ejpmr, 2020,7(12), 82-87

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PREVALENCE OF SOME ENTEROVIRUSES ANTIBODIES AMONG NEONATES OF TAIF GOVERNORATE, SAUDI ARABIA DURING 2018

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Article Received on 01/10/2020 **Article Revised on 21/10/2020** **Article Accepted on 11/11/2020**

ABSTRACT

Objective: Investigate the prevalence of Coxsackievirus and Echovirus antibodies among neonates screened for thyroid stimulating hormone (TSH) of Taif Governorate. **Methods:** A total of 80 cord blood samples were included in this study for screening of thyroid stimulating hormone (TSH), Coxsackievirus and Echovirus antibodies by using ELISA test in neonates of Taif Governorate during the period from January 2018 till May 2018. Samples were classified according to TSH antibodies level into two main groups; diseased group (high TSH) and control group (normal TSH). **Results:** In the control group, of 40 cases, 10 (25%) samples were positive for coxsackievirus IgG and only 3 (8%) samples were positive for coxsackievirus IgM but without any evidence of clinical infection. All samples of the control group were negative for either echovirus IgG or IgM. In the diseased group, of 40 cases, only 10 (25%) samples were positive for coxsackievirus IgM but without any evidence of clinical infection and no samples were positive for coxsackievirus IgG. All samples of the diseased group were negative for either echovirus IgG or IgM. The study revealed a significant correlation between TSH status and coxsackievirus exposure. **Conclusion**: This study declared that no antibodies had been detected for either Echovirus IgM or Echovirus IgG. The prevalence of Coxsackievirus (12.5% for Cox IgG and 16.25% for Cox IgM) is very low among children neonates of Taif governorate when compared to the global prevalence rate.

KEYWORDS: Coxsackievirus, Echovirus, Prevalence, ELISA, Taif.

INTRODUCTION

Enteroviruses (EVs) are members of the genus *Enterovirus* belonging to family *Picornaviridae*. Genus *Enterovirus* includes Rhinoviruses (A-C) and Enteroviruses (A-L). EVs are one of the most frequent human pathogens which cause 10–15 million new infections every year in the USA **(Abedi et al., 2015)**. Most people acquire different EV infections along their life. The four EV species (A, B, C, D) contain 116 different EV types. EVs may cause either asymptomatic infection or acute illnesses ranging from diarrhea to paralysis and encephalitis **(Pallansch et al., 2013).** Enteroviruses are RNA viruses and consists of more than 100 serotypes and characterized by a single positive-strand genomic RNA **(Haston and Dixon, 2015).** EVs have recorded as one of the most important causative agents for encephalitis in children and adults. After the first report of EV encephalitis in 1950, regular outbreaks of EV encephalitis were reported worldwide **(Shikha et al., 2014).** EVs infections occur mostly during the summer and fall epidemics. Enteroviral serotypes cause illness that ranges from nonspecific fevers and rashes to life-threatening myocarditis or central nervous system disease **(Sawyer, 2002).**

Enteroviruses can be transmitted by fecal-oral route such as swallowing contaminated food or water with stool from an infected person, touching a contaminated surface by saliva from an infected person or droplets during sneezing or coughing then touching the mouth and through inhalation of contaminated airborne droplets. Pleconaril is an oral antiviral drug and showed activity against several picornaviruses. It is being investigated for treatment of severe neonatal enteroviral disease **(Greninger, 2015).**

In Saudi Arabia, the epidemiology of EVs still needs more investigation. Many diseases were associated with the isolation of EVs at King Faisal Specialist Hospital and Research Centre in Riyadh after examination of samples submitted for diagnosis during the period from 1989 to 1995, such as herpangina, sepsis-like illness, hand-foot-and-mouth syndrome, laryngotracheitis (croup), aseptic meningitis, pneumonia, and gastrointestinal illness. EVs were isolated all over the year but most epidemics were recorded in winter and early spring **(Al-Hajjar et al., 1997)**.

Considering the seriousness and complexity of Enteroviruses infection beside its negative effects on

public health and its complications, so this study aimed to explore the prevalence of Enteroviruses mainly coxsackie and echoviruses, among neonates screened for thyroid stimulating hormone (TSH) through neonatal program for early discovery of thyroid disease, in the general population of Taif Governorate using ELISA technique.

MATERIAL AND METHODS

A total of 80 sample from newborn babies were included in this study irrespective to the sex, their blood samples were selected from neonatal program for early discover of thyroid disease in Taif Governorate at a period from January 2018 till May 2018. Samples were classified into two main groups according to TSH level into diseased group (high TSH; above 40 mIU/L) and control group (normal TSH) which defined by the neonatal screening program below 40 mIU/L. After birth, 10 ml of cord blood was collected either by syringe or by cutting a segment of the cord and putting the blood in 15 ml tube then sending it immediately to the laboratory. In the laboratory, blood was centrifuged at 3000 rpm for 15 minutes and serum was separated in a dry clean tube and preserved at -20 ^{o}C till sending to the Taif children hospital laboratory which receive samples from all areas of Taif government. All included samples in this study were subjected to Coxsackievirus(Cox) and Echovirus (Echo) antibody screening by ELISA test. Each group was sub-grouped according to Cox and Echo antibodies results into negative and positive, where both of them examined for both types of IgG and IgM for Cox virus antibodies and Echovirus antibodies. Four types of ELISA kits have been used in this study according to the manufacturer's instructions **(SAB, Signalway antibody)**; including Human Coxsackievirus (CoxV) antibody (IgG) ELISA Kit for the qualitative determination of human CoxV antibody (IgG) concentrations in serum, Human coxsackievirus (CoxV) antibody (IgM) ELISA Kit for the qualitative determination of human CoxV antibody (IgM) concentrations in serum, Human Echovirus (EchoV) antibody (IgG) ELISA Kit for the qualitative determination of human Echovirus (Echo) antibody (IgG) concentrations in serum and Human Echovirus antibody (IgM) ELISA Kit for the qualitative determination of human Echovirus (Echo) antibody (IgM) concentrations in serum.

RESULTS

All samples were assessed for the presence of human (CoxV) and (EchoV) antibody, 10 (25%) samples of the control group were positive for coxsackievirus IgG but no samples were positive in the diseased group. Only 3 (8%) samples of the control group without any evidence of clinical infection. No antibodies (IgM and IgG) have been detected for echovirus in both the control and diseased group. Among the total 80 samples, 12.5% were positive for Cox IgG, 16.25% were positive for Cox IgM and no antibodies detected for either Echo IgM or Echo IgG. Data was analyzed statistically using the R-Statistical Software version 3.4.1.

TSH level among the participating neonates

The total number of neonates included in this study was (n=80) newly born babies. In the diseased group, the mean was 50.25 (SD ± 6.75), ranging from 40 (the lowest TSH level) and 60 (the highest TSH level among participants). The median TSH level was 50.5 (Figure 1). In control group, the mean was 4.38 (SD ± 3.03), ranging from 0.1 (the lowest TSH level) and 9.8 (the highest TSH level among participants). The median TSH level was 4 (Figure 1).

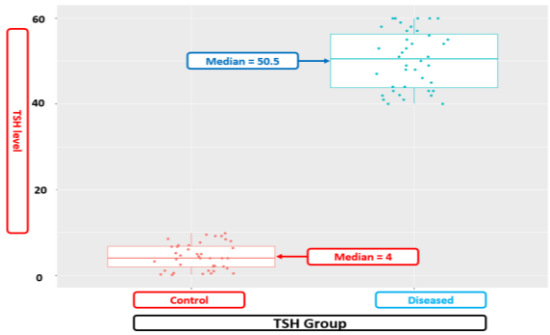

Figure 1: TSH level distribution among the participating neonates.

Coxsackievirus IgG antibodies

In the diseased group, no IgG antibodies had been detected for coxsackievirus. While in the control group, the mean coxsackievirus IgG antibodies was 1.21 (SD = 1.36), ranging from 0.1 (the lowest coxsackievirus IgG antibodies level) and 4.1 (the highest coxsackievirus IgG antibodies among the control group). The median coxsackievirus IgG antibodies level was 0.58 (Figure 2).

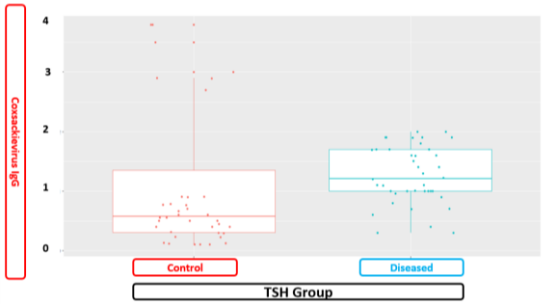

Figure 2: Coxsackievirus IgG antibodies level distribution among the screened neonates.

Coxsackievirus IgM antibodies

Coxsackievirus IgM antibodies in the diseased group mean was 2.03 (SD ± 1.14), ranging from 0.1 (the lowest coxsackievirus IgM antibodies level) and 4.2 (the highest coxsackievirus IgM antibodies among the diseased group). Meanwhile, the median coxsackievirus IgM antibodies level was 1.8 (Figure 3). In the control group, the mean coxsackievirus IgM antibodies was 0.76 (SD ± 0.85), ranging from 0.1 (the lowest coxsackievirus IgM antibodies level) and 3.9 (the highest coxsackievirus IgM antibodies among the control group), while the median coxsackievirus IgM antibodies level was 0.59 (Figure 3).

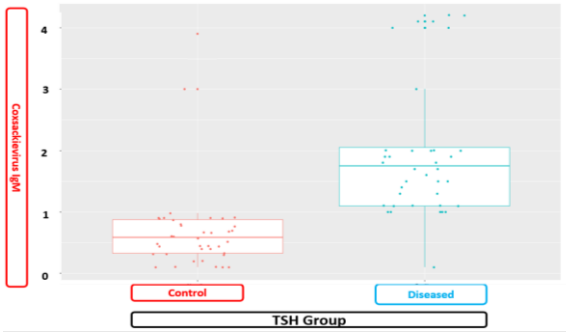

Figure 3: Coxsackievirus IgM antibodies level distribution among the screened neonates.

Echovirus IgG antibodies

No antibodies had been detected for echovirus IgG in either control or disease group (Figure 4).

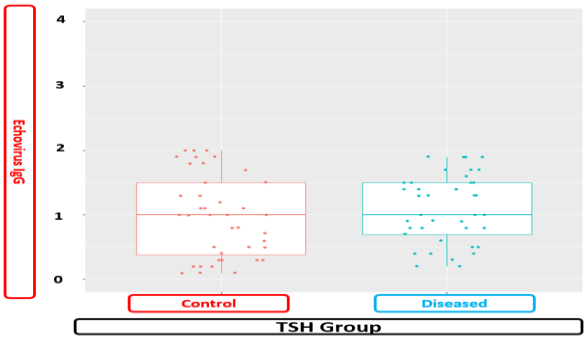

Figure 4: Echovirus IgG antibodies level distribution among the screened neonates.

In addition, no echovirus IgM antibodies were detected in either the 40 neonates of the diseased or the control groups, (Figure 5)

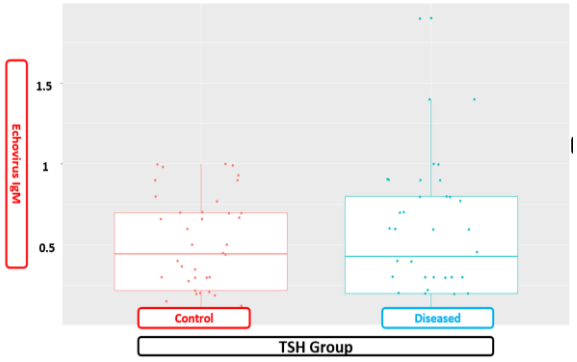

Figure 5: Echovirus IgM antibodies level distribution among the screened neonates.

Table 1: Differences in terms of Coxsackievirus and Echovirus antibodies among TSH positive and TSH negative neonates.

Examined group	TSH positive mean vs TSH negative mean	Difference in means	T test value (Degrees of freedom)	P value
Coxsackievirus IgG	- Vs 1.2720	-	t = 0.256 (df = 49)	0.7994
Coxsackievirus IgM	0.76025 Vs 2.03250	1.27225	t = 5.64 (df = 72)	< 0.001
Echovirus IgG	-	-	t = 0.86534 (df = 76)	0.3896
Echovirus IgM	-	-	t = 1.321 (df = 70)	0.192

Table 1: shows the t-test comparison between the group of TSH positive neonates and the group of TSH negative neonates in terms of mean antibody. Only coxsackievirus IgM antibodies were significantly higher in TSH-negative neonates than in TSH-positive neonates.

Table 2: The positive and negative number of neonates for both Coxsackievirus and Echovirus antibodies in both groups (control and diseased).

ELISA result / Examined Group	Cox Ig G		Cox Ig M		Echo Ig G		Echo Ig M	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Control group (TSH negative, 40)	10 (25%)	30 (75%)	3 (8%)	37 (92%)	0 (0%)	40 (100%)	0 (0%)	40 (100%)
Diseased group (TSH positive, 40)	0 (0%)	40 (100%)	10 (25%)	30 (75%)	0 (0%)	40 (100%)	0 (0%)	40 (100%)
Total	10 (12.5%)	70 (87.5%)	13 (16.25%)	67 (83.75%)	0 (0%)	80 (100%)	0 (0%)	80 (100%)
Chi-Square	9.257		38.9		Not Applicable		Not Applicable	
P value	0.002		< 0.0001		Not Applicable		Not Applicable	

Table 2: Shows the count of neonates broken-up according to TSH level group and virus antibodies. There was significant difference in Chi square of Cox IgM. Also, there was significant correlation between TSH status and coxsackievirus exposure.

DISCUSSION

The genus *Enterovirus* belongs primarily to the family *Picornaviridae* and includes poliovirus (PV), coxsackievirus (CV) and Enterovirus-71 (EV-71) (**Ramsingh, 2008 and Marylynn 2014)**. Enteroviruses (EVs) have been associated with many human diseases; including myocarditis, pancreatitis and chronic inflammatory myopathy (**Rhoades et al., 2011)**. Thyroid stimulating hormone (TSH) concentrations in umbilical cord blood of newly born infants can affect later cognitive function **(Freire et al., 2010).** So, screening for TSH level in blood samples is made at delivery as a routine program of neonatal congenital hypothyroidism, for the exclusion of late appearing transient hypothyroidism **(Tylek-Lemanska et al., 2002)**.

Due to the lack of large-scale studies, the epidemiology of Enterovirus infections in Saudi Arabia, Gulf region and the Middle East is still mysterious **(Al-Hajjar et al., 1997).** Therefore, this study aimed to determine the prevalence of some Enteroviruses (coxsackieviruses and echoviruses) among children neonates in Taif governorate by using ELISA assays for detection of antibodies. In this study, cord blood samples were collected just after birth and this agree with **Halwachs et al. (2002)** who mentioned that screening neonates for virus shedding tests must be done within the first 2–3 weeks after birth.

In this study, a total number of 80 cord blood samples were collected from neonates at Taif Children Hospital and were divided into two groups; 40 samples representing the control group (TSH normal) and 40 samples representing the diseased group (TSH high). Detection of Echovirus IgG (Echo IgG), IgM (Echo IgM), Coxacievirus IgG (Cox IgG) and IgM (Cox IgM) were done in all samples by ELISA assay.

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The prevalence of Coxsackievirus and Echovirus antibodies was investigated in the current study using ELISA assay for detecting antibodies in cord blood samples. The finding in the current study agree with **Ding et al. (2018)** who used ELISA also for detection of antibodies against some Enteroviruses (EV-A, B, C and Rhinovirus-A) among infants and children aged 1 day to 6 years in Shanghai that detected high levels of antibodies in the 1–28 day age group, which reflects maternally derived antibody responses. ELISA assay was used effectively in this study for screening of Coxsackievirus and Echovirus antibodies and it was simple, economic and rapid test. However, some other studies like **Shabani et al. (2018)** had depended on RT-PCR to evaluate the frequency of EVs among neonates (younger than three months) in Ahvaz, Iran, while this technique is not practical for screening program.

During this study, no antibodies had been detected for either Echo IgM, or Echo IgG. This could be explained by absence of recent or previous infection by echovirus in babies and mothers, respectively. On the other hand,

Cox IgG was detected only in 10 samples (12.5%), while Cox IgM was detected in 13 samples (16.25%) of the total samples, but there is no clear serological evidence of a clinically relevant infection in babies or mothers.

In this study, echoviruses antibodies (IgM and IgG) were not detected in any of the examined samples (Table 2) which indicates that mothers of these neonates did not previously acquire echoviruses infection, while **Modlin (1986)** had detected 61 cases of neonatal echovirus infection between the third and fifth day of life in USA. Our study revealed that the prevalence rate of coxsackieviruses and echoviruses antibodies in Taif governorate is very low (12.5% for Cox IgG, 16.25% for Cox IgM and 0% for both Echo IgG and IgM) when compared to that reported by **Boman et al. (1992)** who detected high titers of Enterovirus IgG and IgM in 73% and 68%, respectively in patients with a recent Enterovirus infection by using ELISA technique. This low rate might reflect the application of effective control measures Taif governorate.

The prevalence rate of coxsackie and echovirus that detected by ELISA in our study is nearly the same to that detected by **Wu et al. (2013)** who examined 320 healthy young children (under 5 years old) in Shenzhen, China from 2010 to 2011 and found that 34 cases (10.6%) were positive for EVs by real-time PCR and cell cultures, which declared that ELISA results is comparable to PCR and cell cultures results. **Veronica et al. (2015)** reported a higher rate of EVs (22.8%) among apparently healthy individuals aged 0–53 years (between June 2013 and December 2014) in the Sud-Como region of Cote d'Ivoire, which might be referred to the wide range of age of participating individuals.

In this study, we screened IgM antibodies against Coxsackievirus and Echovirus only in children neonates directly after birth and detected them in 16.25% and 0%, respectively which considered very low rate when compared to that detected by **Elena et al. (2000)**, who detected anti-Enterovirus IgM antibodies in 34% of children younger than 10 years old in Germany by using enzyme immunoassay (EIA), and this might be due to the difference in age. Diagnosis of Enteroviruses was mainly depending on virus isolation and virus identification using neutralization with serotype-specific antisera **(Mahony (2008).** Unfortunately, these methods need more labor, time consuming and the patient often recovered prior to the completion of these assays **(Nurminen et al., 2012).**

Unfortunately, isolation of EVs in tissue culture systems is very boring as it takes long time for cytopathic effects to appear, so ELISA assays are an important tool for diagnosis of coxsackievirus infections. Virus isolation is facing many difficulties due to inability of certain serotypes to grow in the cell cultures. Also, the sensitivity of virus isolation is usually lower than that of PCR techniques **(Wikswo et al., 2009).** While, detection of antibody or virus-specific IgM in serum and/or CSF give evidence of EVs infection as reported by **Rhoades et al. (2011).**

Regarding to all the previous facts, ELISA not only proved to be the test of choice to be reliable for screening of Coxsackievirus and Echovirus antibodies, but also it gave a close view to the current prevalence of some Enteroviruses (Coxsackievirus and Echovirus) among neonates of Taif governorate.

CONCLUSION

Results of this study indicate that coxsackievirus antibodies are present in healthy neonates of Taif governorate which might indicate transplacental transmission from their mothers. This study revealed that there is a significant correlation between TSH status and coxsackievirus exposure. It is recommended to repeat this study in multi-centers and on wide scale of newborn all over the kingdom of Saudi Arabia to know the overall incidence of EVs in order to set up strategic plans to control the spread of EVs.

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