OPEN PEER REVIEW

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Peer Review

Journal of Comprehensive Pediatrics
Article DOI: https://doi.org/10.5812/compreped-130137
Published in: Journal of Comprehensive Pediatrics: 13(4) ; e130137

Peer Review Report for "Diagnostic Virology-Based Spectrum of Neonatal Viral Infections at a Tertiary Center: Time to Switch Over"

Author(s): Vijay Singh Meena, Ram Babu Sharma, Dhan Raj Bagri, Meenakshi Sharma

Review Timeline:

Submit Date:	28 Jul 2022
Revised Date:	26 Oct 2022
Accepted Date:	22 Nov 2022

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE : Reviewer

5 Aug 2022

Dear author
Thank you for submission of this article
Totally: This study gives valuable information about viral sepsis but definition of variables, Type of microbiologic tests for viral infections, differentiation of Torch Syn and viral sepsis are not abvious.
There are some notes
Abstract:
1-The number of words in abstract is 333, plz decrease it to 250 words.
2- what is TLC, HIE and PT Plz give the complete form of abbreviations
3- Is non-viral sepsis equivalent with bacterial sepsis or not plz specify

4- What was the reason of high number of CMV and Rubella cases in neonatal sepsis, did these cases have the symptoms of TORCH?

Introduction: The introduction is very brief and does not describe the concept of your study. Plz give the information about viral sepsis in neonates.

Material and Method:

1- What was the number of cases in sample size calculation?

2- What was the definition of viral and non-viral sepsis in newborn cases?

3- What is the reason of difference between numbers of cases in two groups of your study?

4- What was the method of viral detection, did you use PCR or other methods? Plz explain for each virus.

5- What was the reason of TORCH Panel study? Didn't you excluded the TORCH cases?

6- It is important to mention that cases of viral sepsis are different from TORCH cases and they should be considered separately

7- Plz describe the EOS and LOS

Results:

1- What was the age range in two groups , Was the difference significant?

2- What was the sepsis etiology in Non-viral sepsis group?

3- What is the meaning of "microbiological positive test" and which tests were positive. This should be clearly described .

4- The author mentioned toxoplasma as 0.2% while it is parasite not virus.

5- 42% of viral sepsis cases have hepatosplenomegaly, As you know, hepatosplenomegaly is seen in TORCH cases, how did you differentiate these two groups. Its necessary to completely determine, are cases with TORCH Syn and viral sepsis are considered together?

6- It is strongly recommended to differentiate TORCH with other viral infection in your study.

Discussion:

1- Plz give explanation about each result of your study in comparison with other studies.

2- Plz add the recommendation to the end of conclusion .

3- What is the advantages and limitations of your study .Plz add to discussion part

4- Best Regards

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reviewers' Comments:

Reviewer 1:
I welcome your submission. the subject of manuscript is very important and one of the issues involved in NICU. I reviewed the manuscript and have a few comments: We are not allowed to use any abbreviation word without definition for the first time: HIE, PT, AGA and so on (in abstract and text)

Author Response- We have revised as per instructions- the full forms of names have been added before abbreviations.
Abstract:
1- Study design?

Author response – we have added "*this hospital-based, cross-sectional, observational study" in the methods section.*
Introduction:
2- This section was so week. The introduction should include a better representation of the main rationale of the study and emphasis on the limitations of previous studies.

*Author response- We have revised the introduction as –"*Neonatal *sepsis is a major cause of morbidity and mortality. Of the 6.9 million neonatal sepsis burden, South Asia accounts for 3.5 million cases per year. India, with its 1.6 billion population, claims a large proportion of this disease burden [1]. Although bacterial infections are the most common cause of septicemia in neonates but fungal and viral infections can occur in the setting of prematurity and by vertical transmission from the mother. Neonatal Viral infections are common yet under-recognized. Viral infections are less often suspected and diagnosed than bacterial infections in neonates [2]. There is scarcity of literature and only a few studies have been conducted regarding viral neonatal infections [3-6].*

Viral studies are not routinely obtained in neonates due to the overlapping clinical signs and symptoms, the difficulty in obtaining laboratory confirmation of a viral infection, and the lack of effective therapeutic interventions. Recent developments in rapid and highly sensitive diagnostic methods, such as PCR, have greatly improved diagnosis of virus infections. We have planned this study to find out the incidence, the proportionate distribution of neonatal viral infection and to find a correlation if any, between the type of viral infections with their immediate outcome in newborns admitted to NICUs.

3- In first paragraph, it is suggested that "Sepsis" should be expressed in more detail and one or more sentences must be noted about this issue. For example you note: [sepsis is the main causes of death in infants (the third cause of die). Various infections sources can be the origin of sepsis and infected umbilical cord is one of the sources of sepsis that can lead to cellulitis and eventually sepsis.] You should

be mentioned to this valuable and new article in your references: [Bathing or Not Bathing: Which Is Better for Umbilical Cord Separation Time and Bacterial Colonization in Neonates?, Arch Pediatr Infect Dis. 2021 9(2): e104100. doi: 10.5812/pedinfect.104100.]

Author response- suggested changes done and reference added.
Methods:
Pl explain:
4- If an apparatus or diagnostic lab test is used, its manufacturer's name and address should be given in parenthesis.

Author response- ***Reverse Transcriptase- Polymerase Chain Reaction***

It is one of many variants of polymerase chain reaction (PCR). It was introduced in 1977. The discovery of reverse transcriptase during the study of viral replication of genetic material led to the development of RT-PCR. RT-PCR is used to qualitatively detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA. This technique is commonly used in molecular biology to detect RNA expression.

This technique was started in our institute, SMS medical college, microbiology department in 2009. We have used 7500 Fast Dx RT-PCR in our institute for which time take is 90 min. Various specimens were used from different sources to isolate organism like stool, throat swab, CSF, Blood etc.



7500 Fast Dx Real Time PCR Instrument

Applied Biosystems offers a family of Real-Time PCR instruments with the 96-well block format. The Applied Biosystems 7500 Fast Real-Time PCR System represents the latest innovation in real-time technology—Fast thermal cycling, delivering high-quality results in as little as 30 minutes.

Enzyme Linked Immuno-sorbent Assay

ELISA (Enzyme-linked Immuno-sorbent Assay) is a quantitative immunological procedure in which the Ag-Ab reaction is monitored by enzyme measurements. It is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. The term ELISA was first used by Engvall and Perlma in 1971.

We used DIA PRO kit at microbiology department, SMS Medical College. Time take period for ELISA is 2 hrs. Diagnostic sensitivity and specificity of ELISA for HSV IgM, CMV IgM, Rubella IgM are >98%.



The ELISA test kit provides semi-quantitative in vitro assay for human antibodies of the immunoglobulin class IgM against specific virus in serum and plasma for diagnosis of concern virus.

Principle *- The test kit contains micro titer strips each with 8 breakoff reagent wells coated with specific virus antigens. In first reaction step, diluted patient's sample are incubated in wells. In case of positive samples, specific IgM antibodies (also IgA &IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme- labeled anti human IgM (enzyme conjugate) catalyzing a color reaction.*

5- Statistical methods and general computer programs (software) must be mentioned.

Author response- The sample size was calculated at a 95% confidence level, alpha error of 5% assuming viral infection positively 1.7% among newborns admitted in NICUs, at an absolute allowable error of 1%. Categorical / Nominal variables were expressed as numbers and percentages and were analyzed using the Chi-square

test. All statistical analysis was done using SPSS trial version 20.
Results:
6- Any abbreviation should be explained in tables.

Author Responses- Suggested changes done.

7- In table 2 and in text, you calculated the % of positive test out of total subject (660). It must be calculated by number of group 1 (100 subjects) none total subjects.

Author response- Viral infection was diagnosed in 100 (15.15%) subjects out of 660 studied subjects who were admitted to NICUs. The total number of microbiological positive subjects for viral infection was 100 while the total number of the microbiological positive test for viral infection is 152; this reveals more than one sample positivity in one subject. Out of the total of 152 microbiological positive tests, the most common virus isolated was CMV (60) followed by rubella (30) and HSV (25).

The percentage calculation out of total study participants is done has been done per other similar studies.
8- Authors said that One of propose of study was "to find a correlation if any, between the type of viral infections with their immediate outcome in newborns admitted to NICUs". But I did not see any analysis about these. Pl revises the objects of study or completes the finding, clear and precise.

Author response- we have revised the objectives of the study.
Discussion:
9- In the discussion part you should write the main(s) finding(s) and specially (novelty) at first and then allude to other studies.

Author response- suggested changes done.
10- Any recommendation or limitation must be inserted in last paragraph of discussion, not in separate section after conclusion.

Author response- Suggested changes done.
References:
11- Most of them were so old. The most of references were too old (before 2014, almost before 2010). There have been only a few references from 2010. References need renewal.

Author response- we have added newer references.
12- According to journal rules, references arrange by Vancouver style, not APA or AMA.

Author response- Suggested changes done.
Thank you
Reviewer 2:
Dear author
Thank you for submission of this article

Totally: This study gives valuable information about viral sepsis but definition of variables, Type of microbiologic tests for viral infections, differentiation of Torch Syn and viral sepsis are not abvious.
There are some notes
Abstract:
1-The number of words in abstract is 333, plz decrease it to 250 words.

Author response- we have briefed the abstract.
2- what is TLC, HIE and PT Plz give the complete form of abbreviations

Author response- we have expanded the terms.
3- Is non-viral sepsis equivalent with bacterial sepsis or not plz specify

Author Response- we have specified in revised introduction and discussion.
4- What was the reason of high number of CMV and Rubella cases in neonatal sepsis, did these cases have the symptoms of TORCH?

Author response- TORCH Syndrome results from one of the TORCH agents having crossed the placenta during pregnancy. These infectious agents include Toxoplasma gondii, the single-celled microorganism (protozoa) responsible for Toxoplasmosis; rubella virus; cytomegalovirus; and herpes simplex viruses. (Note: According to some reports, the acronym "TORCH" is sometimes modified to the term "STORCH" to include syphilis, a disease that may result in symptoms similar to those associated with the other TORCH agents. Syphilis is caused by infection with the bacterium Treponema pallidum. In addition, TORCH may encompass other disease-causing agents, such as varicella-zoster virus, which is the virus responsible for chickenpox, and parvovirus.)

We have added folloeing explanation in discussion section-"We found higher rate of virus isolation in our samples as compared to the above studies because of variation of test positivity in different study are different and our laboratory is accredited and designated national laboratory. There had occurred lot of advances in technology and sophistication of newer technique of diagnosis of viruses through last decade. Viral transport media advancement now days improve positivity."

Introduction: The introduction is very brief and does not describe the concept of your study. Plz give the information about viral sepsis in neonates.

Author response- we have revised the introduction.
Material and Method:
1- What was the number of cases in sample size calculation?

Author response- we have added in methods section as -"The sample size was calculated at a 95% confidence level, alpha error of 5% assuming viral infection positively 1.7% among newborns admitted in NICUs, at an absolute allowable error of 1%, the estimated sample size was 660 newborns."
2- What was the definition of viral and non-viral sepsis in newborn cases?

Author response- we have added the following paragraph in introduction-"Neonatal sepsis is defined according to the 2002 International Pediatric Sepsis Consensus Conference, as systemic inflammatory response syndrome in the presence of or as a result of suspected or proven infection. But so far, a worldwide agreement on the definition of early-onset neonatal sepsis (EOS) has not been reached. It encompasses various systemic infections of the newborn such as Septicemia, Meningitis, Pneumonia, Arthritis, Osteomyelitis, and Urinary Tract Infections. A number of bacterial and non-bacterial agents may infect newborn inutero, intra partum or post-partum. Intrauterine transplacental infection is significant to the fetus and/or newborn include syphilis, rubella, CMV, Toxoplasmosis, ParvoB19 and varicella. Although HSV, HIV, Hep B , Hep C and TB can result in transplacental infection, the most common mode of transmission for these agent is intra-partum, during labour and delivery with passage through an infected birth canal (HIV, HSV, Hep B virus) , or postpartum, from contact with an infected mother or care taker."

Reference number 2 added as- Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. PediatrCrit Care Med 2005;6:2-8.

3- What is the reason of difference between numbers of cases in two groups of your study?

Author response- non-viral / bacterial sepsis is the most common cause of NNS. This study included 660 subjects of suspected sepsis which were divided in two groups on the basis of microbiological test positive for viral infection:

1. *Group 1 included 560 newborns which were non-virus sepsis.*
2. *Group 2 included 100 newborns which were positive for virus infection according to microbiological test (virus sepsis).*

Table 1: Distribution of subjects according to gender

Gender	***Number of subjects***	***Percentage (%)***
Male	*490*	*74.25*
Female	*170*	*25.75*
Total	*660*	*100*

Out of total 660 subjects 490 (74.25%) were males and 170 (25.76%) were females.

Table 2: Distributions of subjects according to religion

Religion	***Number of subjects***	***Percentage (%)***
Hindu	*572*	*86.70*
Muslim	*88*	*13.30*
Total	*660*	*100*

Out of total 660 subjects 572 (86.70%) were Hindu and 88 (13.3%) were Muslims.

Table 3: Distributions of subjects according to gestational maturity

Gestational maturity	***Number of subjects***	***Percentage (%)***
Full term	*420*	*63.60*
Pre term	*240*	*36.40*
Total	*660*	*100*

Out of total 660 subjects 420 (63.60%) were full term and 170 (25.76%) were pre term.

Table 4: Distribution of subjects according to gender among two groups

Group	***Gender***				
	Male (%)	***% of male***	***Female (%)***	***% of female***	***Total subjects (%)***
Group 1 (non virus sepsis)	*411 (83.88)*	*73.40*	*149 (87.6)*	*26.60*	*560 (84.8)*
Group 2 (virus sepsis)	*79 (16.12)*	*79.00*	*21 (12.4)*	*21.00*	*100 (15.2)*
Total	*490 (100)*	*74.24*	*170 (100)*	*25.76*	*660 (100)*

Out of total 660 subjects 490 (74.24%) were males and 170 (25.76%) were females.

4- What was the method of viral detection, did you use PCR or other methods? Plz explain for each virus.

Author response- we have added the following in metods section- "Sampling procedures for routine and advanced diagnostic virology tests - 2 ml of blood samples were collected in EDTA vial for CBC and 2ml blood samples were collected for C-Reactive Protein (CRP), viral markers for meningitis, HIV, Hep B, TORCH group of infections in the plane vial. Stool samples were screened for Rotavirus and Adenovirus. Urine samples were sent for CMV infection. Nasopharyngeal and throat swabs for RSV, Metapneumovirus, Para influenza virus, H1N1 virus, Adenovirus, and Influenza virus. 1 ml of CSF sample was sent from suspected newborns with meningitis for Herpes virus, CMV, and Parvovirus. All samples were sent with the maintenance of cold chain temperature between 40C to 80C to Advance Virology Lab., Department of Microbiology, SMS Medical College Jaipur.

Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR)- is used to qualitatively detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA. This technique is commonly used in molecular biology to detect RNA expression. This technique was started in our institute, (microbiology department) in 2009. We have used 7500 Fast Dx RT-PCR, for which

time taken is 90 min. Various specimens were used from different sources to isolate organism like stool, throat swab, CSF, Blood etc.

Enzyme Linked Immuno-Sorbent Assay (ELISA)- is a quantitative immunological procedure in which the Ag-Ab reaction is monitored by enzyme measurements. It is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. We used DIA PRO kit at microbiology department, SMS Medical College. Time take period for ELISA is 2 hrs. Diagnostic sensitivity and specificity of ELISA for HSV IgM, CMV IgM, Rubella IgM are >98%."
5- What was the reason of TORCH Panel study? Didn't you excluded the TORCH cases?

Author response- we have added "The TORCH titers are widely considered for diagnosis of neonatal viral infections. Limitations of TORCH screening in IUGR and SGA neonates have been presented in a few studies [21,22,23]. Routine Screening has low utility and high expenses owing to low incidence in general population. Only 3 out of 23 SGA neonates in a Canadian study,[21] 9 out of 117 SGA neonates in a USA study,[22] and 1 out of 75 SGA in another USA study [23] were found positive. we have excluded the cases which were TORCH positive only and the result didn't percolate on diagnostic virology-based tests. " in discussion section.

6- It is important to mention that cases of viral sepsis are different from TORCH cases and they should be considered separately

Author response – clarified .
7- Plz describe the EOS and LOS

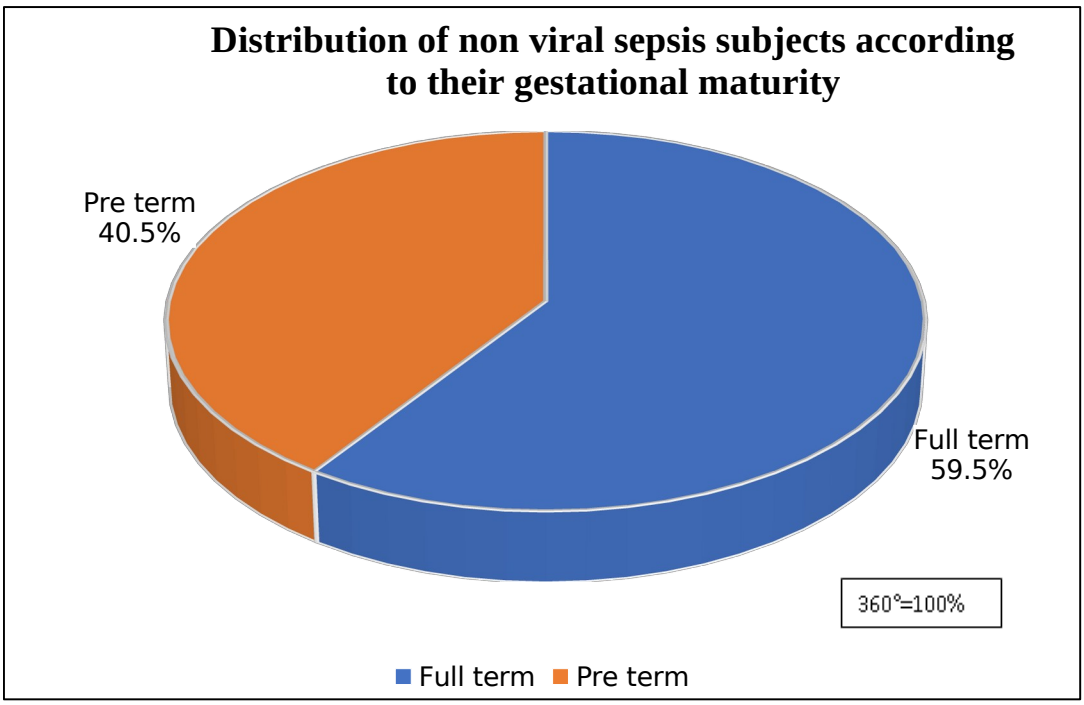
Author response- terms expanded.
Results:
1- What was the age range in two groups , Was the difference significant?

Author response- ***Table : Distribution of subjects according to gestational maturity among two groups***

Group	***Gestational maturity***				
	Full term (%)	***% of full term***	***Pre term (%)***	***% of pre term***	***Total (%)***
Group 1 (non virus sepsis)	*333 (79.3)*	*59.5*	*227(94.60)*	*40.5*	*560 (84.8)*
Group 2 (virus sepsis)	*87 (20.7)*	*87*	*13(05.40)*	*13*	*100 (15.2)*

Total	*420 (100)*	*63.6*	*240 (100)*	*36.4*	*660 (100)*

Out of total 660subjects 420 (63.6%) were full term and 240 (36.4%) were pre term.


Distribution of non viral sepsis subjects according to their gestational maturity
Pre term 40.5%
Full term 59.5%
360°=100%
Full term
Pre term

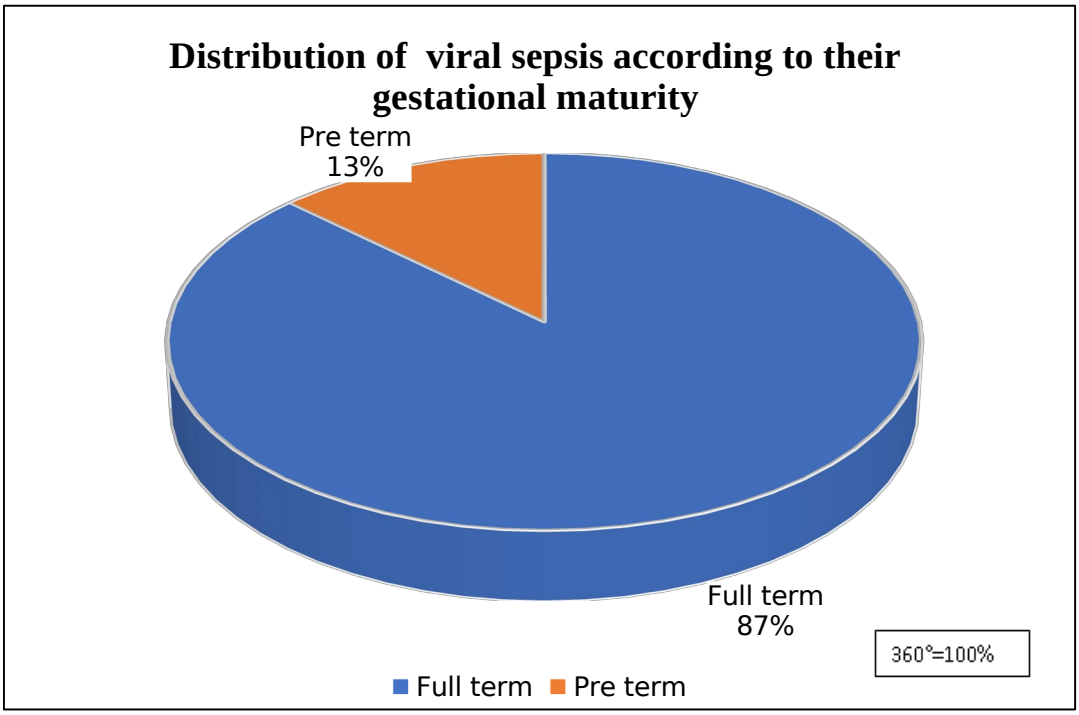

Distribution of viral sepsis according to their gestational maturity
Pre term 13%
Full term 87%
360°=100%
Full term
Pre term

Table : Distribution of subjects according to birth weight in relation to gestational maturity among two groups

Group	***Birth weight in relation to gestational maturity***						***Number of subjects (%)***	***P value***
	AGA (%)	***% of AGA***	***SGA (%)***	***% of SGA***	***LGA (%)***	***% of LGA***		
Group 1 (non virus sepsis)	*539 (88.50)*	*96.25*	*21 (42.00)*	*3.75*	*00*	*00*	*560 (84.80)*	*0.001*
Group 2 (virus sepsis)	*70 (11.50)*	*70.00*	*29 (58.00)*	*29.00*	*01 (100)*	*1.00*	*100 (15.20)*	
Total	*609 (100)*	*92.30*	*50 (100)*	*7.50*	*01 (100)*	*0.20*	*660 (100)*	

Out of 660 subjects, 609 (92.3%) were AGA, 50 (7.5%) were SGA and 1 (0.2%) were LGA.

We have added the following paragraph in results section "Out of 660, 420 were full term and 240 were pre-term, 609 (92.3%) were appropriate for gestational age (AGA), 50 (7.5%) were small for gestational age (SGA), and 1 (0.2%) was large for gestational age (LGA). Out of 609 AGA neonates, 539 (88.5%) had non-viral sepsis & 70 (11.5%) had viral sepsis. Out of 50 SGA neonates, 21 (42%) had non-viral sepsis & 29 (58%) had viral sepsis. One LGA neonate was diagnosed with viral sepsis. Distribution of subjects according to birth weight in relation to gestational maturity among two groups was statistically significant(p-value=0.001) (Table 1)

Distribution of subjects according to gestational maturity among two groups suggested that out of total 660 neonates, 420 (63.6%) were full term and 240 (36.4%) were pre term. Out of 420 full-term babies, 333/420 (79.3%) were diagnosed to have non-viral sepsis and 87 (20.7%) viral sepsis. Out of 240 preterm neonates 227 (94.6%) had non- viral sepsis and 13 (5.4%) had viral sepsis".

2- What was the sepsis etiology in Non-viral sepsis group?

Author response- this was not among the objectives of the study and not described to maintain brevity.
3- What is the meaning of "microbiological positive test" and which tests were positive. This should be clearly described .

Author response- narrated in revised manuscript in discussion section.
4- The author mentioned toxoplasma as 0.2% while it is parasite not virus.

Author response-Corrected as per instructions.
5- 42% of viral sepsis cases have hepatosplenomegaly, As you know, hepatosplenomegaly is seen in TORCH cases, how did you differentiate these two groups. Its necessary to completely determine, are cases with TORCH Syn and viral sepsis are considered together?

Author response- clarified above
6- It is strongly recommended to differentiate TORCH with other viral infection in your study.

Author response- we have included only those cases confirmed by diagnostic virology based tests – RT-PCR, ELISA
Discussion:
1- Plz give explanation about each result of your study in comparison with other studies.

Author response- suggested changes done.
2- Plz add the recommendation to the end of conclusion .

Author response- suggested changes done.
3- What is the advantages and limitations of your study .Plz add to discussion part

Author response- suggested changes done.
4- Best Regards

> Associate Editor's Comments:

Associate Editor 1:
Dear EIC
The reviewer(s) have recommended publication, but also suggest some revisions to the manuscript. Therefore, I invite the author to respond to the reviewer(s)' comments and revise his manuscript.
Kind regards,

> EIC Decision:

Dear Author,

Thank you for submitting your manuscript. According to reviewers' comments, this document needs revision. Please follow their comments as requested. Thanks.

Author response- the constructive comments have really improved the manuscript. We are thankful for the hard work and kind words of honorable reviewers.

Regards

OPEN PEER REVIEW

Revision (1)

REFEREE : Reviewer

26 Aug 2022

Thank you for resubmission and revision of this article
There are still some notes
Abstract:
1- Please define briefly the groups of your study, Give the difference of two groups in abstract.
2- It is recommended that if your study aim is difference in your study groups then your main results may be the results of comparison between these two groups which are positive and negative for viral infection.
3- The abstract has more than 250 words
Material and methods:
1- Pls exactly specify which tests are used to detect viral infections one by one and what is the sensitivity and specificity of each test.
2- Pls exactly define what is the microbiologic positive test?

Results:
1- What is the definition of early onset and late onset sepsis
2- This sentence " The total number of microbiological positive (RT-PCR, ELISA) subjects for viral infection was 100 while the total number of the microbiological positive test for viral infection is 152; this reveals more than one sample positivity in one subject." Is not understandable.
3- In this sentence "Out of the total of 25 microbiological positive test subjects for HSV, isolated HSV was present in 7 subjects (28%), Did isolation means positive viral culture tests?
Discussion:
1- Pls give the reason of differences in items of your results with other results.
2- What are the advantages and limitations of your study?
Tables:
There is no need to have different tables, and charts. If one chart shows important information it is acceptable but this chart or showing different religion groups is not necessary. Plz give the numerical results arranged in one table with showing the P value in it.
Best regards

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Reviewer:

On behalf of my co-authors, we thank you very much for allowing us to revise our manuscript. We appreciate you very much for your positive and constructive comments and suggestions on our manuscript entitled "DIAGNOSTIC VIROLOGY-BASED SPECTRUM OF NEONATAL VIRAL INFECTIONS AT A TERTIARY CENTER: TIME TO SWITCH OVER " (ID: 130137). We have studied the comments carefully and have made a correction which we hope meets with approval. Revised portions are marked in orange on the paper and annotated. The main corrections in the paper and the responses to these comments are as following:

> Reviewers' Comments:

Reviewer 1:
Thank you for resubmission and revision of this article
There are still some notes
Abstract:
1- Please define briefly the groups of your study, Give the difference of two groups in abstract.

Author Response- we have added the line in abstract as- "This study included 660 subjects of suspected sepsis, 560 had non-viral sepsis (group-1) & 100 had viral sepsis (group-2)."
2- It is recommended that if your study aim is difference in your study groups then your main results may be the results of comparison between these two groups which are positive and negative for viral infection.

Author response- The hospital-based, cross-sectional, observational study was aimed to assess patterns of viral infections in newborns.
3- The abstract has more than 250 words

Author Response- We have reworked the abstract and hope it meets your expectations. we have revised the abstract with 230 words-

"Background: Viral infection are important etiology of neonatal sepsis, especially in premature/immune-compromised babies, and are associated with long-term morbidity & mortality. Timely diagnosis of viral infections using newer diagnostic tests in neonates is needed to reduce the global burdens of neonatal morbidity and mortality.

Objectives: The study aimed to assess patterns of viral infections in newborns was done over one year.

Methods- this hospital-based, cross-sectional, observational study included 660 newborns based on inclusion and exclusion criteria, after obtaining pre-requisite clearance from the institutional ethical committee. All newborns admitted in NICUs for suspected clinical sepsis were included except newborns with congenital malformation, hypoxic ischemic encephalopathy (HIE), preterm for care, and negative consent.

Results: This study included 660 subjects of suspected sepsis, 560 had non-viral sepsis (group-1) & 100 had viral sepsis (group-2). In group 1(n=560), there were 73.4% male and 26.6% female newborns; while in group 2(n=100) there were 79% male and 21% female newborns. There was a significant difference found in mean hemoglobin level, mean platelet counts, and mean transaminases level between the two groups. Cytomegaloviruses (CMV) were found in 9.1%, rubella in 4.5%, and herpes simplex virus (HSV) in 3.8% of subjects.

Conclusions: all neonatologists and pediatricians involved in neonatal care must not only suspect a viral agent as a possible cause of sepsis but also should utilize the diagnostic and treatment facilities with antiviral agents whenever and wherever possible."
Material and methods:
1- Pls exactly specify which tests are used to detect viral infections one by one and what is the sensitivity and specificity of each test.

Author response-

Diagnostic methods to detect Neonatal Sepsis:

1. **Blood culture** : 1 ml of peripheral blood was taken under all aseptic precautions in blood culture bottles (McCartney Bottles) containing 5 ml of Brain Heart Infusion (BHI) broth. The blood culture bottles were incubated at 37°C overnight aerobically. Primary subculture was done after 24 hours of incubation on to blood agar and Mac Conkey agar. If there was no growth on plates, then bottles were incubated further and followed up by examining the broth daily and doing a final subculture at the end of day 7 or at appearance of turbidity, discrete colonies on surface of sedimented red cells or haemolysis whichever is earlier. The positive growth were identified by conventional methods according to standard laboratory

protocol, including colony morphology, Gram staining and biochemical reactions. After identification of bacteria, antimicrobial sensitivity testing was done.

2 **RT PCR- Reverse transcription polymerase Chain reaction:**

Reverse transcription polymerase chain reaction (RTPCR) is one of many variants of polymerase chain reaction (PCR). It was introduced in 1977. The discovery of reverse transcriptase during the study of viral replication of genetic material led to the development of RT-PCR. RT-PCR is used to qualitatively detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA. This technique is commonly used in molecular biology to detect RNA expression.

RT-PCR Principles:

There are several types of RT PCR Principles**.**

One step RT PCR:

• RT-PCR can also be carried out as one-step RT-PCR in which all reaction components are mixed in one tube prior to initiation of the reaction.

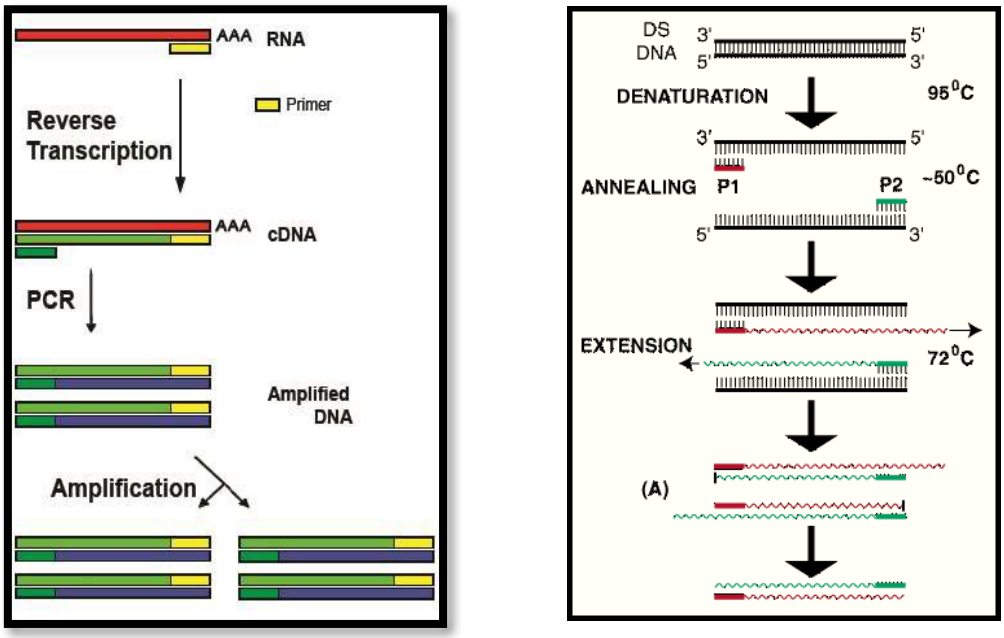
• One-step RT-PCR offers simplicity and convenience and minimizes the possibility for contamination.

• The resulting cDNA cannot be used for detecting multiple messages from a single RNA sample as in two-step RT-PCR.

Two step RT PCR:

- Traditionally, RT-PCR involves two steps: the RT reaction and PCR amplification.
- RNA is first reverse transcribed into complementary DNA (cDNA) using an enzyme, reverse transcriptase.
- The resulting cDNA is used as templates for subsequent PCR amplification using primers specific for one or more genes.

RT-PCR VS PCR



Quantification of RT-PCR products can largely be divided into two categories: end point and real-time.

- **End-point RT-PCR:** measurement approaches of end-point RT-PCR detect gene expression levels by the use of fluorescent dyes like ethidiumbromide, Phosphorus-32labeling of PCR

products using phosphor imager. Endpoint RT-PCR is commonly achieved using three different methods: relative, competitive and comparative.

- **Real-time RT-PCR:** the analysis and detection of PCR products in real-time has consequently led to the widespread adoption of real-time RT-PCR for the analysis of gene expression.
- There are four different fluorescent DNA probes for the detection of PCR products: SYBR Green, TaqMan, Molecular Beacons, and Scorpions. All of these probes allow the detection of PCR products by generating a fluorescent signal.

Application of RT-PCR:

The exponential amplification via reverse transcription polymerase chain reaction provides for a highly sensitive technique in which a very low copy number of RNA molecules can be detected. RT-PCR is widely used in the diagnosis of genetic diseases and semi quantitatively, in the determination of the abundance of specific different RNA molecules within a cell or tissue as a measure of gene expression.

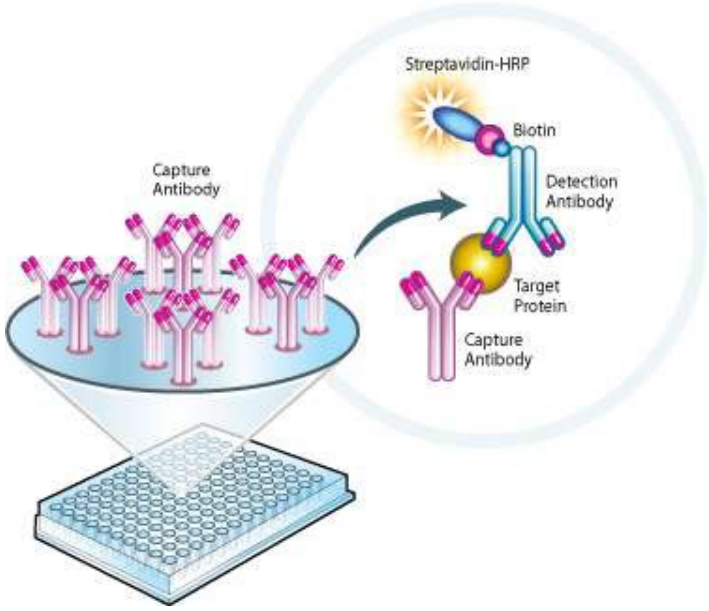
- **Research methods-** For example, Lin et al. used qRT-PCR to measure expression of Gal genes in yeast cells.
- **Gene Insertion-** RT-PCR can also be very useful in the insertion of eukaryotic genes into prokaryotes. RT-PCR is commonly used in studying the genomes of viruses whose genomes are composed of RNA, such as Influenza virus A and retroviruses like HIV.
- **Genetic Disease Diagnosis-** RT-PCR can be used to diagnose genetic disease such as Lesch–Nyhan syndrome. Also can be used as a test for bird flu- H7N9.
- **Cancer Detection-** Scientists are working on ways to use RT-PCR in cancer detection to help improve prognosis, and monitor response to therapy

- **RT-PCR** is commonly used in studying the genomes of viruses whose genomes are composed of RNA, such as Influenza virus A, HSV1, HSV 2, Parvovirus, EBV, CMV, Rotavirus, Enterovirus and retroviruses like HIV.

3.ELISA:

ELISA (Enzyme-linked Immunosorbent Assay) is a quantitative immunological procedure in which the Ag-Ab reaction is monitored by enzyme measurements. It is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. The term ELISA was first used by Engvalland Perlma in 1971.

Principles of ELISA:



The basic steps in ELISA includes:

1. Immobilization of Antigen to a solid surface,
2. Complexation of Antigen with specific antibody (primary),
3. Attachment of primary antibody to enzyme linked secondary antibody,
4. Reaction of substrate with conjugated enzyme to produce a colored product.

ELISA is a popular format of "wet-lab" type assay that uses a solid-phase **enzyme immunoassay** (**EIA**) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.

- It involves detection of an "analyte" (i.e. the specific substance whose presence is being quantitatively or qualitatively analyzed).
- Theanalyte is also called the ligand and it will specifically bind to a detection reagent.
- The ligand-specific binding reagent is "immobilized" onto a stationary solid phase /solid substrate known as the "ELISA plate".
- It is then complexed with an antibody that is linked to an enzyme.
- Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate which form a coloured product.
- The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

Applications:

Used for determining serum antibody concentrations.

- Monoclonal Antibody Screening.
- For Virus test (HIV, West Nile Virus, NDV).
- For Home Pregnancy Test.
- In Food industry for detecting potential food allergens E.g. Milk, Peanuts, Almonds, Eggs and Walnuts.
- Disease diagnosis E.g. HIV, bird flu, common, colds, cholera, STD etc.
- Detection of enterotoxin of E. coli in feces.
- To study drug toxicity.

4 lumber puncture - A lumber puncture (LP) should be performed in neonates undergoing evaluation for sepsis, because clinical signs suggesting meningitis can be lacking in young infants. When infant is critically ill or likely to have cardiovascular or pulmonary compromise from the procedure, the LP can be deferred until the patients status has stabilized.

LP is indicated in neonates with clinical finding concerning for sepsis. In addition, LP should be performed in infants with any of the following:

- A positive blood culture
- Laboratory data suggestive of sepsis
- Worsening clinical status while on antibiotic therapy

Cerebrospinal fluid (CSF) should be sent for Gram stain, routine culture, cell count with differential, and protein and glucose concentration. Interpretation of CSF needs to account for variations due to gestational age, chronologic age, and birth weight.

5 C-reactive protein- C-reactive protein (CRP) is increased in inflammatory conditions, including sepsis. A variety of noninfectious inflammatory conditions can also cause elevated CRP, including maternal fever, fetal distress, stressful delivery, perinatal asphyxia, meconium aspiration, and intraventricular hemorrhage.

A single measurement of CRP is not a useful aid in the diagnosis of neonatal sepsis because it lacks sufficient sensitivity and specificity. However, sequential assessment of CRP values may help support a diagnosis of sepsis. If the CRP level remains persistently normal (<1mg/dl), neonatal bacterial sepsis is unlikely.

CRP levels can be helpful in guiding the duration of antibiotic therapy in suspected neonatal bacterial infection. Infants with elevated CRP levels that decrease to <1mg/dl 24 to 48 hours after initiation of antibiotic therapy typically are not infected and generally do not require further antibiotic treatment if cultures are negative. However, routine use of serial CRP measurements can be associated with longer length of stay.

An elevated CRP level alone does not justify continuation of empiric antibiotics for more than 48 hours in well-appearing infants with negative culture results. Additional evaluation may be warranted to investigate alternative explanations for persistently elevated CRP values.

WE HAVE BRIEFLY SUMMARIZED IT AS "2 ml of blood samples were collected in EDTA vial for CBC and 2ml blood samples were collected for C-Reactive Protein (CRP), viral markers for meningitis, HIV, Hep B, TORCH group of infections in the plane vial. Stool samples were screened for Rotavirus and Adenovirus. Urine samples were sent for CMV infection. Nasopharyngeal and throat swabs for RSV, Metapneumovirus, Para influenza virus, H1N1 virus, Adenovirus, and Influenza

virus. 1 ml of CSF sample was sent from suspected newborns with meningitis for Herpes virus, CMV, and Parvovirus. All samples were sent with the maintenance of cold chain temperature between 40C to 80C to Advance Virology Lab., Department of Microbiology, SMS Medical College Jaipur.

Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR)- is used to qualitatively detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA. This technique is commonly used in molecular biology to detect RNA expression. This technique was started in our institute, (microbiology department) in 2009. We have used 7500 Fast Dx RT-PCR, for which time taken is 90 min. Various specimens were used from different sources to isolate organism like stool, throat swab, CSF, Blood etc. PCR to detect viral DNA has sensitivity of 75% to 100% and specificity of 71% to 100%.

Enzyme Linked Immuno-Sorbent Assay (ELISA)- is a quantitative immunological procedure in which the Ag-Ab reaction is monitored by enzyme measurements. It is a plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. We used DIA PRO kit at microbiology department, SMS Medical College. Time take period for ELISA is 2 hrs. Diagnostic sensitivity and specificity of ELISA for HSV IgM, CMV IgM, Rubella IgM are >98%."
2- Pls exactly define what is the microbiologic positive test?

Author response-we have added the definition in methods section as (highlighted in orange colour) - technique commonly used in molecular biology to detect microbes in various specimens from different sources to isolate organism like stool, throat swab, CSF, Blood etc ; and the tests based on detection of RNA expression (RT-PCR) or quantitative immunological procedure (ELISA) are considered as microbiological tests.

Results:
1- What is the definition of early onset and late onset sepsis

Author response- we have added the definition in introduction section (highlighted in orange colour) as-"EOS refers to sepsis in neonates at or before 72 hours of life, and LOS is defined as sepsis occurring at or after 72 hours of life."
2- This sentence " The total number of microbiological positive (RT-PCR, ELISA) subjects for viral infection was 100 while the total number of the microbiological positive test for viral infection is 152; this reveals more than one sample positivity in one subject." Is not understandable.

Author response- we have revised above sentence in results section (highlighted in orange) as- The total number of microbiological positive (RT-PCR, ELISA) subjects for viral infection was 100 while the total number of the microbiological positive test for viral infection is 152. In present study out of 660 subjects, 100(15.15%) subjects were microbiological confirmed viral sepsis. Out of total 60 microbiological positive test subjects for CMV, isolated CMV was present in 29 subjects (48.33%) and in 31 (51.67%) subjects CMV was present in association with other viruses. Out of total 30 microbiological positive test subjects for rubella, isolated rubella was present in 2

subjects (6.67%) and in 28 (93.33%) subjects' rubella was present in association with other viruses. Out of total 25 microbiological positive test subjects for HSV, isolated HSV was present in 7 subjects (28%) and in 18 (72%) subjects HSV was present in association with other viruses. Out of total 8 microbiological positive test subjects for H1N1, isolated H1N1 was present in 7 subjects (87.5%) and in 1 (12.5%) subject H1N1 was present in association with other viruses. Out of total 5 microbiological positive test subjects for EBV, isolated EBV was present in 3 subjects (60%) and in 2 (40%) subjects EBV was present in association with other viruses. 1 subject metapneumo and 1 subject toxoplasma these two viruses were found in association with other viruses only.

3- In this sentence "Out of the total of 25 microbiological positive test subjects for HSV, isolated HSV was present in 7 subjects (28%), Did isolation means positive viral culture tests?

Author response- yes
Discussion:
1- Pls give the reason of differences in items of your results with other results.

Author response- we have added new paragraph in discussion(highlighted in orange) as – "Multiple test positivity for viruses can be due to poor socioeconomic status and overcrowding seen in India due to high population load. Hence, the high overall incidence of mixed infection can be explained in our study . Studies conducted in western world shows decrease incidence of multiple viral infection due to overall less population, better hygiene and high education level of mother."

2- What are the advantages and limitations of your study?

Author response- we have added strengths and limitations of the study.
Tables:
There is no need to have different tables, and charts. If one chart shows important information it is acceptable but this chart or showing different religion groups is not necessary. Plz give the numerical results arranged in one table with showing the P value in it.

Author response- suggested changes done. Finally, we hope the revised manuscript is now acceptable to you. If not, we are glad to receive any further feedback which we shall continue to apply our best effort to address. Once again, thank you very much for your comments and suggestions.

Best regards
Reviewer 2:
comment for authors:
I welcome your submission. the subject of manuscript is very important and one of the issues involved in NICU. I reviewed the manuscript, again.

all comments were revised by authors.
Thank you

> Associate Editor's Comments:

Associate Editor 1:
Dear EIC,
The author could not satisfy one of reviewers. We can give a chance to authirs for revising manuscript again.

> EIC Decision:

Dear EIC,
The manuscript still needs revision. Please revise it based on reviewers comments accuratly.

OPEN PEER REVIEW

Revision (2)

REFEREE : Reviewer — 4 Sep 2022

Dear Author
Thank you for resubmission
Totally a lot of organisms are tested in this article, but which organism is identified with which test is not specified in the method and result part
There are some notes
Abstract:
1- It is recommended to give important results in the result part. Information about the gender of two groups are not important
Methods:
2- Based on methods explanation, the results are obtained from different samples but it is not obvious which specimens are used for which organism?
3- It is not obvious which test was used to identify which virus or other pathogens.
Results:
4- it is recommended to make a table and give the results by different specimens in the result part
best regards

Parisa Bahmani : Associate Editor — 5 Sep 2022

Dear EIC
Based on reviewer comments, article still needs correction.

Mohammad Reza Boloursaz : EIC — 5 Sep 2022

Dear Author,
Pleaee revise the manuscript accuratly.

OPEN PEER REVIEW

Revision (3)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reviewer 1:

Dear Author

Thank you for resubmission

Totally a lot of organisms are tested in this article, but which organism is identified with which test is not specified in the method and result part

There are some notes

Abstract:

1- It is recommended to give important results in the result part. Information about the gender of two groups are not important

Author response- we have removed gender related information.

Methods:

2- Based on methods explanation, the results are obtained from different samples but it is not obvious which specimens are used for which organism?
Author response- we have tabulated above information to maintain brevity.

Clinical presentation	Kind of sample	Virus Isolated
1. Bronchiolitis	Nasopharyngeal swab or nasal aspirate or throat swab	RSV, Metapneumovirus, Para influenza, H1N1 virus, Adenovirus, Influenza virus
2. Abdomen distension , diarrhoea, GI symptoms	Stool	Rota virus, Adeno virus
3. Seizure or encephalitis	CSF, Urine	Herpes, CMV
4. Sepsis like illness	Blood	Enterovirus, Parvovirus, Ebstein-Barr virus.
5.History of fever with rash in mother	CSF	Varicella zoster virus
6.Hepatosplenomegaly,cholestasis, chorioretinitis, Microcephaly , cataract, micro opthalmia	Blood	TORCH group
7. Microcephaly, cutis gyrata, fetal brain cleft or hydrocephalus	Blood, Urine	Zika virus
8.Mother HBsAg positive with hepatitis	Blood	Hepatitis B virus
9.Maternal HIV infection present	Blood	HIV

10.Localised skin, eye, mouth or mucocutaneous membrane lesion, Seizure, shock, respiratory distress and DIC	Stool, Urine & CSF	Herpes simplex virus

3- It is not obvious which test was used to identify which virus or other pathogens.
Author response- we have provided this information in the table.

Results:

4- it is recommended to make a table and give the results by different specimens in the result part

Author response- thanks for the constructive comment. We have added following table in the results section.

Table: Distribution of subjects according to type of sample tested and test positivity

Type of sample	**Over all Negative samples (%)**	**Sample specific negative samples (%)**	**Over all Positive samples (%)**	**Sample specific positive samples (%)**	**Total number of samples (%)**
Blood	374 (66.80)	85.60	63 (63)	14.40	437 (66.20)
CSF	118 (21.10)	96.70	04 (04)	3.30	122 (18.50)
Throat swab	049 (08.70)	77.80	14 (14)	22.20	63 (9.54)
Stool	016 (02.90)	66.66	08 (08)	33.40	24 (3.60)
Urine	003 (00.50)	21.40	11 (11)	78.60	14 (2.10)
Total (%)	560 (100)	84.85	100(100)	15.15	660 (100)

Out of total 660 subjects, blood samples were analyzed in 437 (66.20%) subjects , CSF samples were analyzed in 122 (18.5%) subjects, throat swab samples were analyzed in 63 (9.54%) , stool samples analyzed in 24 (3.6%) and urine samples were analyzed in 14 (2.1%).

5- best regards

OPEN PEER REVIEW

Revision (3)

REFEREE : Reviewer

6 Oct 2022

Dear author:
Thank you for your correction
There are few notes:
Results:
1-In sentence "…. Subject metapneumo and 1 subject toxoplasma these two viruses were found in association with other viruses only." Please write the complete name of virus and toxoplasma is not virus. Plz note.
Discussion:
1- Please remove the limitation title. Don't give your recommendations in this part. It can move to conclusion part.
Best regards

OPEN PEER REVIEW

Revision (4)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reviewers' Comments:

Reviewer 1:

Dear author:

Thank you for your correction

There are few notes:

Results:

1-In sentence “…. Subject metapneumo and 1 subject toxoplasma these two viruses were found in association with other viruses only.” Please write the complete name of virus and toxoplasma is not virus. Plz note.

AUTHOR RESPONSE- SUGGESTED CHANGES DONE. ABOVE SENTENCE REMOVED.

Discussion:

1- Please remove the limitation title. Don’t give your recommendations in this part. It can move to conclusion part.
AUTHOR RESPONSE- SUGGESTED CHANGES DONE.

Best regards

OPEN PEER REVIEW

Revision (4)

REFEREE : Reviewer — 22 Nov 2022

Dear Author
Thank you for your correction
This is a complete investigation on viral pathogens in neonates
There is a notes:
1- There are no information about weight and gestational age in table 2
Please check it
Best regards

Parisa Bahmani : Associate Editor — 22 Nov 2022

Dear EIC,
The manuscript revised succesfully and it is acceptable.

Mohammad Reza Boloursaz : EIC — 22 Nov 2022

Dear Author,
Thank you for your efforts in revising the manuscript. It is acceptable now.