these meninges assume a significant role in the developmental processes of the brain and cranium during the embryonic phase of evolutionary history (2).

Meningitis, conversely, represents an inflammatory condition affecting these meningeal layers. Etiologically, meningitis manifests in two principal forms: septic meningitis and aseptic meningitis (1). Septic meningitis, a subtype of meningitis, results from the virulent activity of pus-producing bacteria (3) and aseptic meningitis which is not caused by bacterial infection but rather by viral, fungal, or non-infectious agents (4). This condition primarily affects the central nervous system and can lead to severe complications if not properly diagnosed and managed (5). The significance of studying aseptic meningitis lies in its impact on public health (6). Aseptic meningitis is a relatively common condition, with thousands of cases reported worldwide each year. It affects individuals of all ages, but children and young adults are particularly susceptible (7). The symptoms of aseptic meningitis, including fever, headache, neck stiffness, and photophobia, can be debilitating and significantly impact the quality of life of affected individuals (8).

The exact annual incidence of aseptic meningitis is uncertain due to inadequate reporting (9). The overall estimated frequency is about 11 per 100,000 individuals annually in the United States, with a rate of 7.5 per 100,000 among adults. On the other hand, septic meningitis has an incidence rate of 0.9 per 100,000 individuals to 80 per 100,000 individuals per year and has a mortality rate as high as 54% (3). In aseptic meningitis, males are three times more likely to experience it than females, with no

specific inclination towards age or racial differences (10). This condition leads to 26,000 to 42,000 hospitalizations each year in the US (8). Additionally, Mount and Boyle has indicated rates of 70 per 100,000 for children under one year, 5.2 per 100,000 for children aged one to fourteen, and 7.6 per 100,000 in adults (9).

One of the key challenges in managing aseptic meningitis is the difficulty in accurately diagnosing the specific causative agent (11). Unlike bacterial meningitis, which can be identified through bacterial cultures, aseptic meningitis requires specialized laboratory techniques to detect the viral or non-infectious agents responsible. *Enteroviruses*, such as *coxsackievirus* and *echovirus*, are the most common viral causes of aseptic meningitis. However, other viruses, including *herpes simplex virus*, *varicella-zoster virus*, and *mumps virus*, can also be implicated (12). Understanding the prevalence and distribution of *Enteroviruses* genotypes associated with aseptic meningitis is crucial for several reasons (10). it allows for the identification of specific viral strains that may be more virulent or associated with more severe clinical outcomes. By studying the genotypes, researchers can determine if certain strains are more prevalent in certain regions or populations, providing valuable insights into the epidemiology of the disease (13).

knowledge of the prevalent *Enteroviruses* genotypes can aid in the development of effective diagnostic tools and treatment strategies and can inform public health interventions (14). Different genotypes may exhibit variations in their response to antiviral medications, and accurate identification of the specific genotype can guide appropriate treatment decisions. Additionally, understanding the genotypic diversity can inform the development of vaccines or preventive measures targeted at the most prevalent strains (15). Understanding the prevalence and distribution of *Enteroviruses* genotypes provides valuable epidemiological insights. By studying the genotypes in different regions or populations, researchers can identify patterns and trends. This information can help in identifying high-risk areas, understanding transmission dynamics, and implementing targeted preventive measures (10). Furthermore, studying the prevalence of *Enteroviruses* genotypes associated with aseptic meningitis can contribute to the overall understanding of viral evolution and transmission patterns. Viruses are known to undergo genetic changes over time, and monitoring the prevalence

of different genotypes can provide insights into the dynamics of viral spread and evolution within a population or region (16).

In the context of the West Bank, Palestine, studying the prevalence of *Enteroviruses* genotypes associated with aseptic meningitis is particularly important. The region has its unique demographic, environmental, and socioeconomic factors that may influence the prevalence and distribution of viral strains (17). By conducting research in this specific context, researchers can generate data that is relevant to the local population and contribute to the development of targeted public health interventions.

1.2 Etiology

1.2.1 *Enteroviruses es es*

Non-polio *Enteroviruses* (EV) and human par*echovirus*es (HPeV) are compact RNA viruses, belonging to the Picornaviridae family (18). They are responsible for frequent infections in newborns. EV are among the most common viral pathogens worldwide and are associated with a wide range of clinical manifestations, including aseptic meningitis. HPeV is another group of picornaviruses that cause meningitis. In the United Kingdom, the collective occurrence of pediatric viral meningitis (PVM) caused by EV and HPeV is reported as 0.79 per 1,000 live births for EV and 0.04 per 1,000 live births for HPeV (19), with HPeV-3 represents the primary type of HPeV causing aseptic meningitis in children (20). EV are responsible for the majority of cases of aseptic meningitis, accounting for up to 50% of all cases (21). EV are classified into several genotypes. Originally, EV used to be categorized into subgroups using their behavior during replication in either cell cultures or animal models (22). These subgroups encompassed *polioviruses* (PV), *coxsackievirus* (CV) as well as *echovirus*es (ECV). Later, newly discovered EV were labeled with numbers (EV 68–71) (22).

The most common EV genotypes associated with aseptic meningitis include CVs and EVs (5). These genotypes exhibit variations in their genetic makeup, antigenic properties, and clinical manifestations. CV are divided into two groups: CV A group (CV-A) and CV B group (CV-B) (23). They have been classified into 23 serotypes within group A (A1–A22, A24) and six serotypes within group B (B1–B6) (24). CV-A are primarily associated with mild respiratory and gastrointestinal symptoms, while CV-B are more commonly associated with aseptic meningitis and other severe clinical

manifestations (25). EV, on the other hand, are a diverse group of EV that can cause a wide range of clinical conditions, including aseptic meningitis (26). CV-A, CV-B, EV-A71, EV-B69, and EV-B73 are the predominant EVs responsible for inducing PVM (27, 28). The relationship between *Enterovirus* genotypes and disease severity in aseptic meningitis is an area of ongoing research. Some studies have suggested that certain genotypes may be associated with more severe clinical outcomes, including prolonged hospitalization, neurological complications, and even death. For example, *Enteroviruses* genotypes such as CV-B5 and EV-30 have been associated with more severe cases of aseptic meningitis (29).

1.2.2 Other viruses

Herpesviruses. Human *Herpesviruses* (HHVs) consist of eight known viruses, six of them have been reported to causes aseptic meningitis, namely; *Herpes simplex virus* 1 and 2 (HSV-1 and HSV-2), *Epstein Barr virus* (EBV), *varicella-zoster virus* (VZV), *cytomegalovirus* (CMV), and HHV 6 (HHV-6) (30-32). One of the distinctive pathogenic attributes of meningitis associated with HHVs is their ability to establish a latent presence within human neurons subsequent to the initial infection, leading to meningitis upon reactivation (33). HSV-2 was reported to cause a higher number of recurrent aseptic meningitis in children than HSV-1 (32).

Influenza Viruses. *Influenza Viruses* can reach the CNS through the olfactory route or via the cranial nerve pathway (34). Some studies have reported the involvement of type A (subtypes A-H5N1 and A-H1N1) and B *Influenza Viruses* in aseptic meningitis (35, 36).

Arboviruses. The incidence of aseptic meningitis caused by arthropod-borne viruses such *arboviruses* is highly correlated with their geographical and seasonal existence (4). The *West Nile Fever virus* (WNFV) which was reported in USA and some countries and the Japanese encephalitis virus (JEV) which reported particularly in Southeast Asian region, are the most commonly *arboviruses* causing aseptic meningitis (37, 38).

1.3 Diagnosis

Clinical presentation, disease history, and physical examinations constitute the potential diagnostic tools for aseptic meningitis.

1.3.1 Clinical presentation and physical examination

The relationship between clinical presentation and disease severity of aseptic meningitis is complex and multifactorial (39). Factors such as the host immune response, viral load, and individual susceptibility can also influence the clinical course and severity of aseptic meningitis. Additionally, the geographic and temporal variations in the prevalence of genotypes can further complicate the understanding of their association with disease severity (4).

The clinical presentation of aseptic meningitis can vary in severity, with some cases being relatively mild and self-limiting, while others may require medical intervention and close monitoring. The non-specific signs include fever, chills, fatigue, nausea, headache, malaise, neck stiffens, and vomiting, these signs can be totally or partially observed aseptic meningitis patients (11). EV meningitis patients can experience additional symptoms like skin rash, muscle pain, photophobia, cough, sore throat, respiratory symptoms, and gastrointestinal discomfort (40). Newborn with meningitis caused by EV (particularly ECV-11) and who developed a more severe and chronic form of infection, might not display obvious or distinctive symptoms of meningitis. Instead, they could exhibit symptoms commonly associated with other systemic infections, such as dermatomyositis, myocarditis, hepatitis, or arthritis (41).

The clinical presentation of EV and HPeV meningitis is usually non-specific and can present with the same clinical presentation, usually fever. Even though both viruses are belonging to Picornaviridae family, but their diagnosis require two different molecular tests. A recent case report (42) of a 9 days girl with two successive episodes of viral meningitis, found that relying on the clinical signs and laboratory indices is unreliable for etiological diagnosis of meningitis. In this case, she presented with two different clinical presentation and laboratory test results between the first and the second episodes. In the first episode, she presented with fever (38.4) and irritability, mild increase in the protein level and normal glucose level were found in the CSF sample. The molecular detection revealed that the CSF was positive for EV (*echovirus* 11) by reverse transcription-polymerase chain reaction (RT-PCR) of CSF. In the second episode, four weeks after the first one, she presented with fever (38.9), vomiting, and diarrhea for 36 hours, skin mottling, tachycardia (180/min), tachypnea (60/ min), without bulging fontanel or hypotonia. The molecular detection revealed that the CSF

was positive for HPeV (HPeV-3) by RT-PCR. Clinically, HPeV meningitis often presents with a sepsis-like clinical presentation, which can mimic a serious bacterial infection Additionally, HPeV infection can be linked to a range of abnormalities detected in laboratory tests (42).

Over years, clinicians have used physical signs of meningeal irritation such as Brudzinski's signs, Kernig's signs, nuchal rigidity, or head jolt accentuation of headache (Table 1) to help diagnose meningitis at bedside and to decide need for lumbar puncture (43). However, some studies revealed that these physical signs do not help clinicians rule in or rule out meningitis accurately. Regardless of the presence or absence of physical signs, its recommended that meningitis suspected patients should undergo a lumbar puncture (43, 44). Afhami et al. found that a positive head jolt accentuation of headache has a good diagnostic accuracy and showed better sensitivity and specificity rates compared to other signs (45).

Table 1

Physical signs of meningeal irritation

Physical sign	Method	Positive test
Brudzinski's signs	In the supine position, the doctor flexed the patient's neck and observed for the occurrence of flexion in both lower limbs.	Flexion of the knees and hips
Kernig's signs	In the supine position, the doctor raised the patient's knee while keeping it flexed until they achieved the maximum hip flexion. Subsequently, the doctor extended the leg at the knee while assessing for any resistance encountered during this movement	Resistance to extension at the knee to >135◦ or pain in the lower back or posterior thigh
Nuchal rigidity	In the supine position, the doctor gently flexed the patient's neck, instructing them to bring their chin down towards their sternum.	Resistance to flexion
Head jolt accentuation of headache	The doctor instructed the patients to horizontally rotate their heads at a rate of 2 to 3 rotations per second.	Worsening of the base line headache

1.3.2 Diagnostic investigations

Laboratory investigations served as a crucial tool for confirming the diagnosis of aseptic meningitis following a clinical examination of suspected patients. They were also employed to differentiate between aseptic meningitis and septic meningitis by providing valuable diagnostic information. The examination of the CSF for the presence of

bacterial growth, coupled with clinical presentation and physical examination, serves as a potent method for distinguishing between the two primary etiological types of meningitis (46). The most characteristic laboratory feature of aseptic meningitis is the presence of lymphocytic pleocytosis in the CSF of affected individuals, but this may not be observed in young children and neonates (4) and this type pleocytosis can be accompanied with low glucose levels. CSF WBC in viral meningitis typically fall within the range of 20 to 500 cells per milliliter (ml). However, in some cases, they may occasionally rise to levels as high as 1000 cells per ml (4).

It was observed that the laboratory indicators associated with aseptic meningitis largely depended on the specific virus responsible for the infection. Aseptic meningitis cases caused by EVs were characterized by an increase in neutrophil leukocyte count in the CSF, while a decline in CSF glucose levels was primarily detected in cases caused by *mumps virus* and HSV-1 (4). Additionally, lymphocyte counts and protein concentrations in the CSF of patients showed significant variations between cases caused by EVs and VZV. These findings underscore the importance of evaluating laboratory abnormalities in both CSF and serum samples of patients with viral meningitis, not only for distinguishing between viral and bacterial meningitis but also for determining the specific causative virus in cases of viral meningitis. In addition, Martinot et al. was also observed that monocyte leukocytosis in the CSF of patients with viral meningitis was detected (47). Azadfar et al. observe that in cases of viral meningitis caused by HSV-1 and 2, both the RBC count and erythrocyte sedimentation rate (ESR) tended to be higher than the typical values (48). Furthermore, one of the most prominent laboratory features associated with viral meningitis cases is a significant elevation in serum amylase levels, particularly in cases caused by Coxsackie A and B viruses and *mumps virus* (40).

Isolating viruses from CSF, blood, or urine through tissue culture is considered the gold standard for diagnosing many viral pathogens that cause meningitis (4). However, this diagnostic procedure has some limitations. It can be slow, as it may take time for the virus to grow in culture, making it less suitable for rapid diagnosis. It can also be expensive and is not always sensitive, meaning that it may not always successfully detect the virus, especially in cases with low viral loads or when the virus is present in only small quantities in the samples. Therefore, clinicians often use a combination of

diagnostic methods, including molecular tests like PCR, serological assays, and clinical evaluation, to enhance the accuracy of meningitis diagnosis. RT-PCR assays exhibited a remarkable sensitivity rate of 100% and demonstrated clear superiority over the virus isolation technique in detecting both EVs and HPeV meningitis cases (49). Numerous advancements in PCR technology have enhanced both the specificity of detection and the efficiency of performing assays such as multiplex nested PCR, Real-time PCR, and Time-resolved fluorometric PCR (49). Rapid diagnosis through CSF RT-PCR assays had several significant benefits in clinical practice. For instance, it has led to reductions in both hospital stays and the unnecessary use of antibiotics in patients admitted with enteroviral meningitis (21). In addition to traditional molecular tests, advanced techniques such as next-generation sequencing (NGS) and metagenomic next-generation sequencing (mNGS) have been employed in numerous studies for the diagnosis of infectious meningitis (50, 51). These molecular approaches have proven highly useful because they allow for the identification of a broad spectrum of pathogenic organisms in a single assay. This includes the detection of viral, bacterial, fungal, and parasitic causes of meningitis (51). The versatility and comprehensive nature of mNGS make it a valuable tool for diagnosing infectious etiologies, particularly in cases where the causative agent may not be readily identified by conventional methods.

Lumbar puncture (LP) has been employed for dual diagnostic and therapeutic objectives in clinical instances of aseptic meningitis especially in patients with fever and seizures, unconsciousness or altered mental status (28). When CSF is collected from patients using a LP, several laboratory investigations are routinely conducted with the specific aim of confirming infection and distinguishing between bacterial meningitis and viral meningitis such as gram staining, RBC and WBC count, and glucose and protein levels (4).

The CSFs were the routinely used samples for virus isolation. When obtaining CSF sample is difficult, culture of throat and stool samples can be used especially with EV suspected infections (52). The primary limitation associated with the virus isolation method as a diagnostic tool for viral meningitis is the poor sensitivity of certain cell cultures to support the propagation of specific virus strains. Some viruses may not readily grow or may not reach detectable levels in these cultures (40). Additionally, the

timing of sample collection is a crucial factor affecting the sensitivity of virus isolation, as virus titers in body fluids may not be detectable at all stages of infection (21). In response to these limitations, the utilization of serology has been employed as an alternative approach. Serological methods involve the detection of viral antigens or virus-specific antibodies in samples obtained from viral meningitis patients. While serological tests have proven to be valuable tools for diagnosing viral meningitis , it's important to note that false-negative results can occur, especially during the early stages of the illness (8). Radiological investigation using computed tomography and MRI can be used. Such radiological investigation only necessary if there are clinical features of encephalitis or if indicated before lumbar puncture. MRI is more sensitive at detecting demyelination or edema associated with encephalitis (21).

1.4 Prevention

Prevention strategies for viral meningitis are essential to reduce the risk of this potentially serious condition, which can result from various viral infections. While it's not always possible to prevent every case of viral meningitis, several measures can help mitigate the risk and protect individuals, especially those in high-risk groups (53).

Vaccination stands as one of the most powerful tools in preventing viral meningitis. Many vaccines are available to protect against specific viruses that can lead to meningitis, (4, 41, 53, 54) including: (1) *Measles, Mumps,* and *Rubella* (MMR) vaccine, which provides protection against measles and mumps, two viruses that can cause viral meningitis. (2) *Japanese B Encephalitis* Vaccine, this vaccine helps prevent Japanese encephalitis, which can lead to viral encephalitis and, in some cases, viral meningitis. It's typically recommended for long-term residents in endemic areas and travelers at high risk, it may have a relatively low rate of severe adverse reactions, but it is typically reserved for individuals with specific risk factors (37) (3) Tick-Borne Encephalitis Vaccine, this vaccine offers protection against tick-borne encephalitis, which can lead to viral meningitis, which it's essential for individuals traveling to regions where this disease is prevalent (55). (4) Rabies Vaccine, this vaccination is essential for individuals at risk of exposure to the rabies virus, as rabies can also cause viral encephalitis and meningitis (56). (5) Influenza Vaccine, while the *influenza virus* primarily causes respiratory illness, severe cases can sometimes lead to viral meningitis. (6) Polio

Vaccine, in the UK, the inactivated polio vaccine combined with diphtheria and tetanus is used(41).

Good hygiene practices play a pivotal role in preventing viral infections that can potentially lead to meningitis. Practicing proper hand hygiene by washing hands regularly with soap and water helps reduce the risk of transmission of various viruses, including *Enteroviruses*, which are common culprits in viral meningitis cases. In regions where mosquito-borne or tick-borne viruses are a concern, individuals can take preventive measures to minimize the risk of infection. This includes using insect repellents, wearing protective clothing, and utilizing bed nets when necessary (9). These precautions can be particularly important for travelers visiting areas where these viruses are endemic. Avoiding close contact during contagious period of viruses can help reduce the risk of transmission. This is especially important in settings like schools and healthcare facilities where viral infections can spread easily (11).

Pregnant women should take specific precautions to prevent congenital infections that can lead to viral meningitis in newborns. One such example is *Lymphocytic Choriomeningitis Virus* (LCMV), which can be transmitted through contact with rodents. Pregnant women are advised to avoid potential contact with rodents and take steps to rodent-proof their homes (57).

Raising awareness about viral meningitis, its causes, symptoms, and prevention strategies is crucial. Healthcare providers, public health agencies, and educational institutions play a vital role in disseminating information about vaccinations and hygiene practices to the general public. Increased awareness can lead to higher vaccination rates and better adherence to preventive measures (1). While prevention is paramount, it's essential to recognize the signs and symptoms of viral infections promptly. Early diagnosis and appropriate treatment can prevent complications, including viral meningitis, from progressing to severe stages. Individuals experiencing symptoms such as high fever, severe headache, neck stiffness, and sensitivity to light should seek medical attention promptly (21).

1.5 EV Meningitis Worldwide

Several worldwide studies have investigated the incidence of aseptic meningitis, including the incidence of positive EV meningitis, including studies from Finland,(58) England(59) and Wales,(60) South Korea,(61) Brazil,(62) USA,(63) Spain,(64) China,(65) South Africa, (66) and Ethiopia (13) (Table 2).

Table 2

Previous studies on the incidence of EV meningitis worldwide

Study	Period	Country	Patients	EV meningitis infection rate	M/F	Most two common clinical manifestation	Peak
Kupila et al.	1999-2003	Finland	Adults	26% (38 cases)	23/15	Headache and photophobia	Summer
McGill et al.	2011-2014	England	Older than 16	20% (127 Cases)	48/79	Headache and photophobia	Summer
Han et al.	2008-2013	South Korea	Older than 16	38.5% (68 cases)	36/32	Headache and fever	Summer
Vidal et al.	2005-2006	Brazil	All age groups	10.6% (48 cases)	26/22	NI	Summer
Kadambari et al.	2004-2013	England and Wales	All age groups	52% (5133 cases)	NI	NI	Summer
Dupuis et al.	2004-2007	USA	All age groups	47.6% (81 cases)	NI	Fever	Summer
Ory et al.	2008-2009	Spain	All age groups	43.8% (149 cases)	NI	NI	Summer
Wang et al.	2018-2019	China	1 month to 13 years	21.2 % (107 cases)	71/36	Fever and headache	Summer
Nkosi et al.	2018-2019	South-Africa	All age groups	21% (742 cases)	405/337	Fever and headache	Spring
Wami et al.	2020	Ethiopia	All age groups	26.7% (39 cases)	19/20	Vomiting and fever	NI

1.6 EV meningitis in the Middle East and North Africa (MENA) Region

Several studies have investigated the incidence of aseptic meningitis in the MENA region, including the incidence of positive EV meningitis, these studies were conducted in Egypt,(67) Iran,(68) Syria,(69) Tunisia,(70) Jordan,(71, 72) Qatar,(73) Saudi Arabia,(74) and Kuwait(75) (Table 3).

Table 3

Previous studies on the incidence of EV meningitis in the MENA region

Study	Year	Country	Patients	EV meningitis positive rate	M/F	Most two common clinical manifestation	Peak
Raouf et al.	May 2017 to April 2019	Egypt	All age groups	7.4% (7 cases)	NI	Fever and convulsions	Spring and summer
Shahroodi et al.	Mar to Sep 2007	Iran	Neonates and children	36.2% (21 cases),	NI	NI	NI
Yahia et al.	Nov. 2011 to Nov. 2013	Syria	All age groups	46.5% (60 cases)	35/25	NI	Spring
Rmadi et al.	May 2014 to May 2017	Tunisia	Children	12% (24 cases)	14/10	Fever and vomiting	Spring
Masri et al.	Jan 2016 to Aug 2020	Jordan	1 to 14 years	60% (60 cases)	NI	Fever and headache	NI
Meqdam et al.	June to November 1999	Jordan	Children	8.2% (32 cases)	23/9	Fever and vomiting	NI
Mathew et al.	2015 t0 2018	Qatar	All age groups	68.7% (503 cases)	290/213	Fever	Spring
Aldriweesh et al.	Jan 2018 to Jan 2020	KSA	Adults	25% (60 cases)	32/28	Fever and headache	NI
Dalwai et al.	Sep 2003 to Aug 2006	Kuwait	0-12 years	24% (92 cases)	NI	Fever and flu-like symptoms	Summer and fall

As summarized, the prevalence of aseptic meningitis in the MENA region, including the incidence of positive EV meningitis varied between studies in different countries in the MENA region, while most studies included patients form all age groups, some studies included younger and specific age groups, including neonates. The Iranian study of

Shahroodi et al. (68) included neonates and children and found that EV meningitis infection rate was 36.2%, with 21 EV positive cases, among them, 13 were neonates, with 37.1% EV infection rate of this age group compared to 8 children (34.7%).

Dalwai et al. (75) analyzed 387 CSF samples from suspected aseptic meningitis cases among children of various age groups in Kuwait, and found that the overall EV infection rate was 24% for the included children between 0 to 12 years. For patients less than 2 years old, 281 CSF samples were analyzed in this age group, and found that the EV infection rate was 27% with 75 EV positive cases, compared to 21% (14 cases) in the among 68 analyzed CSF samples in the 2-4 age group. Finally, for patients between 4 and 12 years old, among 38 CSF analyzed, the EV infection rate was 8% (3 cases), with a statistically significant difference ($P = 0.011$) regarding the EV infection rate of <2 years and 4-12 years age groups, with 27% and 8% EV positive rate, respectively.

1.7 Aseptic Meningitis in Palestine

Meningitis in Palestine poses a significant health concern. In the Palestine-2020 Annual Health Report (76), it is evident that aseptic meningitis stands as one of the endemic diseases in the region, displaying some seasonal fluctuations. Notably, more than 72% of cases occurred during the spring and summer seasons, amounting to a total of 361 reported cases in the year 2020. These cases were distributed with 67 in the West Bank and 294 in the Gaza Strip. The incidence rate for viral meningitis was recorded at 7.5 cases per 100,000 populations, but it exhibited regional disparity, standing at 2.4 per 100,000 populations in the West Bank and significantly higher at 14.4 per 100,000 populations in the Gaza Strip.

On the other hand, the report reveals that bacterial meningitis had a distinct pattern in 2020. Notably, no cases of meningococcal meningitis were reported, a testament to the efficacy of the ministry of health's immunization policy. Furthermore, the years 2017, 2018, and 2019 witnessed zero occurrences of *Haemophilus influenza* meningitis, with only a singular case reported in 2020. In the context of other bacterial meningitis types, the year 2020 recorded a total of 111 cases, resulting in an incidence rate of 2.3 cases per 100,000 populations. These cases were distributed, with 23 occurring in the West Bank and 88 in the Gaza Strip. The incidence rate in the West Bank was noted at 0.8 per

100,000 populations, while the Gaza Strip reported a relatively higher incidence rate of 4.3 per 100,000 populations.

Dumaidi and Al-Jawabreh (15) conducted a study in Palestine, enrolling patients from diverse age groups who displayed symptoms suggestive of sepsis-like illness and/or aseptic meningitis upon admission to three governmental hospitals between 2012 and 2015. The hospitals involved were Jenin Government Hospital in the northern city of Jenin, Rafidia Government Hospital in Nablus in the north, and Al-Khalil Government Hospital in the southern city of Al-Khalil. The researchers utilized nested RT-PCR targeting the 5'UTR to examine CSF samples, revealing that 18.5% (66 out of 356) of the samples tested positive for EVs RNA. A majority of the positive cases (62%) were infants less than one year old. Among the 12 successfully genotyped cases, seven distinct EVs genotypes were identified, all belonging to the HEV-B species. Notably, the most prevalent genotype was *echovirus* 6 (E6), accounting for 42% of the cases.

In a separate investigation by Dumaidi et al. (17) in 2017, 249 CSF samples from suspected meningitis cases were collected from Rafidia Governmental Hospital in Nablus and Jenin Governmental Hospital in Jenin. Through RT-PCR targeting the 5'UTR, they detected EVs in 22% (54 out of 249) of the CSF samples. After successful genotyping of 26 cases, four different EVs types were identified, all of which belonged to the HEV-B species. The most prevalent detected EVs were E18, accounting for 50% of the cases, followed by *coxsackievirus* B5 (CVB5) at 34.5% (9 cases), E25 at 11.5% (3 cases), and CVB2 at 3.8% (1 case).

In the Gaza Strip, Ghuneim et al.(77) conducted a cross-sectional study to report the epidemiology of meningitis cases in the Gaza Strip between December 2013 and January 2014. Out of the 129 reported meningitis cases, 80 cases (62%) cases were diagnosed as aseptic meningitis, 32 cases (24.8%) were diagnosed as septic meningitis, and 17 cases (13.2%) were diagnosed as meningococcal meningitis. Out of the 129 cases, 57% of the cases were males, and 48.8% were infants. Only 20 CSF samples were undergoing examination to determine the causes of the meningitis, 7 cases (35%) were positive for EVs.

1.6 Problem Statement

The molecular epidemiology of *Enteroviruses* and their genetic variability in Palestinian patients exhibiting sepsis-like illness and/or aseptic meningitis across various age groups has been examined by Dumaidi and colleagues in their studies (15, 17). Nevertheless, prior investigations have not explored the prevalence of *Enterovirus* in CSF samples from neonate patients admitted with aseptic meningitis in different Palestinian regions, specifically focusing on cases with negative bacterial culture. To fill this knowledge gap on this age group, the present study employs PCR as an assessment tool to shed light on this age group.

1.7 Study aims and questions

The objective of this study is to investigate the prevalence of *Enterovirus* associated with aseptic meningitis in different regions of the West Bank, Palestine between June 2022 and May 2023. The specific research objectives are as follows:

1. To determine the overall prevalence of *Enterovirus* among neonate aseptic meningitis cases in the West Bank, Palestine.

2. To assess the variations in the prevalence of *Enterovirus* among neonate aseptic meningitis cases across different demographic characteristics.

Research questions

1. What is the overall prevalence of *Enterovirus* among neonate aseptic meningitis cases in the West Bank, Palestine between June 2022 and May 2023?
2. Are there variations in the prevalence of *Enterovirus* among neonate aseptic meningitis cases across different demographic characteristics in the study population?

These research questions will guide the data collection, analysis, and interpretation of the study findings. By addressing these questions, the study aims to provide valuable insights into the prevalence and characteristics of *Enterovirus* associated with aseptic meningitis in the West Bank, Palestine, contributing to the existing knowledge in the field.

1.8 The study significance

This study holds significant significance and can make valuable contributions to the field of aseptic meningitis research. The prevalence of *Enterovirus* associated with neonate aseptic meningitis patients in the West Bank, Palestine in between June 2022 and May 2023 represents a research gap or knowledge deficit that needs to be addressed. Currently, there is limited information available on the prevalence of aseptic meningitis caused by EVs among neonate patients in this region and their impact on the occurrence and severity of aseptic meningitis cases. This research gap highlights the need for a comprehensive study to fill this knowledge deficit.

Firstly, understanding the prevalence of EV associated with aseptic meningitis among neonate patients in this region is crucial for accurate diagnosis and appropriate treatment targeted this age group. By identifying the prevalence rate, healthcare professionals can tailor their diagnostic approaches and treatment strategies to target the specific strains causing the infection. This can lead to improved neonate patient outcomes and more efficient use of healthcare resources.

Secondly, studying the prevalence of EVs associated with aseptic meningitis among neonate patients in the West Bank, Palestine can contribute to the development of region-specific prevention strategies. Different viruses may exhibit variations in their virulence and clinical outcomes in different age groups and demographical areas. By identifying the prevalence rate, public health authorities can implement targeted prevention measures, such as vaccination campaigns or hygiene promotion, to control the spread of specific viruses among neonate patients. This can help in reducing the overall burden of aseptic meningitis among neonate patients in the region.

Thirdly, the research findings can have implications for public health planning and resource allocation. Understanding the prevalence of EVs associated with aseptic meningitis among neonate patients can guide public health authorities in prioritizing resources, implementing surveillance systems, and developing response strategies targeted this age group. This can enhance the preparedness and response capabilities of the healthcare system, leading to more effective control and management of aseptic meningitis cases.

Furthermore, the research findings can contribute to the global knowledge base on aseptic meningitis. By adding to the existing body of literature, the study can provide valuable insights into the epidemiology of EVs associated with aseptic meningitis among neonate patients This information can be used by researchers worldwide to compare and analyze the prevalence EVs meningitis cases in different regions and different age groups, leading to a more comprehensive understanding of the disease.

1.9 Study Hypotheses

The study hypothesis suggests that there will be a variation in the prevalence of *Enterovirus* associated with aseptic meningitis among neonate patients in different regions of the West Bank, Palestine, in 2022. Additionally, it proposes that there may be variations in the prevalence of *Enterovirus* meningitis across different demographic characteristics within the study population.

Chapter Two

Methodology

2.1 Study Design and Setting

This cross-sectional study was conducted during the period of June 2022 to May 2023 to collected the cerebrospinal fluid (CSF) samples of neonate patients admitted for Aseptic meningitis to 3 governmental hospitals in three cities in west bank, Palestine.

Nablus: Rafidia Governmental Hospital

Tulkarm: Thabet-Thabet Governmental Hospital

Jenin: Jenin Governmental Hospital

2.2 Study Population and Sample Size

The study population was delineated as individuals of Palestinian descent residing in specific regions of the North West Bank, namely Tulkarm, Nablus, and Jenin. The research focused on individuals who were diagnosed by medical professionals in the previously specified medical facilities, and CSF samples were obtained using lumbar puncture. The CSF samples underwent examination by culture and gram stain techniques at hospital microbiology departments, resulting in the absence of common bacterial infections. A convenience sampling method was employed to choose all accessible CSF samples that met the specified criteria from the aforementioned government hospitals.

Inclusion criteria:

neonate patients (< 60 days) with clinical manifestation of aseptic meningitis.

Exclusion criteria:

Patients older than 60 days .

Patients admitted for infections other than aseptic meningitis.

Patients with meningitis proved to be caused by bacterial pathogens.

All CSF samples are suspected to be affected during the transportation and storage process.

- **Sample size**

All samples obtained during the collection period

Storage of samples:

Following the successful permission for sample collection from hospitals, the aforementioned criteria were disseminated to technicians working in hospital laboratories for the purpose of collecting samples for our study. The CSF samples were preserved at a temperature of -80°C until they underwent molecular testing at the Medical Laboratory Sciences Department, which is part of the Faculty of Medicine and Health Science at An-Najah National University.

According to numerous studies, it is widely accepted that a freezer operating at temperatures of -70°C to -80°C is preferable for long-term storage of RNA due to its enhanced stability over extended periods of time. While a standard freezer set at -20°C may be suitable for certain applications, the lower temperatures provide a more optimal environment for preserving RNA integrity(78, 79).

2.3 Study Variables

2.3.1 Background variables

Age: From the date of birth to the date of admission, in days.

Gender: Male or Female.

Place of residence: Tulkarem , Nablus and Jenin

2.3.2 Clinical Variables

The meningitis case was defined as a patient with fever or headache with at least two additional symptoms with the laboratory results of; glucose (normal), protein < 15 mg/dl, cell count <2000 cell / mcl predominate lymphocytes. The clinical symptoms and signs of aseptic meningitis were as follows: fever, headache, vomiting, convulsion, lethargy, neck stiffens, fatigue, photophobia.

Sign and symptoms such as: fever, headache, vomiting, convulsion, lethargy, neck stiffens, fatigue, photophobia, and any other signs and symptoms will be collected from the patient medical file.

2.3.3 Outcome Variables

Positive result of polymerase chain reaction (PCR) for *Enterovirus*.

2.4 Measurement tool and data collection

The extraction of viral RNA from the CSF samples was performed using the ReliaPre™ Viral Total Nucleic Acid Purification Kit. These extraction procedures were performed in accordance with the protocol recommended by the manufacturer.

1. To begin, carefully transfer a volume of 20µl of Proteinase K (PK) Solution into a microcentrifuge tube with a capacity of 1.5ml.
2. Thoroughly homogenise the cerebrospinal fluid (CSF) and thereafter introduce 200µl of the homogenised CSF into the tube holding the protein kinase (PK) Solution. In this concise discourse, we shall proceed to amalgamate many elements.
3. Next, introduce 200 microliters of Cell Lysis Buffer into the tube. The objective of this experiment is to observe the behaviour of a cap and vortex for a minimum duration of 10 seconds.
4. The samples should be incubated at a temperature of 56°C for a duration of 10 minutes.
5. During the incubation period, it is necessary to carefully position a ReliaPrep™ Binding Column into a CollectionTube that is devoid of any contents.
6. The tube should be detached from the heating block. To initiate the binding process, 250µl of Binding Buffer should be added to the tube. The tube should then be capped and mixed by vortexing for a duration of 10 seconds.
7. The contents of the sample tube should be added to the ReliaPrep™ Binding Column. The column should then be capped and centrifuged for a duration of 1 minute at the maximum speed. Ensure that all lysate has fully traversed the membrane.Continue centrifugation for a further minute in order to eliminate any leftover lysate.
8. Place the binding column into a new collecting tube.

9. To initiate the washing process, introduce 500 microliters (µl) of Column Wash Solution into the column. Proceed to centrifuge the column at maximum speed for a duration of 3 minutes. The concept of flow through should be disregarded.
10. Perform step 9 two additional times, resulting in a cumulative total of three washes.
11. To begin, position the ReliaPrep™ Binding Column into a sterile 1.5ml microcentrifuge tube.
12. Next, introduce a volume of Nuclease-Free Water ranging from 50 to 200 microliters into the column. The sample should be subjected to centrifugation for a duration of one minute at the highest achievable speed.
13. The ReliaPrep™ Binding Column should be discarded and the eluate should be retained.
14. The RNA that was extracted was kept at a temperature of -20 degrees Celsius until the performance of the PCR technique.

Primers

The selection of primers targeting the conserved 5' untranslated region (UTR) of the Enterovirus genome was based on previously published studies, as indicated in Table 4. The primers utilised in this study were synthesized by Metabion international AG, a company based in Germany. The primers were prepared at a concentration of 10 nmol/ml in sterile distilled water and thereafter stored at a temperature of -20°C.

Table 4

Primers from the 5' UTR of the Enteroviruses genome used in the RT-PCR assay

Name	Sequence (5'_3')	Map position
Outer primer (P1)	GTA CCT TTG TGC GCC TGT T	65-84
Outer primer(P4)	GAT GGC CAA TCC AAT AGC TA	619-639
Inner primer (P2)	TGG CTG CGT TGG CGG CCT G	358-377
Inner primer (P3)	ACA CGG ACA CCC AAA GTA GTC GG	561-539

***Enteroviruses* Diagnoses by RT-PCR**:

The diagnosis of EV was conducted following the methodology outlined in earlier investigations by Dumaidi et al.(15, 17). The detection of Enteroviral RNA was conducted by employing two distinct primer sets that specifically targeted the 5' untranslated region (UTR). The cDNA synthesis and initial round of RT-PCR

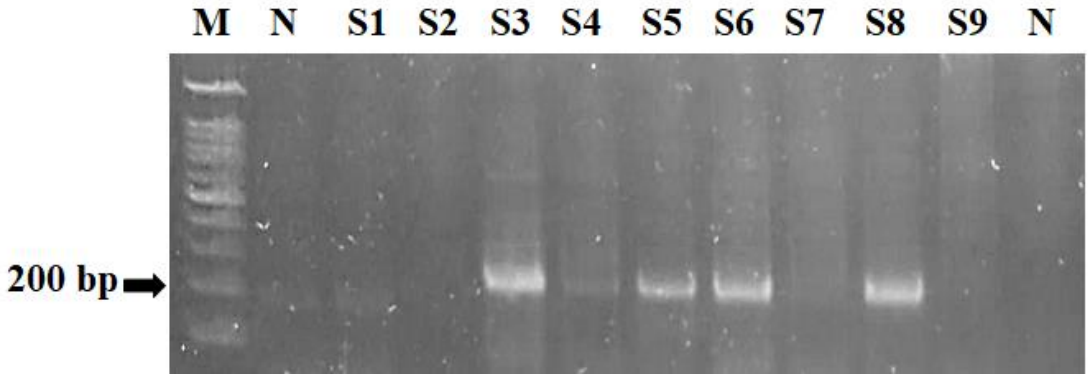
amplification were conducted using a 25µl reaction mixture. The mixture included of 4 µl of viral RNA extraction, 10 U of Reverse transcriptase , 10 pmol of the outer primers (P1 and P2), and 12.5 µl of PCR Reddy master mix (Thermo Scientific), then two microliters from the first round were further amplified in 25 µl reaction mixture containing 10 pmol of the inner primers (P2 and P3) and 12.5 µl of the PCR master mix (Thermo Scientific) and RT-PCR first and second round program (Thermal cycles) in Appendix D

Interpretation:

The electrophoresis of five microliters of PCR product on a 2% agarose gel containing ethidium bromide was performed, A positive result was indicated by a 203 bp band.

Figure1

gel electrophoreses, M: Marker, N: negative control, and S: 10 random samples



2.4.1Quality control

Positive and negative controls were conducted with each iteration of sample runs to ensure procedural verification and confirmation of outcomes. A CSF sample was found to be positive for all genes, and it was utilised as a positive control. In addition to the utilisation of an appropriately sized molecular DNA ladder, it is imperative to ensure accurate interpretation of gel banding.

2.5 Safety Precautions

Cerebrospinal fluid is considered a potentially infectious substance, hence necessitating the adherence to normal precautions.

The aforementioned criteria are applicable in this context. It is imperative to utilize suitable personal protection equipment. Semi-automated micropipettes and single-use plastic counting chambers are considered the most secure choice for doing manual cerebrospinal fluid (CSF) counts. It is imperative that all discarded materials be appropriately disposed of by placing them in a biohazard container. It is imperative to engage in a comprehensive handwashing procedure following the conclusion of the cerebrospinal fluid (CSF) study. In order to ensure proper disposal, spinal fluid samples that are intended to be disposed should be stored in biohazard containers.

2.6 Ethical approval

This research proposal was submitted to the university IRB committee, and get their approval, in addition to contact with Palestinian ministry of health to get their acceptance for sample and. Patient's data collection from records; Confidentiality of patient's information was maintained and secured. Samples given codes; no names were used.

Chapter Three

Result

3.1 Statistical analysis

Statistical Package for Social Science (SPSS) software version 25 was used for data analysis. Descriptive and frequency statistics were used to summarize different variables and presented in the form of texts, charts and tables, Categorical variables were described as frequencies and proportions, and the associations between the categorical variables was assessed by Chi-square and Fisher exact tests.

3.2 Socio-demographic characteristics among neonates' patients

In this study, 150 CSF samples were collected of neonate patients admitted for meningitis in different hospitals in three cities in west bank. The response rate was 100%, and the distributions of samples among the cities were as follow: 40 sample (26.7 %) was from tulkarem, 70 samples (46.7%) was from Nablus and 40 sample (26.7 %) was from Jenin, as shown in Figure 1. In terms of sex distribution, there were 60 males, accounting for 40% of the sample, while the remaining 90 individuals were females, representing 60% of the total population. The study found that the mean age of the participants was 34.98 days, with a standard deviation of 14.24 days. The participants were divided into two groups based on their age. The first group consisted of neonates aged 30 days or less, which accounted for 40% of the sample. This group included 60 patients. The second group comprised neonates older than 30 days, representing 60% of the sample. This group consisted of 90 patients (Figure 2, Table 7).

Collecting the samples was conduct through the different four seasons with different numbers for each season as follow: 27 samples (18%) were collected in fall, 33 samples (22%) also collected in spring, 55 samples (36.7%) collected in summer and finally 35 samples (23.3%) collected in winter. The majority of neonates were suffering from poor feeding, 108 neonates (72%) suffered from poor feeding and just 42 (28%) of neonate weren't had poor feeding (Table 7).

According to our RNA test the vast majority 127 (84.7%) of neonates didn't have the *EV* and but 23 of them (15.3%) had the *EV* (Figure 3 and Table 7).

Figure 2

The distribution of samples among the three cities

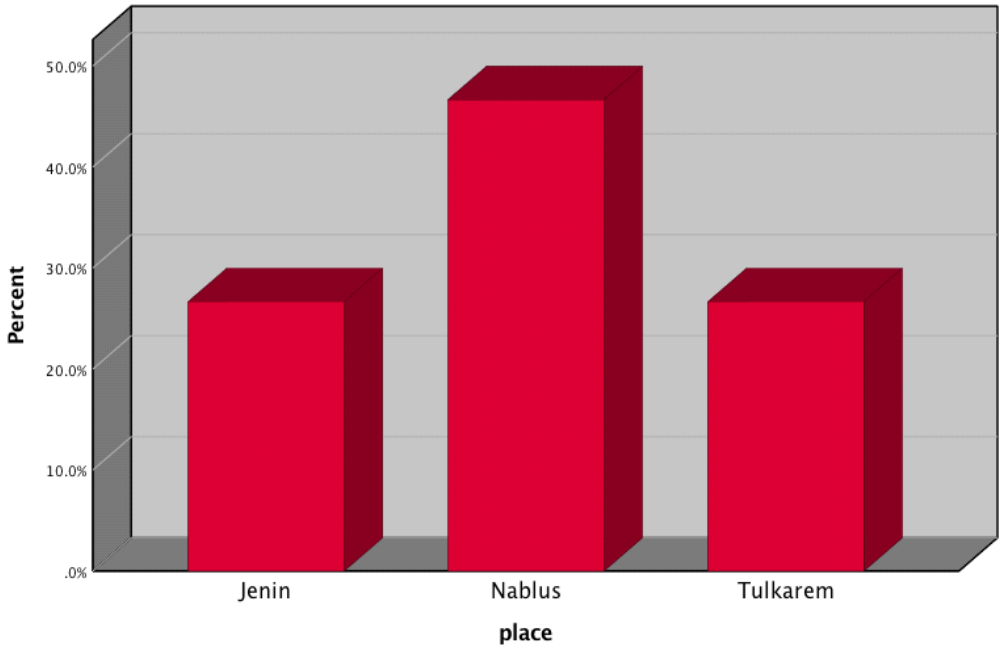


Figure 3

Distribution of age levels among patients

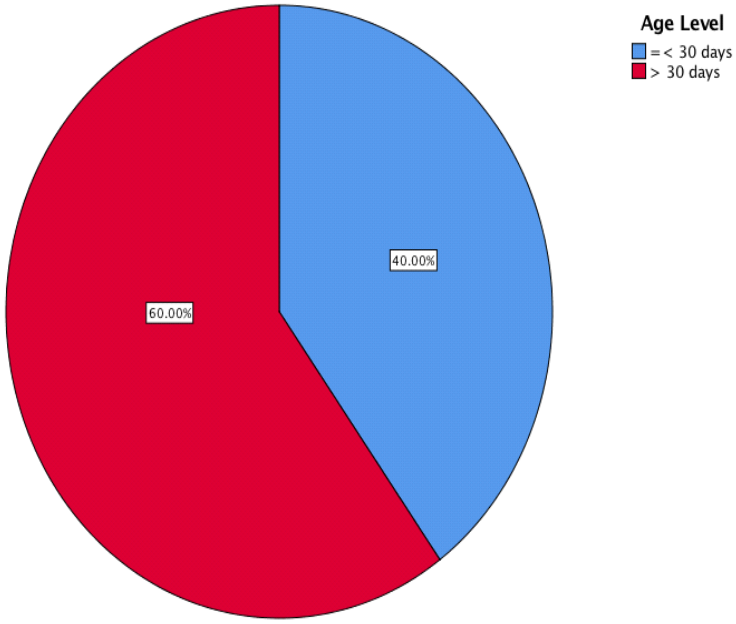


Figure 4

The percent of positive and negative samples

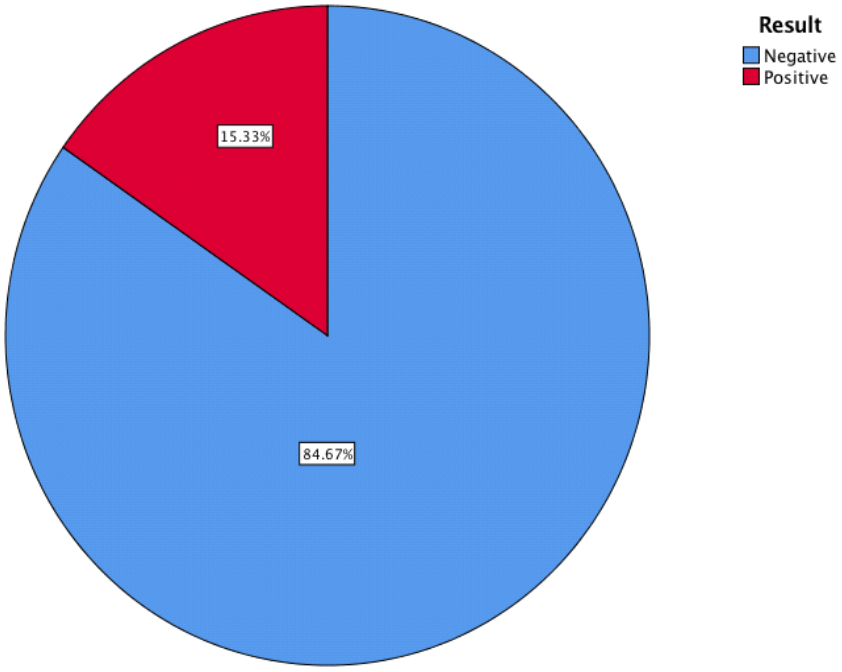


Table 5

Distribution of patients' socio-demographic data (N = 150)

Variable	Values	Frequency	Percentage
Sex	Female	90	60 %
	Male	60	40 %
Age	=< 30 days	60	40 %
	> 30 days	90	60 %
	Mean ± Standard deviation	34.98 ± 14.24	
Place		26.7 %	
Tulkarem	40	46.7 %	
Nablus	70	26.7 %	
Jenin	40		
Season			
Fall	27	18 %	
Spring	33	22 %	
Summer	55	36.7 %	
Winter	35	23.3 %	
Poor feeding			
No	42	28.0 %	
Yes	108	72 %	
Result of presence of Virus			
Negative	127	84.7 %	
Positive	23	15.3 %	

3.3 The distribution of EV results (positive and negative) among other variables

The distribution of EV results between socio-demographic variables was as follow: in place 30.4% of positive results was in Jenin, 52.2% of positive results was in Nablus (Rafidia), 17.4% of positive results was in Tulkarem (Figure 4). However, the distribution of *Enterovirus* results among gender as follow: 56.5% of positive results were among male, in addition to 43.5% of positive results were among female

Figure 5

Distribution of Enterovirus result among place

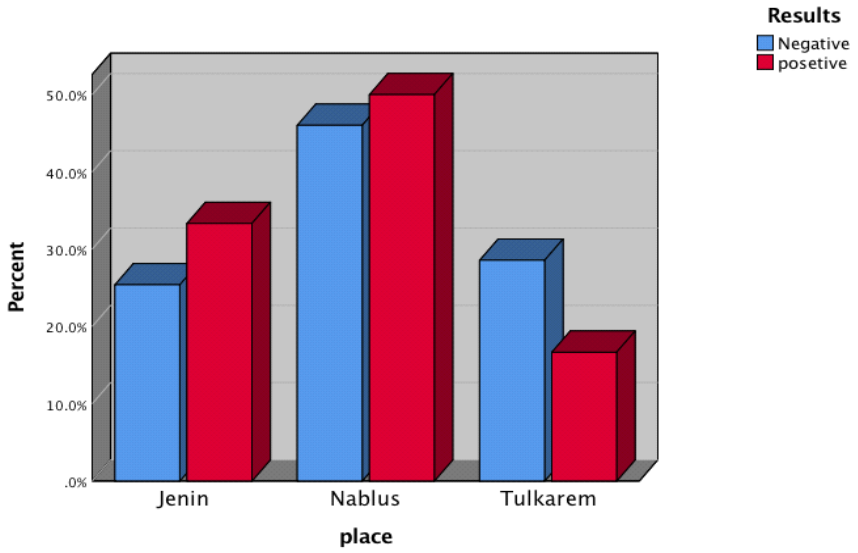
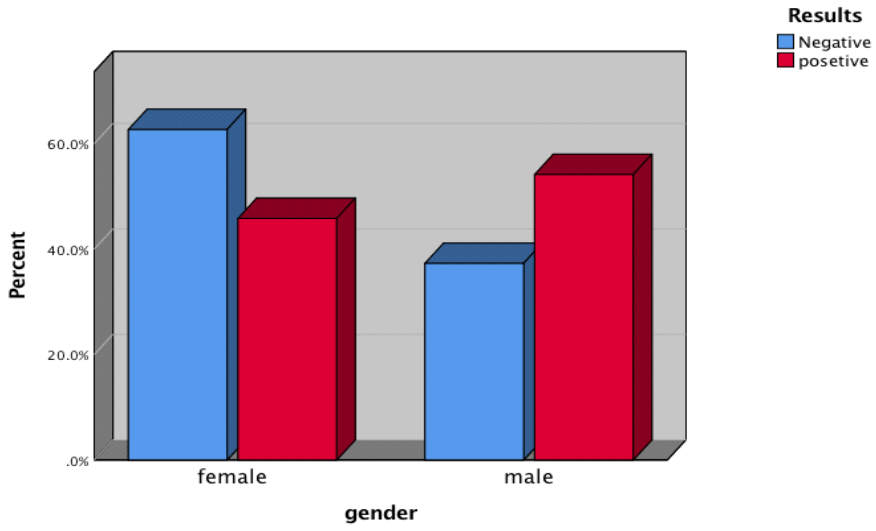


Figure 6

Distribution of Enterovirus results among gender



About the distribution of EV results between lab and clinical finding were as follow: 74.8% of patients in the negative results and 73.9% of patients in positive results were had the CSF WBCs less than 6/mm^3, opposed to it 12.6% of patients in the negative results and 13.0% of patients in positive results were had the CSF WBCs more than 6/mm^3, but 12.6% of patients in the negative results and 13.0% of patients in positive results the results of CSF WBCs weren't available (Figure 7). Meanwhile, 38.6% of patients in the negative results and 39.1% of patients in positive results were had the blood WBCs more than 11 * 10^3/ l, in contrast to, 61.4% of patients in the negative results and 60.9% of patients in positive results were had the blood WBCs less than 11 * 10^3/ l (Figure 8).

Figure 7

Distribution of Enterovirus results among lab result CSF WBC >6 /mm3

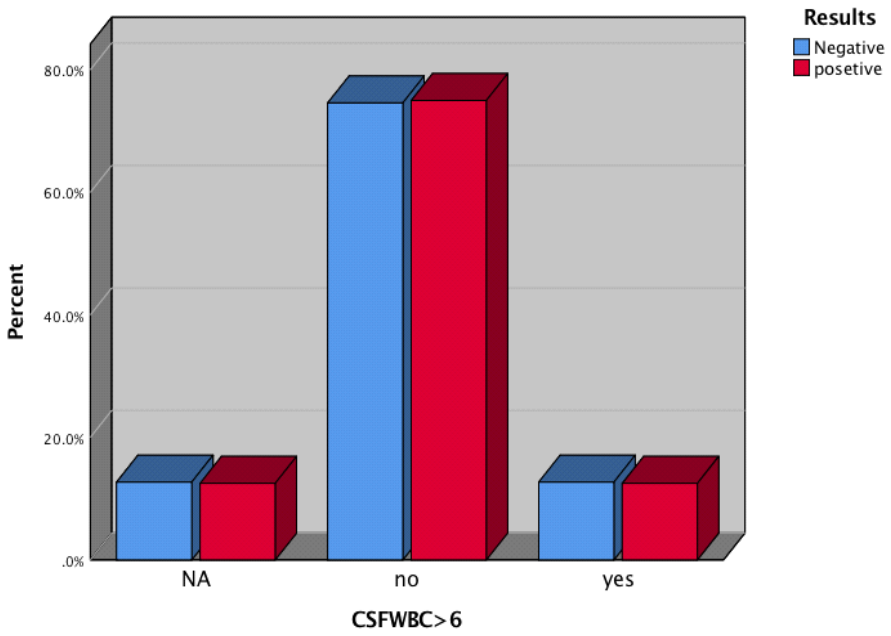
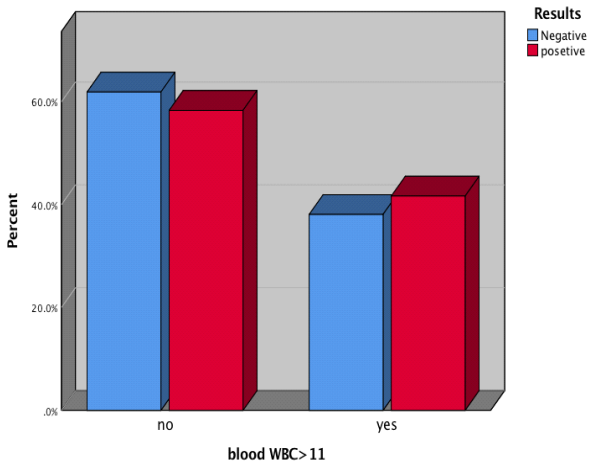


Figure 8

Distribution of Enterovirus results among lab result blood WBC >11 × 10^9/L



3.4 The distribution of patients' age levels (equal or less than 30 days and more 30 days) between other variables

The distribution of age levels between socio-demographic variables was as follow: in place 28.3% of age levels of patients equal or less than 30 days old and 25.6% of age levels more than 30 days was in Jenin, 50% of less than 30 days and 44.4% of positive results was in Nablus (Rafidia), also 21.7% of less than 30 days and 30% of more than 30 days was in Tulkarem (Figure9). However, the distribution of virus results among gender as follow: 66.7% of age levels of patients equal or less than 30 days and 33.3% of age levels more than 30 days were among male, in addition to 55.6% of age level equal or less than 30 days and 44.4% of age levels more than 30 days were among female (Figure 10).

Figure 9

Distribution of age levels between the places

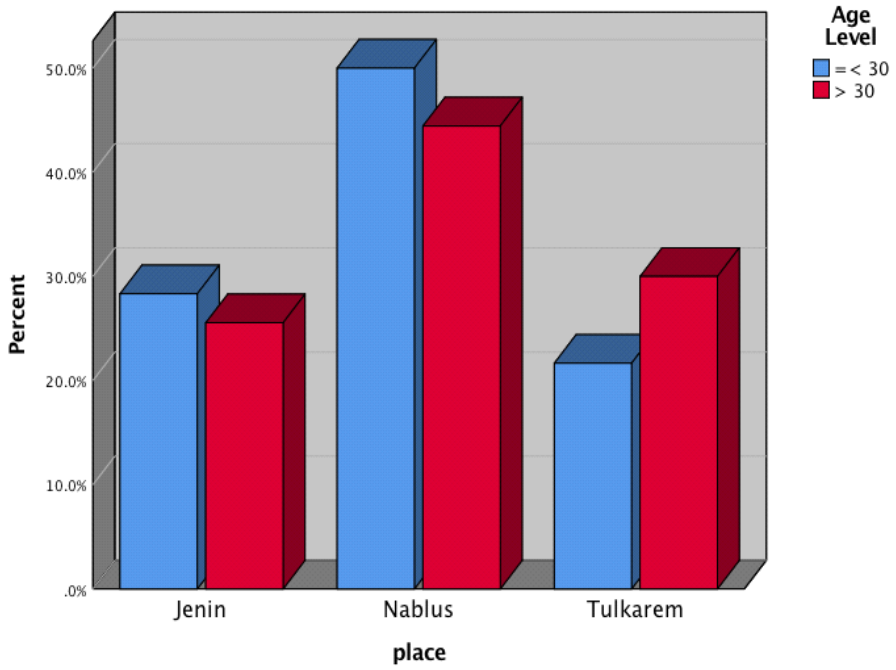
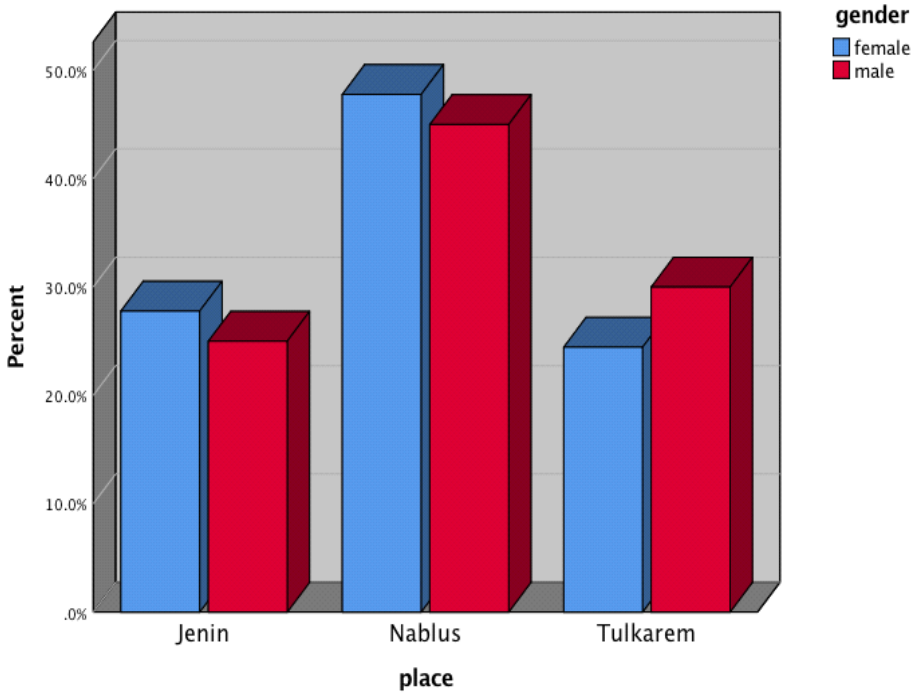


Figure 10

Distribution of age levels among gender



3.5 Signs, symptoms and lab results of neonates' patients

On the other hand, the descriptive and frequency analysis of clinical finding (signs and symptoms) among neonates' patients are display in Table 8. All of the patients (100%) had fever more than 37C°. According to gastroenteritis symptoms the majority of them 109 (72.7%) weren't suffered from vomiting but 41 (27.3%) were, and most of them 93 (62%) hadn't diarrhea but 57 (38%) were had. With regard to photophobia the vast majority 148 (98.7%) of the patients didn't complain of it, however two of them (1.3%) complain. As well as, the vast majority 138 (92%) of the patients didn't complain of stiff neck, in contrast to twelve of them (12%) complain from stiff neck. Also, it is the same for irritability the vast majority 130 (86.7%) wasn't had it and just twenty (13.3%) had the irritability (Table 8).

According to the lab results, the distribution of patients who had number of white blood cells (WBCs) in cerebrospinal fluid (CSF) more than $6/mm^3$ as follow: 19 (12.7%) was yes, 112 (74.6%) was no and 19 (12.7%) of them the results weren't available. While the distribution of patients who had number of white blood cells (WBCs) in peripheral blood more than $11 * 10^3/ l$ as follow: 58 (38.7%) was yes and 92 (61.3%) was no. Meanwhile the mean ± standard deviation of protein in the CSF was 49.49 ± 26.44 and 60.14 ± 13.2 for glucose in the CSF (Table 8).

Table 6

The signs, symptoms and lab results of the patients (N = 150)

Signs, symptoms and lab results	Characteristics	Number (n)	Percentage (%)
Fever > 37	Yes	150	100
	No	0	0
Vomiting	Yes	41	27.3
	No	109	72.7
Diarrhea	Yes	57	38.0
	No	93	62.0
Photophobia	Yes	2	1.3
	No	148	98.7
Stiff neck	Yes	12	8.0
	No	138	92.0
CSF WBC > 6 /mm3	NA	19	12.7
	Yes	19	12.7
	No	112	74.6
Blood WBC > 11 10^9/L	Yes	58	38.7
	No	92	61.3
Irritability	Yes	20	13.3
	No	130	86.7
CSF protein (N = 127)	Mean ± Standard deviation		49.49 ± 26.44
CSF glucose (N = 131)	Mean ± Standard deviation		60.14 ± 13.2

3.6 *Enterovirus* results in association to socio-demographic, laboratory results and clinical parameters

We were made a comparison between EV results and other laboratory results and clinical findings; Chi-Square and Fisher exact tests were conducted to assess that association.

There were a total of 23 patients who had the EV (positive results) and 127 who didn't have (negative results). We found that there were significant associations between *Enterovirus* presence with some of clinical symptoms as follow: there was highly significant association between vomiting and the positive or negative results for virus with P-value 0.002, the most of patients 99 (78.0%) in negative results didn't suffer from vomiting but the most of patients 13 (56.5%) in positive result suffered from vomiting. It was the same for diarrhea; the majority of patients 87 (68.5%) in the negative results didn't suffer from diarrhea, in contrast to the majority of patients 17 (73.9%) in the positive results suffered from diarrhea. The last variable had a significant association with the virus positive and negative results was photophobia, all the patients 127 (100%) in the negative results were not had photophobia, and the vast majority of

patients 21 (91.3%) in the positive results also were had the photophobia (Table 9 and 10).

Finally, there weren't any significant associations between the *EV* results (negative and positive) and the rest of variables: gender, place, season, stiff neck, poor feeding, irritability, CSF WBCs > 6 /mm3 and blood WBCs > 11 × 10^9/L (Table 9 and 10).

Table 7

Comparison of clinical and biological finding with Enterovirus results

Parameters		Results		P-Value
		Negative (127)	Positive (23)	
Vomiting	Yes	28 (22.0%)	13 (56.5%)	0.002
	No	99 (78.0%)	10 (43.5%)	
	Total	127 (100.0%)	23 (100.0%)	
Diarrhea	Yes	40 (31.5%)	17 (73.9%)	0.000
	No	87 (68.5%)	6 (26.1%)	
	Total	127 (100.0%)	23 (100.0%)	
Photophobia	Yes	0 (0.0%)	2 (8.7%)	0.023
	No	127 (100.0%	21 (91.3%)	
	Total	127 (100.0%)	23 (100.0%)	
Stiff neck	Yes	8 (6.3%)	4 (17.4%)	0.090
	No	119 (93.7%)	19 (82.6%)	
	Total	127 (100.0%)	23 (100.0%)	
Poor feeding	Yes	90 (70.9%)	18 (78.3%)	0.635
	No	37 (29.1%)	5 (21.7%)	
	Total	127 (100.0%)	23 (100.0%)	
Irritability	Yes	17 (13.4%)	3 (13.0%)	1.000
	No	110 (86.6%)	20 (87.0%)	
	Total	127 (100.0%)	23 (100.0%)	

Table 8

Comparison of socio-demographic and lab results with Enterovirus results

Parameters		Results		P-Value
		Negative (127)	Positive (23)	
Gender	Male	47 (37.0%)	13 (56.5%)	0.127
	Female	80 (63.0%)	10 (43.5%)	
	Total	127 (100.0%)	23 (100.0%)	
Place	Jenin	33 (26.0%)	7 (30.4%)	0.549
	Rafidia	58 (45.7%)	12 (52.2%)	
	Tulkarem	36 (28.3%)	4 (17.4%)	
	Total	127 (100.0%)	23 (100.0%)	
CSF WBC > 6 /mm3	NA	16 (12.6%)	3 (13.0%)	0.996
	No	95 (74.8%)	17 (73.9%)	
	Yes	16 (12.6%)	3 (13.0%)	
	Total	127 (100.0%)	23 (100.0%)	
Season	Fall	25 (19.7%)	2 (8.7%)	0.520
	Spring	28 (22.0%)	5 (21.7%)	
	Summer	44 (34.6%)	11 (47.8%)	
	Winter	30 (23.6%)	5 (21.7%)	
	Total	127 (100.0%)	23 (100.0%)	
Blood WBC > 11 × 10^9/L	Yes	49 (38.6%)	9 (39.1%)	1.000
	No	78 (61.4%)	14 (60.9%)	
	Total	127 (100.0%)	23 (100.0%)	

Table 9

Comparison of clinical and biological finding with Enterovirus results

Parameters		Age levels		P-Value
		<= 30 days (60)	>30 days Positive (90)	
Vomiting	Yes	14 (23.3%)	27 (30%)	0.369
	No	46 (76.7%)	63 (70%)	
	Total	60 (100%)	90 (100%)	
Diarrhea	Yes	23 (38.3%)	34 (37.8%)	0.945
	No	37 (61.7%)	56 (62.2%)	
	Total	60 (100%)	90 (100%)	
Photophobia	Yes	59 (98.3%)	89 (98.9%)	0.771
	No	1 (1.7%)	1 (1.1%)	
	Total	60 (100%)	90 (100%)	
Stiff neck	Yes	4 (6.7%)	8 (8.9%)	0.623
	No	56 (93.3%)	82 (91.1%)	
	Total	60 (100%)	90 (100%)	
Poor feeding	Yes	41 (68.3%)	67 (74.4%)	0.414
	No	19 (31.7%)	23 (25.6%)	
	Total	60 (100%)	90 (100%)	
Irritability	Yes	12 (20%)	8 (8.9%)	0. 086
	No	48 (80%)	82 (91.1%)	
	Total	60 (100%)	90 (100%)	

3.7 Age level in association to socio-demographic, laboratory results and clinical parameters

According to Chi-Square and Fisher Exact tests, we didn't find any significant association between age levels (equal or less than 30 days and more than 30 days) and clinical symptoms (vomiting, diarrhea, photophobia, stiff neck, poor feeding and irritability), the p-value was more than 0.05 (Table 11).

It was the same for comparison the association between age levels and both socio-demographic and lab results variables (gender, place, season, CSF WBCs > 6 and blood WBCs > 11) (Table 12).

Table 10

Comparison of socio-demographic and lab results with Enterovirus results

Parameters		Results		P-Value
		=< 30 days (60)	>30 days Positive (90)	
Gender	Male	20 (66.7%)	50 (55.6%)	0.174
	Female	40 (33.3%)	40 (44.4%)	
	Total	60 (100%)	90 (100%)	
Place	Jenin	17 (28.3%)	23 (25.6%)	0.527
	Rafidia	30 (50%)	40 (44.4%)	
	Tulkarem	13 (21.7%)	27 (30%)	
	Total	60 (100%)	90 (100%)	
CSF WBC > 6 /mm3	NA	8 (13.3%)	11 (12.2%)	0.746
	No	43 (71.7%)	69 (76.7%)	
	Yes	9 (15%)	10 (11.1%)	
	Total	60 (100%)	90 (100%)	
Season	Fall	10 (16.7%)	17 (18.9%)	0. 738
	Spring	11 (18.3%)	22 (24.4%)	
	Summer	23 (38.3%)	32 (35.6%)	
	Winter	16 (26.7%)	19 (21.1%)	
	Total	60 (100%)	90 (100%)	
Blood WBC > 11 × 109/L	Yes	23 (38.3%)	35 (61.7%)	0.945
	No	37 (38.9%)	55 (61.1%)	
	Total	60 (100%)	90 (100%)	

Chapter Four

Discussion and Conclusion and recommendations

Aseptic meningitis is a relatively common condition caused most commonly by EVs such as PV, CV, or ECV.(1) De Corm et al.(49) reported that RT-PCR assays displayed a 100% sensitivity rate, clearly outperforming the virus isolation technique in identifying EVs and HPeV meningitis cases. Understanding the regional and seasonal patterns of EV meningitis has implications for public health strategies.(54) Enhanced surveillance during peak seasons and targeted interventions in regions with higher prevalence can contribute to the early detection and management of cases.(4) This study aimed to advance our understanding of EV in neonates with aseptic meningitis, providing valuable insights into regional and demographic variations. The identified associations with clinical symptoms underscore the importance of a nuanced approach to diagnosis and management in neonatal EV meningitis cases.

The present study described the prevalence of EVs associated with aseptic meningitis in neonates admitted for aseptic meningitis from three governmental hospitals in three northern cities in West Bank, Palestine between June 2022 to May 2023. Opting to collect this study samples from three governmental hospitals rather than private hospitals carries several advantages rooted in the broader representation of the population, yields a more representative sample, enhancing the generalizability of findings according to Basu et al.(80) Moreover, such hospitals often serve as frontline institutions for public health issues, aligning research outcomes more closely with public health interventions and policies.(81) Accessibility is another key factor, as governmental hospitals are typically more reachable for underprivileged or underserved populations, addressing health disparities and promoting inclusivity in the study population.(82) The comprehensive analysis of socio-demographic characteristics, distribution of EV results, signs, symptoms, and lab results provides valuable insights into the epidemiology of EV meningitis in this vulnerable population. In this study, during a ten-month period, 150 samples of neonate patients admitted for aseptic meningitis were collected from three major governmental hospitals, all patients were aged less than 8 weeks with a mean age of 34.98 days. All of these cases were confirmed to be aseptic meningitis cases after rolling out the bacterial nature of the infection as all cases were tested by culture and gram stain techniques and none of these

cases were found to have a positive finding to be septic meningitis cases. 15.3% of this sample were found to have a positive finding to be EV meningitis cases. Compared to previous studies, this rate was lower than the prevalence rate of EV meningitis in Dumaidi et al. studies which reported a rate of 18%(15) and 22%(17). Previous studies from other countries reported lower prevalence than our study, such as studies conducted in Brazil (10.6%),(62) Egypt (7.4%),(67) and Tunisia (12%).(70) However, several previous studies have reported a higher prevalence than our study, such as studies conducted in Syria (46.5%),(69) Spain (43.8%),(64) England (20%),(59) Finland (26%),(58) China (21.2%),(65) and South Africa (21%).(66)

In general, this study has features other than previously published studies done in Palestine by Dumaidi et al.(15, 17), while this study exclusively included neonate patients, in the first investigation by Dumaidi et al,(15) 356 CSF samples from Rafidia, Jenin, and Al-Khalil governmental hospitals during 3 years collection period between 2012 and 2015, the included patients were with different age group and suspected of having sepsis-like illness and/or aseptic meningitis the inclusion of Al-Kalil hospital differed than our study, in which we also included Tulkarem governmental hospital, this hospital was never has been a part of any investigation on the topic. The second investigation by Dumaidi et al.(17) was closer in its methodology to our study compared to the first investigation,(15) In this 2nd investigation, 249 CSF samples from Rafidia and Jenin governmental hospitals for children admitted to aseptic meningitis during 7-month period in 2017, the median age of these children were 6 months, these two hospitals were also included in our study as they are the two main governmental hospitals in the two cities. Regarding the infection rate of EV meningitis between our study and Dumaidi et al. studies,(15, 17) a infection rate of 15.3% was found in the study compared to 18.5%(15) and 22%(17) in the 1st and 2nd investigations of Dumaidi et al. While Dumaidi et al.(15, 17) included a higher male-to-female ratio with 58.1%(15) and 62.5%(17) of the samples were males compared to 40% in our study, however, all studies found an almost a similar infection rate in the male group, with an infection rate of 21.6% in our study compared to 20.2%(15) and 21.1%(17) in their studies. In the female group, despite having a higher female ratio (60%) in our sample compared to 41.9%(15) and 37.5%(17) in their studies samples, however, the infection rate of the female group in our study was lower than the infection rate in their study with 11.11% in our study compared to 16.1%(15) and 22.5%(17).

The relation between the gender and number of EV meningitis cases has been poorly reported in previous investigations. In our study, out of 23 EV meningitis cases, 13 were males compared to 10 females, these results were in agreement with Dumaidi et al. studies(15, 17, 70) and several previous studies regarding the higher male-to-female ratio among the EV-positive cases, such as studies conducted in the MENA region in Jordan,(72) Syria,(69) and Saudi Arabia(74) or worldwide studies in South Korea,(61) Brazil,(62) China,(65) and South Africa(66). Ramadi et al.(70) found almost a similar male-to-female ratio among EV positive cases, with 14 males and 10 females in Tunisia.(70) it should be noted that this male-to-female ratio was not statistically significant different between the two groups, which aligns with some previous studies.(13, 65, 74) However, this higher male-to-female ratio could be related to influenced by genetic, biological, or behavioral factors(11), these factors are needed to be further investigated in future studies.

The inclusion of neonates from different northern cities within the West Bank is crucial for understanding the regional epidemiology of EV meningitis. The infection rate could not truly represent the relation between the demographic area and the risk of having EV meningitis, while the EV infection rate could be a more accurate indicator for the risk of the infection rather than the number of cases in each city, as increasing the sample form one city could be related to increasing number of cases, but it could not represent the risk of infection which can be accurately identified using the EV positive rate. The EV infection rate was the highest in Jenin with 21.1% followed by the cities of Nablus and Tulkarem; 20.6% and 11.1%, respectively. Compared to Dumaidi et al.(17) study, Jenin reported a lower EV infection rate in our study compared to their study (21.1% Vs 30.1%), however, both studies reported that Jenin had a higher EV infection rate than any other included cities in both studies. In contrast, compared to the previous study,(17) increase and decrease in the EV infection rate were reported in Nablus and Jenin in our study, respectively, with an EV infection rate of 20.6% compared to 16.6%(17) in Nablus, and 21.1% compared to 30.1%(17). Such differences in the EV infection rate could be related to the age group included in our study and its relation to the demographic area, in which only neonates were included in our study, this suggests that neonates in jenin could be at higher risk of having EV meningitis than neonates in Nablus and potential regional variations in healthcare infrastructure, access, or environmental factors influencing the incidence of EV meningitis in these cities. The

highest EV infection rate was reported in Al-Khalil by Dumaidi et al.(15) with a infection rate of 42%, compared to Nablus's (21%) and Jenin's (15%), Al-Khali infection rate was higher than the highest infection rate in our study which was reported in Jenin, with a difference of 10%. This difference could be related to the differences in the included age group between the two studies and geographical differences between the two cities, which differ in the community diversity, number of residents, and number of cases in Al-Khali compared to Jenin or other cities such as Nablus and Tulkarem. In addition, this difference could be related to the longer collection period of samples in their study of 4 years compared to 10 months in our study.

Among neonates, previous studies focusing on this age group are lacking. While some previous studies focused on adults or adolescents only,(58, 59, 61) other studies have included this age group with other age groups in their investigation.(15, 17, 64, 66-70, 74) To the best of our knowledge, studies that focuses on this age group solely are lacking. Several previous investigations found that the prevalence of EV meningitis was higher among children and neonates than any other age groups. In Egypt, Raouf et al.(67) found that out of the 13 included neonates, 4 of them were EV-positive cases with a prevalence rate of 30.7%. Aldriweesh et al.(74) found that among 60 EV meningitis cases, 41 of them were between 0 to 4 years old. Mathey et al.(73) found that among EV meningitis cases, 85.1% were children up to 9 years old followed by adolescents (8.94%), and adults (5.77%) among samples from many different nationalities in Qatar. For patients younger than 3 months, Kadambari et al.(60) found that among 2121 cases, 92% (1952 cases) of them were EV meningitis, with an incidence of 76.6/100,000 live births in 2013. Shahroodi et al.(68) included 35 neonates older than 44 days and 23 children up to 17 years in their study, for the neonate group, the EV-infection rate was 37.1% (13/35 cases). Yahia et al.(69) found that among 60 Syrian EV meningitis cases, 46 of them were younger than 6 years old. Dalwai et al.(75) found that among aseptic meningitis cases aged less than 2 years, 27% were positive EV meningitis, compared to 21% and 8% for 2-4 years and 4-12 years groups, respectively. Wami et al.(13) found that among 39 EV meningitis cases, 28 of them were less than 1 year old, among them 16 out of 28 were less than 28 days, with a prevalence rate among this group of 28% (16/57). Although the prevalence of EV meningitis is likely to vary between different age groups. Potential differences can be seen between neonates and children, while several studies have investigated the difference in the incidence of

clinical manifestation between the different age groups. The variation in the EV-infection rate among age groups could be related to different factors such as immune status, seasonal trends, and diagnostic sensitivity.(4) In addition, higher population density, as seen in certain age-specific settings such as schools or daycare centers, may contribute to increased transmission and outbreak.(71)

In this study, neonate patients were stratified into two different age groups less than 30 days, and between 30 to 60 days, with 60% of the sample in the 2nd age group. interestingly, this age stratification did not reveal significant associations with clinical symptoms, sociodemographic factors, or laboratory results. 66.7% of EV-positive meningitis belonged to the age group of equal or less than 30 days. This suggests that EV prevalence and its associations with symptoms are consistent across different age groups in neonates. Due to the lack of studies that compare such age stratification in these age groups, discussing these results could not be feasible with other age groups included in several previous studies. However, these results aligned with previous studies that found no significant differences between smaller age groups.(13, 74)

The EV infection cases were equally distributed having a similar incidence in spring and winter, with an incidence of 21.7% for each season, and less incidence (8.7%) was noted in fall, while the higher incidence was noted in Summer (47.8.7%). These results were in disagreement with Dumaidi et al.(15) who found a slight seasonal pattern with almost similar incidence in summer and spring; 30.3% and 27.3%, respectively, and a similar incidence (21.2%) in fall and winter. These results showed a relatively similar pattern of EV meningitis peaked in May and June as reported by 4 year period study in Jordan.(83) Similarly, the ten-year period study of Kadambari et al.(60) found a seasonal trend with a peak during summer and winter. In this study, the increased incidence in the summer was in agreement with Sadeghi et al.(84) who reported 66.7% incidence in summer among 6 months to 13 years Iranian children, and Turner et al.(85) who reported 59% incidence in summer among British pediatric patients. Vidal et al.(62) observed a similar finding with higher incidence among Brazilian EV meningitis patients during the period between January and March with a median temperature of 21.5°. while other studies reported an increase during summer and fall in the USA.(63) In Tunisia, Rmadi et al.(70) found that half of the EVs positive cases were reported between May and August.

Studying the relation between EV meningitis cases and clinical and laboratory findings is important, as such relation could guide treatment decisions and improve patient outcomes,(8) in which specific clinical and laboratory features associated with EV meningitis aid in differentiating it from other types of meningitis,(71) and such relation could contribute to public health surveillance and to our epidemiological understanding of the disease, and aid in the development of preventive strategies.(9) Fever more than 37° was reported in all the included patients (150/150; 100%), followed by diarrhea and vomiting which these symptoms were reported in 38% and 27.3% of the sample, respectively. These results were in disagreement with Raouf et al.(67) who found that fever, convulsions, and lethargy were the most clinical manifestations among neonate meningitis patients. These results were partially in agreement with Dumaidi et al.(15) found that fever, headache, and diarrhea were the most prevalent clinical manifestations. Fever was not reported separately for each group in this study, however, Aldriweesh et al.(74) reported a statistically significant higher temperature in the EV-positive group compared to EV-negative group (37.8 vs. 37.4).

The associations between EV meningitis positive results and clinical symptoms of vomiting, diarrhea, neck stiffens, poor feeding, irritability, and photophobia were investigated in this study, by comparing the incidence of occurrence of these symptoms among positive and negative EV meningitis cases. In this study, diarrhea was found to have the statistically strongest association with EV-positive meningitis patients, followed by vomiting and photophobia, in which only these three clinical symptoms were found to have a statistically significant association with EV-positive results, in which 73.9% of EV-positive meningitis patients suffer from diarrhea compared to only 31.5% of EV-negative meningitis patients, while 56.5% of EV positive meningitis patients suffer from vomiting compared to only 22% of EV negative meningitis patients. For photophobia, 2 patients representing 8.7% of EV positive meningitis patients suffer from photophobia, while no cases (0.0%) with photophobia were reported among EV-negative meningitis patients. Other clinical symptoms of a stiff neck, poor feeding, and irritability all failed to have a statistically significant difference between the two groups, with almost a similar incidence of poor feeding and irritability between the two groups, while the stiff neck was found in 17.4% of EV-positive meningitis patients compared to 6.3% among EV-negative meningitis patients, however, this difference was not statistically significant. In the study of Dumaidi et al.,(17) headache, followed by

vomiting and nausea, and diarrhea were found to have a statistically significant higher prevalence rate among EV positive than EV-negative meningitis cases. Similarly, wang et al.(65) found that the difference in the incidence of headache, neck stiffness, dizzy, and convulsion was significant between the EV positive and negative groups. On the other hand, Wami et al.(13) did not find any significant differences between the two EV positive and negative groups in any clinical symptoms. The prospective observational study of Rodríguez et al.(86) who included 39 EV meningitis infant patients (56.2% are males) to compare the clinical and analytical characteristics of neonate patients less than 28 days (16 patients) to other infant patients up to 3 months (26 patients), found that except the less development of exanthema in the neonates group, no significant differences were found between both groups.

The WBC of CSF and blood results were available for 131 and all aseptic meningitis patients in this study, respectively. The incidence of having CSF WBC more than 6/mm^3 was similar in the aseptic, EV-positive, and EV-negative meningitis patients, in which 112 out of 131 aseptic meningitis patients (85.5%) and 17 out of 20 EV-positive meningitis patients (85%), and in 95 out of 111 EV-negative meningitis patients (85.5%) were having CSF WBC more than 6/mm^3. These results were contradictory to Dumaidi et al.(17) who found a statistically significant higher CSF WBC in EV-positive meningitis patients with a median of 379 compared to a median of 19 in EV-negative meningitis cases. Similarly, Wang et al.(65) observed a significantly higher prevalence of having CSF WBC of more than 10 in the EV-positive compared to EV-negative meningitis group (92.7% vs 69.3%). Kadambar et al.(19) reported that 52% of EV meningitis patients had normal CSF WBC among infants aged less than 90 days.

For blood WBC, the incidence of having blood WBC of more than 11 was similar in the EV positive and negative meningitis patients, in which 9 out of 23 EV-positive meningitis patients (39.1%), and 49 out of 127 EV negative meningitis patients (38.6%) were having CSF WBC more than 11. Wami et al.(13) reported that relatively a higher blood WBC was observed among EV-positive meningitis patients. Among the whole included patients, the CSF protein and glucose results were available for 127 and 131 patients, respectively. The mean CSF protein was 49.49 and CSF glucose was 60.14. The mean CSF protein in this study was lower than the CSF protein of both neonates and children with EV-positive meningitis in the Shahroodi et al. study,(68) 75.4 and

65.7, respectively, while the mean CSF glucose was lower than the neonate group and higher than the children group in their study, 65.9 and 55.7, respectively. Wang et al.(65) observed a significantly higher prevalence of having CSF protein of more than 40 in the EV positive compared to EV-negative meningitis group (19.6% vs 9.3%).

These associations with clinical symptoms, particularly vomiting, diarrhea, and photophobia, are consistent with the established manifestations of EV infections.(5) The correlation between these symptoms and EV-positive results emphasizes the importance of considering clinical presentation in diagnostic decision-making. The lack of significant associations with other variables, such as gender, place, season, stiff neck, poor feeding, irritability, CSF WBCs > 6, and blood WBCs > 11, suggests a nuanced clinical picture. It highlights the need for a multifaceted approach in diagnosing and managing neonatal meningitis, considering both clinical and laboratory parameters. Overall, only the three clinical symptoms of diarrhea, vomiting, and photophobia, were statistically associated with EV meningitis cases, while none of the demographics and laboratory findings were statistically associated with EV meningitis cases. While Wami et al.(13) found no association between EV-positive cases and the demographics, clinical, and laboratory findings, other studies observed an association with some clinical and lab findings.(65, 66, 74)

The genotyping of some not all CSF samples was reported in several studies, Kadambari et al.(60) were able to genotype only 2.6% of EV meningitis cases, most of these cases were EV30 (26 cases), EV22 and EV6 (22 cases each). Ramadi et al.(70) reported that genotyping was possible for only 12 out of 24 EV-positive meningitis cases. Dumaidi et al.(15, 17) were able to genotype only 12 out of 66(15) and 26 out of 54(17) EV-positive meningitis cases. The genotyping of EVs from CSF samples is not routinely conducted for several practical reasons. Firstly, the process involves molecular techniques that can be resource-intensive, and many laboratories may lack the necessary capacity to perform genotyping on every CSF sample. In routine clinical practice, the identification of the specific *Enterovirus* genotype may not always be essential for immediate patient management, as the focus is primarily on diagnosing and treating the infection.(68) Additionally, some *Enterovirus* types are more prevalent and associated with common clinical presentations, making genotyping less critical in certain

cases.(86) The time required for genotyping processes and potential challenges in interpreting the data further contribute to its limited routine use.

Other factors related to treatment effectiveness, complications, and outcomes were not investigated in this study, considering these factors in future studies is important for a better understanding of EV meningitis cases. Shahroodi et al.(68) found that among EV meningitis, neonates have a longer mean length of hospital stay of 4.3 days compared to 1.6 days in children, such results could suggest differences between different age groups and treatment effectiveness, complications, and outcomes among EV meningitis cases. Wang et al.(65) found no significant difference in the mean length of hospital stay between EV positive and negative meningitis patients aged between 1 month to 13 years.

4.1 Conclusion

Among 150 CSF samples of neonates younger than 8 weeks collected from three northern regions of the West Bank, Palestine, between June 2022 and May 2023, 15.3% (23 cases) of these neonates tested positive for EV. No significant association between EV positivity and several demographic factors such as gender, geographical location, and seasonality. Moreover, most clinical symptoms and laboratory findings did not show a significant association with EV positivity**.** However, the study did identify significant associations between EV positivity and specific clinical symptoms: vomiting, diarrhea, and photophobia, in which a significant higher incidence of theses clinical symptoms was observed among EV positive group compared to EV negative group.

The distribution of aseptic meningitis cases was across different cities and seasons within the study cities**.** Furthermore, no significant correlations were found between EV results and other variables like stiff neck, poor feeding, irritability, and certain laboratory findings such as CSF and blood WBCs.

This study's findings emphasize the importance of routine testing for EV in neonates presenting with aseptic meningitis, particularly when specific symptoms like vomiting, diarrhea, or photophobia are present.

4.2 Recommendations

Considering the notable presence of EV in many aseptic meningitis cases, it seems wise to routinely test neonates who show symptoms like vomiting, diarrhea, and photopia. Setting up dedicated surveillance in hospitals, particularly targeting EV detection in newborns, could lead to quicker identification and response to any outbreaks, enhancing patient care and safety.

Neonatal units need to adopt more stringent infection control practices. This includes isolating infants who are suspected or confirmed to have EV aseptic meningitis, a vital step in preventing the spread of the infection within hospital environments.

It is essential to engage in public health efforts aimed at educating parents and caregivers. By understanding the symptoms of aseptic meningitis, especially in newborns, they can seek timely medical help, which is crucial for the health and recovery of the infants.

Policymakers should look into the insights provided by this study to refine neonatal care guidelines. Placing a greater focus on the early detection and effective management of neonates with EV aseptic meningitis, can significantly improve care for newborns.

4.3 Limitations and recommendation for future research

Acknowledging the limitations of the study, including the relatively small sample size, the inclusion of only governmental hospitals in the northern West Bank, and the short collection period of ten months, could limit the generalizability of the results. In addition, the genotyping of the CSF samples of EV-positive meningitis was not done in this study. The data regarding treatment, complications, and outcomes of the included patients was not retrieved in this study, and such relation was not investigated.

Future studies that include a higher number of cases obtained by a longer collection period, at different Palestinian cities in the Gaza Strip and West-Bank such as Ramallah and Bethlehem, and that investigated the relation between the outcome of patients regarding treatment, complications, and outcomes. A larger sample would make it possible to have genotyping of the EV cases, to examine which genotypes are responsible for these cases, and to examine such relation between these genotypes with demographic, clinical, and lab results, in addition to the relation to the treatment outcomes.

Longitudinal studies tracking EV prevalence among meningitis patients over multiple seasons and years can provide a more comprehensive understanding of the temporal dynamics and trends in neonatal EV meningitis.

List of Abbreviations

Abbreviation	Meaning
EV	Enterovirous
PCR	Polymerase chain reaction
HPeV	human par*echovirus*es
PVM	pediatric viral meningitis
PV	*polioviruses*
CV	*coxsackievirus*
ECV	*echovirus*es
HHV	*Human Herpesviruses*
HSV-1 and HSV-2	*Herpes simplex virus* 1 and 2
EBV	*Epstein Barr virus*
VZV	*varicella-zoster virus*
CMV	*cytomegalovirus*
CSF	Cerebrospinal Fluid
WNFV	*West Nile Fever viru*
RT-PCR	reverse transcription-polymerase chain reaction
NGS:	next-generation sequencing
mNGS	metagenomic next-generation sequencing
RBC	Red Blood Cell
WBC	White Blood cell
MMR	*Measles, Mumps,* and *Rubella*
LCMV	*Lymphocytic Choriomeningitis Virus*
MENA	Middle East and North Africa
UTR	untranslated region
DNA	deoxyribonucleic acid
RNA	Ribonucleic acid
cDNA	complementary DNA
RT-qPCR	reverse transcription- quantify polymerase chain reaction

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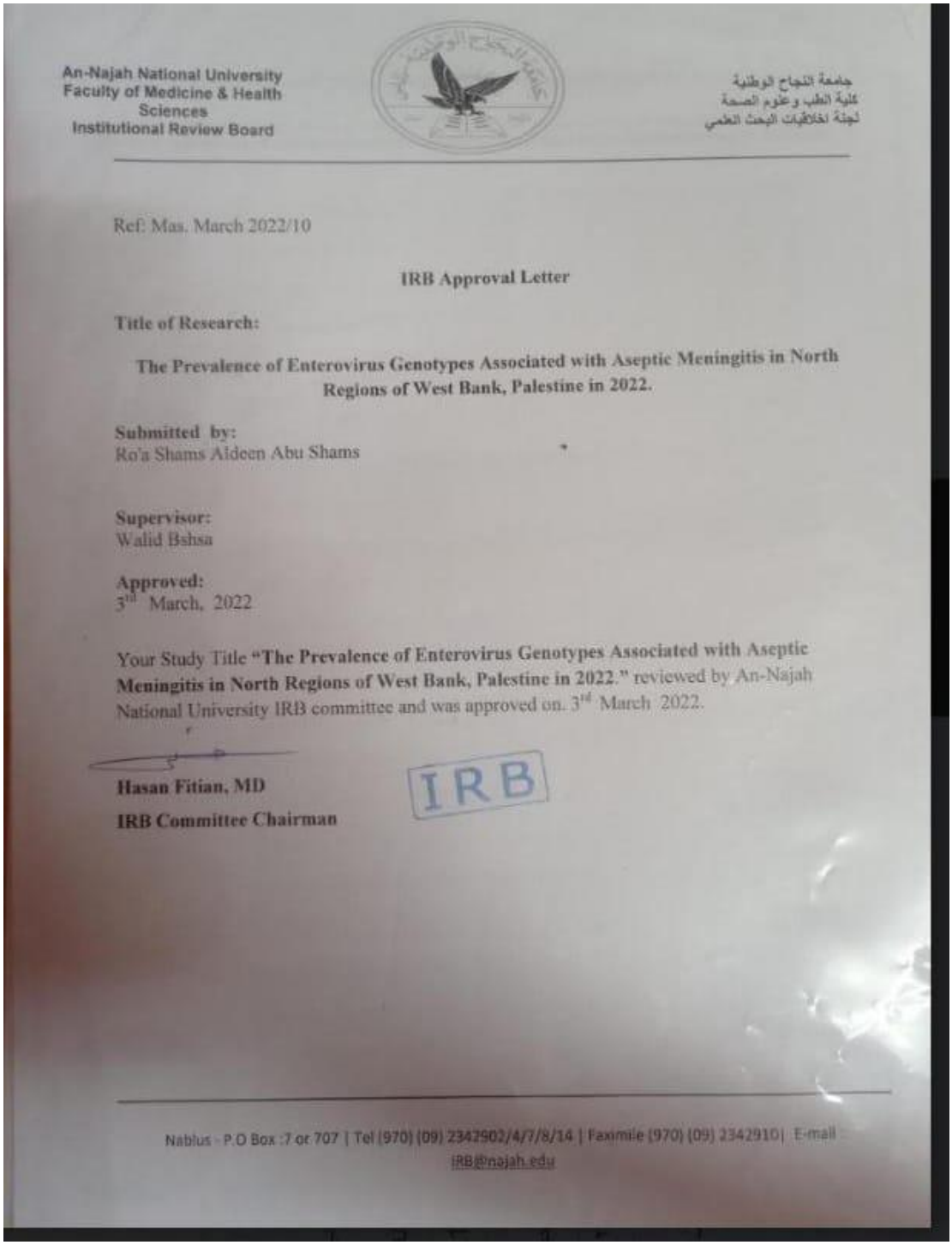
Appendices

Appendix A

IRB –approval



An-Najah National University
Faculty of Medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas. March 2022/10

IRB Approval Letter

Title of Research:

The Prevalence of Enterovirus Genotypes Associated with Aseptic Meningitis in North Regions of West Bank, Palestine in 2022.

Submitted by:
Ro'a Shams Aldeen Abu Shams

Supervisor:
Walid Bshsa

Approved:
3rd March, 2022

Your Study Title **"The Prevalence of Enterovirus Genotypes Associated with Aseptic Meningitis in North Regions of West Bank, Palestine in 2022."** reviewed by An-Najah National University IRB committee and was approved on. 3rd March 2022.

Hasan Fitian, MD
IRB Committee Chairman

IRB

Nablus - P.O Box :7 or 707 | Tel (970) (09) 2342902/4/7/8/14 | Faxmile (970) (09) 2342910| E-mail IRB@najah.edu

Appendix B

Ministry of health correspondence

State of Palestine
Ministry of Health
Education in Health and Scientific
Research Unit

دولة فلسطين
وزارة الصحة
وحدة التعليم الصحي
والبحث العلمي

Ref.: .....
Date:.....

الرقم: ٢٠٢٢/١٠٥٤
التاريخ: ٢٠٢٢/٦/٢٩

عطوفة الوكيل المساعد المدير التنفيذي لمجمع فلسطين الطبي المحترم،،،
الأخ مدير عام الادارة العامة للمستشفيات المحترم،،،
تحية واحترام،،

الموضـــوع: تسهيل مهمة بحث

يرجى التكرم بتسهيل مهمة الطالبة: رؤى شمس الدين محمد ابو شمس- ماجستير امراض معدية

- جامعة النجاح، لعمل بحث بعنوان:

The Prevalence of Enterovirus Genotypes Associated with Aseptic Meningitis in ' North Regions of West Bank, Palestine in 2022

حيث ستقوم الطالبة بجمع معلومات حول عينات في المختبر مسحوبة من المرضى سابقا وتم فحصها ونتائج فحوصات من ملفات المرضى، بدون استخدام المعلومات الشخصية، وذلك في:

- مستشفى رفيديا – مستشفى جنين – مستشفى الوطني – مستشفى طولكرم
- مجمع فلسطين الطبي

مع العلم أن مشرف الدراسة: د. وليد باشا.

على ان يتم الالتزام بالمحافظة على اخلاقيات البحث العلمي وسرية المعلومات.
على ان يتم الالتزام بجميع تعليمات واجراءات الوقاية والسلامة الصادرة عن وزارة الصحة بخصوص جائحة كورونا، وتحت طائلة المسؤولية. وابراز شهادة التطعيم قبل دخول مرافق وزارة الصحة.
على ان يتم تزويد الوزارة بنسخة PDF من نتائج البحث، التعهد بعدم النشر لحين الحصول على موافقة وزارة الصحة.

مع الاحترام،،

د. عبد الله القواسمي
رئيس وحدة التعليم الصحي والبحث العلمي

نسخة: عميد كلية الدراسات العليا المحترم/ جامعة النجاح

Telfax.:09-2333901 scientificresearch.dep@gmail.com تلفاكس: 09-2333901

Appendix C

QUESTIONNAIRE FOR *Enterovirus* Infection

Date: / /

General characteristic

Hospital…… Clinic…….
Age …….. Gender • Male • Female
Place of residence …….
Season ……..

Suspected Diagnosis …….

Clinical symptoms	1. headache	2. Fever > 38^{o}	3. Photophobia
	4 Vomiting and nausea.	5. Stiff neck	6. Diarrhea
	7. poor feeding	8.irritability	9.
	10.	11.	12.

Date of sampling ……/……/……

Lab. Test Results
Gram stain …….

Bacterial …….
CSf Protein ……mg/dl

CSF Glucose …….mg/dl

CSF WBC >6 /mm3 …….
Blood WBC >11 × 10^ 9/L ……

Appendix D

Tables of Study

Table D.1

RT-PCR first and second round program (Thermal cycles)

Temperature	Time	Cycles
37 °C	60 minutes	1
94 °C	5 minutes	1
94 °C	30 second	34
52 °C	30 second	34
72 °C	1 minutes	34
72 °C	5minutes	1
4 °C	Hold	Hold
Second round		
94 °C	5 minutes	1
94°C	1second	34
62 °C	1second	
72 °C	1second	
72 °C	5 minutes	1
4 °C	Hold	Hold

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

انتشار الفيروس المعوي في السائل الدماغي للأطفال المصابين بالتهاب السحايا الغير التهابي في فلسطين- شمال الضفة الغربية 2022-2023

إعداد

رؤى شمس الدين محمد أبو شمس

إشراف

د. وليد الباشا

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في مكافحة وضبط العدوى، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس- فلسطين.

2024

انتشار الفيروس المعوي في السائل الدماغي للأطفال المصابين بالتهاب السحايا الغير التهابي في فلسطين- شمال الضفة الغربية 2022-2023

إعداد

رؤى شمس الدين محمد أبو شمس

إشراف

د. وليد الباشا

الملخص

الخلفية: الفيروس المعوي (EV) هو سبب رئيسي لالتهاب السحايا العقيم في جميع أنحاء العالم. لم يتم دراسة انتشار EV في الرضع في الضفة الغربية، فلسطين من قبل.

الأهداف: هدفت هذه الدراسة إلى تحديد مدى انتشار EV في الرضع الذين تم تشخيصهم بالتهاب السحايا العقيم في ثلاثة مستشفيات حكومية رئيسية في المناطق الشمالية من الضفة الغربية، فلسطين، مع النظر في العلاقات مع مختلف النتائج الديموغرافية والسريرية والمخبرية.

المنهجية: تم جمع ما مجموعه 150 عينة من السائل الدماغي النخاعي (CSF) من الرضع الأصغر من 8 أسابيع المصابين بالتهاب السحايا العقيم من المستشفيات الثلاثة بين يونيو 2022 ومايو 2023. تم الكشف عن RNA لفيروس EV في السائل الدماغي النخاعي باستخدام تقنية النسخ العكسي PCR-وتم تقييم الارتباط مع البيانات الديموغرافية والأعراض السريرية والنتائج المخبرية. تم استخدام اختبارات Chi-Square و Fisher Exact للتحليل الإحصائي.

النتائج: من بين 150 عينة، وُجِد أن 23 (15.3%) من الرضع قد جاءت نتائجهم إيجابية لفيروس EV. لم تجد الدراسة أي علاقة كبيرة بين إيجابية EV والجنس أو الموقع أو الموسمية أو معظم الأعراض السريرية والنتائج المخبرية. ومع ذلك، تم ملاحظة اختلافات كبيرة مع القيء والإسهال وحساسية الضوء. تفاوتت توزيع حالات التهاب السحايا العقيم عبر المدن والفصول.

الخلاصة: كان فيروس EV موجودًا في أقلية من حالات التهاب السحايا العقيم في الرضع في المناطق الشمالية من الضفة الغربية. تُبرز الدراسة أهمية الاختبار الروتيني لفيروس EV في الرضع المصابين بالتهاب السحايا العقيم، خاصةً في الحالات التي تظهر أعراضًا معينة مثل القيء والإسهال وحساسية الضوء. هناك حاجة إلى المزيد من الدراسات للتحقيق في الوبائيات الجزيئية لفيروس EV بين هذه الفئة العمرية.

الكلمات المفتاحية: التهاب السحايا العقيم، السائل الدماغي النخاعي، الفيروس المعوي، الرضع، فلسطين.