Immunological Responses to Varicella-Zoster Virus (VZV) in Basrah with Special Emphasis on the Pattern of Exposure

Wijjdan Taha Yassien[1], Hassan J. Hasony[2]

ABSTRACT

The study aimed on the study of pattern of exposure and immunological responses to varicella-zoster virus (VZV) infection. Methods: A seroepidemiological study was carried out on 92 blood samples collected from children in primary health care centers and outpatient clinics of Al-Sader Teaching and Basrah maternity and children hospital during the period from January 2007 till March 2008. Serological responses were investigated by an enzyme-linked immunosorbent assay (ELISA) to detect VZV IgG antibody and cellular responses was detected by Erythrocyte rosette (E-Rosette) formation test. *Results:* The overall VZV-antibody prevalence was 53.3% for both serologic and cellular responses. The degree and pattern of exposure to VZV cases plays important role in the seroconversion and responses to VZV infections. The greatest responses observed in those with direct contact (household) with chickenpox cases (68.6% and 74.3%) and the direct contact acquired from VZV infected mothers (80% for both serologic and cellular responses). Exposure to typical chickenpox cases confers higher serologic and cellular responses (75% and 94.4% respectively). Cellular responses to VZV infections lasted longer than that for serologic responses. Sever VZV infections confers significantly higher levels of immunity (100%) and the complication post VZV infections (Pneumonia and encephalitis) mostly associated with sever cases at earlier age groups rather than the mild VZV cases. The differences in immune responses to VZV infections between male and females was significant (P<0.05). The rates of exposure to VZV infections in urban areas are significantly higher than in rural areas (P<0.05). There was a significant decline in VZV immunity over time post infections and losses of protective immunity levels were quite evident few years post infection which stress the need for vaccination. (MJBU,30,2: 2012, Page 106-114)

مديات تأثير التعرض لحمات الجدري المائي لدى الأطفال على الاستجابة المناعية بعد التعرض

د.وجدان طه ياسين1 و أ.د.حسن جابر حسوني2

[1]طبيبة/دائرة صحة ميسان، [2] فرع الأحياء المجهرية/كلية الطب/جامعة البصرة

الهدف وطريقة العمل: دراسة مصلية – مناعية شملت 92 عينة دم اخذت من الأطفال في مراكز الرعاية الطبية الأولية ومستشفى الولادة والأطفال في البصرة للفترة من ك2 2007 الى آذار 2008 وكان الهدف من الدراسة تقييم مديات التعرض لحمات الجدري المائي على مستوى الاستجابة المناعية لها حيث استخدمت تقنية المقايسة المناعية المرتبطة بالإنزيم للتخري عن مستضدات الحمات وفحص تلازن الكريات الحمراء لتحديد المناعة الخلوية. *النتائج:* لقد كان مستوى انتشار مستضدات الحمات المصلية والخلوية 53.3% لكليهما وكان لمستوى التعرض اثر كبير على مستوى الاستجابة المناعية ووجد أعلى مستوى تمنيع لدى ذوي التعرض المباشر للمصابين بالحمات (الملامسين) وبنسب 68,6% و 74,3% للمناعة المصلية والخلوية على التوالي واظهر التلامس المباشر للام المصابة بنسبة 80% لكلا النوعين من المناعة. التعرض المباشر لحالات الجدري المائي الشديدة أظهرت أعلى استجابة مناعية لدى المتعرضين وبنسب 75% و 94,4% للمناعة المصلية والخلوية على التوالي وان المناعة الخلوية دامت لفترات أطول من المناع المصلية لدى المتعرضين وان شدة الإصابة تتناسب طرديا مع المستوى المناعي بعد التعرض (100%). لوحظ بان الاختلاطات المصاحبة للإصابات كانت ذات الجنب والتهاب الأغشية الدماغية حيث اقتصرت هذه الاختلاطات على الحالات الشديدة وبمستوى إحصائي يعتد به. *الاستنتاجات:* ان مستويات التعرض أعلى لدى القاطنين في المناطق الحضرية منها في المناطق الريفية وبمستوى إحصائي يعتد به وان هذه المستويات من التمنيع تنخفض بنسب تنازلية مع ابتعاد زمن التعرض مما يقتضي اللجوء الى التحصين باللقاحات اللازمة لحصول التمنيع الكافي.

[1]MBchB, MSC,

[2]MPhil, PhD, Department of Microbiology, Collage of Medicine, University of Basrah, Iraq.

INTRODUCTION

Varicella is a childhood disease due to a primary exposure to varicella-zoster virus (VZV), with a wide spread skin lesions[1,2] Varicella is a highly contagious disease. [3] Attack rates for VZV may reach 90% for susceptible household contacts. In healthy persons, clinical illness after re-exposure is rare, such illness is more likely to occur among immunocompromised persons.[2] However, as with other viral diseases, re-exposure to a wild-type varicella virus often lead to reinfection that boosts antibody titers without causing clinical illness or detectable viremia. VZV remains dormant in sensory nerve and may be reactivated at a later time causing herpes zoster. [3] Herpes zoster is an endemic sporadic disease that occurs mainly in the elderly and the immunocompromised.[4] Herpes zoster usually manifests as painful vesicules covering a distinct patch of skin and can include nerve damage. [1] Before 1995 when a vaccine for chickenpox became available in the USA about 4 million Americans, mostly children, contracted chickenpox each year. The number of cases and hospitalizations is down dramatically after the introduction of the VZV vaccine. [3] Chickenpox usually lasts about two weeks and rarely causes complications, but the disease can be serious, even in healthy children.[3,4] Primary VZV subclinical infection is rare for persons of all ages.[3] There is immunological evidence to suggest that subclinical reinfection with VZV is common, although it is unknown what reinfection plays in the maintenance of protective antibody levels. [5,6] Maternal varicella with viremia can spread transplacentally to the fetus. [6] Family members who have never had chickenpox have a 90% chance of becoming infected when another family member in the household is infected. [6] By the age of 10 years, 80% of children had varicella, 95% of adults are immune, and 25% of adults with no history of varicella are susceptible. [5,6] However, different parts of the world can show significant variations in the age distribution of infection.[6,7] In temperate climate more than 90% of people have had clinical or subclinical infection by adolescence. [3] Herpes zoster occur in 20% of the population and with more than one recurrence in 4%. [7,8] The most common complication is varicella pneumonia which occurs in 1-5 per 10000 cases, encephalitis occur in older teenager and adults in 1 per 3000 cases. [5] The role of VZV antibody alone in the containment of primary infection is unclear. Specific varicella virus antibody modifies the disease pattern but does not prevent infection.[9] However, increased severity and risk for herpes-zoster associated with cellular responses dysfunction.[1,10,11] Although T-cells memory of VZV decreases with age, several factors may serve to periodically boost the immune system, as exposure to individuals with varicella or herpes zoster or a subclinical reactivation of the virus.[1] The objectives of this study are to estimate the prevalence of VZV-antibody and to detect the cellular responses among children below 15 years, to evaluate the impact of pattern of exposure to chickenpox on immunological responses and the rate of the over time antibody decline.

MATERIALS AND METHODS

- Study population:

Ninety two immune competent children below 15 years of age (52 females and 40 males) from the attendance of primary health care centers and the outpatients clinics of Al-Sader teaching hospital and Basrah maternity and children hospital during the period from January 2007 to March 2008 from various areas in Basrah governorate after taking a formal permission from the family member who brought the child. All required informations were collected on special questionnaire form including many variables; beside the demographic information, the history of chickenpox or exposure to

chickenpox cases (household contact, mothers, exposure to chickenpox school episodes), severity of disease which was scored as mild, moderate or severe considering the area distribution of vesicularlesions and degree of complains which was all stated in by the examining physician in the child health record booklet, and any evident of post VZV infection complications.

Methods:

Blood specimens were drawn from the veins aseptically and divided into two portions. One part used for collection of serum which was stored at -20°C until use for estimation of VZV antibody by ELISA commercial kit (DRG Varicella-Zoster IgG: EIA-3523). All the procedures were done according to the manufacturers instructions. The second part of blood was kept in heparinized tube and used for separation of lymphocytes and testing for Erythrocytes rosette (E-Rosette) forming cells according to the standard methods.[12] The number of active lymphocytes was estimated by the number of rosette forming cells multiplied by total number of lymphocytes divided by 100.[13] Statistical analysis was done by using

RESULTS

The main characteristics of the studied children are presented in (Table-1). The majority of the studied children (65.2%) were living in urban area and 34.8% inhabiting the rural areas. Half of the study population were with a history of skin rash and the majority of them had mild illness (91,3%) among them 10.9% had complications including encephalitis (20%) and pneumonia (80%) based on clinical and laboratory investigations as stated in the child health cards. Fifty percent of the study children with a history of skin rash had VZV infections since 1-5 months before sampling, 10.9% of them had skin rash since 6-11 months before sampling, 23.9% had skin rash 1-3 years before sampling and 15.2% had history of skin rash before more than 3 years of sampling. Among the contact with chickenpox cases, 46(50%) had history of previous VZV infection, 13(14.1%) have a history of contact with VZV infection (9 cases to VZV infected mother and 4 cases to other household contact). However, 33(35.9%) of children neither of a history of VZV infection nor a history of contact.

Table 1. the characteristics of the study population.

Character	No. Tested (%)
1- Age	92 (100)
<1-3 years	29 (31.5)
4-6 years	17 (18.48)
7-9 years	16 (17.39)
10-15 years	30 (32.6)
2- Sex	92 (100)
Female	52 (56.5)
Male	40 (43.47)
3-Residency	92 (100)
Urban area	60 (65.2)
Rural area	32 (34.78)
4-History of skin rash	92 (100)
Yes	46 (50)
No	46 (50)
5- Severity of the disease	46 (50)
Mild and moderate	42 (91.3)
Severe	4 (8.7)
6- Complications of the disease	5 (10.87)
Encephalitis	1 (20)
Pneumonia	4 (80)
Skin complications	None
7- Duration of infection	46 (50)
1-5 Months	23 (50)
6-11 Months	5 (10.87)
1-3 Years	11 (23.9)
>3 Years	7 (15.2)
8- History of previous VZV infection	46 (50)
History of contact with VZV infection	13 (14.1)
(mothers=9, others =4)	
9- Neither history of VZV nor a history of contact	33 (35.9)

Table-2 shows the prevalence of anti-VZV antibody and cellular responses (E-rosette) among children in relation to age. The overall immunologic responses were observed in 53.3%. These responses was presented more frequent at age group 4-6 years which was 58.8% and 64.7% for serologic and cellular responses respectively. Followed by the exposure at early age of < 1-3 years, and by late age of 10-15 years in whom the seropositivity increased from 43.8% to 50%. This difference was statistically not significant (P>0.05).

Table 2. The prevalence of serological and cellular responses to VZV among children according to their age.

Age	Total No. (%)	Serologic response No. (%)	Rosette forming cells No. (%)
<1-3 Years	29 (31.5)	17 (58.6)	15(51.7)
4-6 Years	17 (18.48)	10 (58.8)	11 (64.7)
7-9 Years	16 (17.39)	7 (43.75)	6 (37.5)
10-15 Years	30 (32.6)	15 (50)	17 (56.67)
Total	92 (100)	49 (53.25)	49 (53.25)

Typical chickenpox cases confers high immunological responses at the time of presentation (75% and 94.4% for serological and cellular responses respectively) as shown in (Table-3). Patients with non-specific rash showed greater cellular responses (50%) than VZV specific antibody responses (30%). However, the responses that obtained from unexposed individuals were significantly with lower seropositivity to VZV(41.3%) and 21.7% for rosette formation test.

X^2 = 0.376 d.f = 3 P>0.05

Table 3. the distribution of serologic and cellular responses to VZV among children according to history of skin rash.

History of skin rash	Total No. (%)	Serologic response No. (%)	Rosette forming cells No. (%)
Typical chickenpox cases	36 (39.1)	27 (75)	34 (94.4%)
Non-specific skin rash	10 (19.9)	3 (30%)	5 (50%)
None	46 (50)	19 (41.3%)	10 (21.7%)
Total	92 (100)	49 (53.25%)	49 (53.25%)

X^2 = 4.1 d.f = 2 P> 0.05

(Table-4), shows the responses to VZV infections in relation to the duration and persistency of serologic and cellular activity post exposure. The cellular responses persist longer than that for serologic responses which did not last more than three years in less than the protective levels, while the cellular responses persist in rosette forming activity for more than three years with higher percentage of activity at early periods of exposure. The serologic responses declined by three years and more post exposure (42.9%). The differences between the levels of responses at early and long period post exposure was statistically significant (P<0.05). The degree and pattern of contact to VZV cases play important role in the seroconversion and responses to VZV infection where the greatest responses observed in those with direct contact (household) with chickenpox cases (68.6% and 74.3%) and the direct contact acquired from VZV infected mothers (80% for both serologic and cellular responses). However, history of contact since long periods and mild exposure to chickenpox school episodes confers lower responses.

Table 4. the distribution of serologic and cellular responses to VZV among children according to the duration between skin rash and sampling.

Duration between skin rash and sampling	Total No. %	Serologic responses No. (%)	Rosette forming cells No. (%)
1-5 Months	23 (25)	17 (73.87)	22 (95.65)
6-11 Months	5 (5.4)	4 (80)	4 (80)
1-3 Years	11 (11.96)	6 (54.5)	8 (72.7)
>3 Years	7 (7.6)	3 (42.86)	5 (71.4)

X^2 = 19.89 d.f = 3 P< 0.05

(Table-5). On the other hand, individuals reported no history of any contact showed a very mild type of responses to VZV in both serologic (27.3%) and cellular levels (18%) which might be due to subclinical infection.

Table 5. The distribution of serologic and cellular responses to VZV among children according to history of contact with chickenpox.

Pattern of exposure to chickenpox	Total No. (%)	Serologic response No. (%)	Rosette forming cells No. (%)
Household contact	35 (38.04)	24 (68.6)	26(74.3)
Chickenpox in the mother	5 (5.4)	4 (80)	4 (80)
Exposure to school episode of chickenpox	15 (16.3)	8 (53.3)	9 (60)
***All**	4 (4.35)	4 (100)	4 (100)
None	33 (35.87)	9 (27.27)	6 (18)
Total	92 (100)	49 (53.25)	49 (53.25)

X^2 =1.1 d.f =4 P>0.05

***All: exposed to all factors.**

There was a positive relation between the severity of illness and immunologic responses to VZV infection (Table-6) as those with severe illness showed 100% serologic and cellular responses in contrast to those with mild presentation (P<0.05).

Table 6. the distribution of serologic and cellular responses to VZV among children according to severity of the disease*

Severity of the disease	Total No. (%)	Serologic response No. (%)	Rosette forming cells No. (%)
Mild	42 (91.3)	26 (61.9)	35 (83.3)
Severe	4 (8.7)	4 (100)	4 (100)
Total	46	30 (65.2)	39 (84.78)

X^2 =16.78 d.f =1 P< 0.05

***Based on health booklet records and physician notes. Only one case scored as moderate classified with mild group.**

Table-7 showed that pneumonia occurs in 8.7% as a complications associated with VZV mostly occur among infants and young children which was attributed to 9.38% and encephalitis to 3.1%, those were less frequent among older age group of 10-15 years, but the difference was statistically not significant (P>0.05). However, both mild and severe cases of VZV infections confers high antibody titers, although the complications associated with severe infections only (Table-8). The difference in the occurrence of complications between mild and severe cases was statitistically not significant (P>0.05). None of the study cases reported a skin complication.

Table 7. The distribution of varicella complications among children in different age groups

Age group	Total No. (%)	Encephalitis No. (%)	Pneumonia No. (%)	Skin complications No.(%)
<1-9 Years	32 (69.57%)	1 (3.13%)	3 (9.38%)	None
10-15 Years	14 (30.4%)	None	1 (7.14%)	None
Total	46	1 (2.17%)	4 (8.7%)	None

X^2 =0.313 d.f =1 P> 0.05

Table 8. The relationship between severity and complications of VZV infection and antibody titer.

Severity of VZS infection	Complications			AB titer
	Encephalitis No. (%)	Pneumonia No. (%)	Skin complications	
Mild	None	1 (2.38)	None	*High
Severe	1(25)	3 (75)	None	High

X^2 =0.313 d.f =1 P>0.05

***High IgG titer equivalent to > 15 IU/ml**

DISCUSSION

Age related susceptibility to varicella in various regions of the world is well recognized.[13] Our findings are close to a study in the UAE which showed 45.8% susceptibility to VZV in children less than 10 years old and similar to the results of survey in tropical areas at least for children under 10 years old.[14] According to the literature, a majority of (about 85%) VZV infections occur during early childhood by the age of nine with strong association between varicella infection and VZV seropositivity. [15] Varicella is a highly contagious disease, particularly after the close contact as household contact or in collection environments such as day care centers.[16] In our locality, the life pattern of large families sharing same house, play a role in increasing the incidence of VZV infection among the urban areas. This results is consistent with a study done in Turkey which reported that varicella seropositivity was higher in large families with five or more members (91.2%) than small families with four or fewer members (80%).[17] However, our results in contrast to studies done in south America [18] and Switzerland[19] which were documented no seroprevalence differences between rural versus urban areas. On the other hand, a study conducted in India reported marked differences in susceptibility in adult living in urban and rural areas[20] In the current study 78.3% of cases of varicella were with typical skin rash, among them 75% were seropositive and 94.4% had positive cellular immune responses. While 21.7% of varicella cases were presented with non-specific skin rash, of them 30% were seropositive and 50% had positive cellular responses. We did not find any study for comparison. However, among children with no history of skin rash 41.3% were seropositive to VZV and 21.7% had positive cellular immune responses, this may be attributed to the subclinical infection among this group. This observation is in agreement with a study carried in Brazil showed that among cases with no history of varicella 33.1% were seropositive.[21] Cell-mediated immunity (CMI) play a crucial role in the protection from VZV infection and also against reactivation.[22] Although CMI has been shown to decline with increasing age, the presence of VZV-specific antibodies does not.[22,23] However, this was not observed in this study, we found both serologic and cellular immune responses were decreased gradually with increasing the period between skin rash and sampling, but the cellular responses persist in their activity for longer period, therefore, it can be concluded that many factors may affect the immune responses in our community like life style, low educational levels and exposure to many abnormal situations as wars. Our findings is in contrast to a study carried in Colombia[22] showed that serologic responses persist in 91.3% of cases for 10 years after infection, while cellular responses decreases to 76.1%. Contact with varicella cases is important in responses to VZV, where the highest immune responses were among those with history of chickenpox in their mothers. This observation is

inconsistent with a study carried out in Spain[24] showed that 78.2% of cases of varicella had history of infection in their mothers. Exposure to a chickenpox school episodes confers 53.3% seropositivity and 60% cellular responses. These findings is in agreement with CDC reports 2004 on Nevada unvaccinated children and the CDC reports on Oregon unvaccinated children where the attack rate was 43% in 2001.[25] In the current study, mild VZV cases represent 91.3%, of them 61.9% were seropositive to VZV and 83.3% had positive cellular responses and sever cases represent 8.7% all of them (100%) were VZV seropositive with cellular immune responses. These figures are consistent with an Australian study which reported that severe varicella attributed to 8.2% mainly among 5-14 years old.[26] The main VZV infection complications observed in this study was pneumonia (8.7%) and encephalitis (2.17%) with no any skin manifestations. These complications mostly associated with severe cases with low T-cell activity (rosette formation) among children below 9 years of age, although pneumonia also associated with mild VZV cases. These results almost consistent with that reported in Australia[26] and Canada.[27] However, higher percentages for these complications reported in studies from Quebec[28] and Sri Lanka[29] with high rate of skin manifestations (64.4%) and neurologic disorder.[6] Also it has been reported that VZV complications and reactivation of latent infection, associated with low T-cell activity (cellular responses).[27]

In conclusion, VZV infections are common in all age groups and the pattern of exposure plays an important role in seroconversion to infection. Serologic responses seems to be not adequate to confer protection against infection or reactivation without the effect of cellular responses. Because of the evident decline in immunity post exposure, the implementation of VZV vaccination program especially among risky groups is highly recommended. Also further study is required to elucidate the role of vaccines in maintaining the protective levels of immunity.

REFERANCES

1. **CDC. Prevention of varicella: Recommendation of the advisory committee on immunization practice (ACIP). MMWR. Recommendations and Reports, 12,1996/45(RR11); 1-25.**
2. **Gnann J.W. Varicella-zoster virus: atypical presentations and unusual complications.J. Infect. Dis. 2002; 186:91-98.**
3. **CDC. Decline in annual incidence of varicella-selected states, 1990-2001. MMWR 2003; 52(37): 884-885.**
4. **Facanha MC, Celia A, Monroe P. Occupational and nosocomial transmission of varicella. The Brazilian Journal of Infectious Diseases 2006; 10(2):156-158.**
5. **Cameron JC, Allan G, Johnston F, et al. Severe complications of chickenpox in hospitalized children in the UK and Ireland. Archives of Disease in childhood 2007; 92:1062-1066.**
6. **Marchetto S, de Benedictis FM, de Martino M, et al. Epidemiology of hospital admissions for chickenpox in children: an Italian multicenter study in the pre-vaccine era. Acta Pediatrica. 2007; 96(10): 1490-1493.**
7. **Dominic ED, Cunningham AL. Herpes simplex and varicella-zoster virus infections. eMJA. The Medical Journal of Australia. MJA 2002; 177(5): 267-273.**
8. **Fleming DM, Schellevis FG, Falcao I, et al. The incidence of chickenpox in the community. European Journal of Epidemiology 2007; 17: 1023-1027.**
9. **Charles Grose. The immunology of VZV infection. Children's Hospital of Iowa, University of Iowa, Iowa City, IA, USA. 2006. Available at: www.ihmf.org/journal/download/13s1grose(9a)13s9a.pdf Accessed in: 3/2/2008.**
10. **Yilmaz C, Caksen H. Severe neurological complications of chickenpox. Eur.J. Gen. Med. 2005; 2(4):177-179.**
11. **Heininger U, Seward JF. Varicella. Health line, Elsevier Inc. 2005. Lancet. Available at: http://www.healthline.com/elseviercontent/lancet-varicella Accessed in: 11/2/2008.**
12. **Mendes NF, Tolnal MEA, Silveira NPA, et al. Technical aspects of the rosette tests used to detect human complement receptor (B) and sheep erythrocyte binding (T) lymphocytes.J. Immunol., 1973; (111)3: 860-867.**
13. **Lisse IM, Qureshi K, Poulsen A, et al. T-lymphocyte subsets and eosinophil counts in acute and convalescence chickenpox infection: a household study in Guinea-Bissau. Journal of Infection 2005; 50(2):125-129.**
14. **Sharifi Z, Ghanjin SE. The seroepidemiology of varicella-zoster virus (VZV) in different age groups in Tahran, Iran. Iranian Journal of Allergy, Asthma, and Immunology. 2005; 4(2): 95-98.**

15. Motamedifar M, Handjani F, Hadi N, et al. Seroprevalence of varicella-zoster virus in children from Shiraz–Iran. Iran J. Immunol. 2006; 3(1): 43-46.
16. Bricks LF, Pannuti CS, Sato HK, et al. Reliability of information on varicella in preschool children. Clinics Sao Paulo. 2007; 62(3): 180-191.
17. Kurugol Z, Koturoglu G, Aksit S, et al. Varicella seroprevalence in Turkish population in Cyprus. Acta Paediatrica . 2007; 96(6):861-863.
18. Dayan GH, Panero MS, Debbag R, et al. Varicella seroprevalence and molecular epidemiology of varicella-zoster virus in Argentina, 2002. Journal of Clinical Microbiology (JCM). 2004; 42 : 5698-5704.
19. Heininger UC, Braun-Fahrlander D. Champs D, et al. Seroprevalence of varicella zoster virus immunoglobulin G antibodies in Swiss adolescents and risk factor analysis for seronegativity. Pediatr. Infect. Dis.J. 2001; 20:775-778.
20. Mandal BK, Mukherjee PP, Murphy C, et al. Adult susceptibility to varicella in tropics is a rural phenomenon due to lack of previous exposure. J. Infect. Dis. 1998; 178: 53-54.
21. Maria Isabel DE, Moraes P, Yin L, Weck X. Prevalence of IgG varicella zoster virus antibodies in the Kuikuro and Kaiabi indigenous communities in Xingu National Park, Brazil, before varicella vaccination. Rev. Inst. Med. Trop. S.Paulo May-June, 2005; 47(3):139-142.
22. Jawetz E, Melnick M, Adelberg's M. Medical Microbiology. 23rd ed. GEO.F. Brooks and Lange. California; 2004: 438-442.
23. Liyanage NPM, Sirimali F, Malavige GN, et al. Seroprevalence of varicella zoster virus in Colombia. Indian Journal of Medical Sciences 2007; 61(3):128-134.
24. Fleming DM, Schellevis FG, Falcao I, et al. The incidence of chickenpox in the community. European Journal of Epidemiology 2007; 17:1023-1027.
25. Tugwell BD, Lee LE, Gillette H, et al. Chickenpox outbreak in a highly vaccinated school population. Pediatrics. 2004; 113:455-459.
26. Gidding HF, Macintyre CR, Burgess MA, et al. The seroepidemiology and transmission dynamics of varicella in Australia. Cambridge Journals. Epidemiology and Infection. 2003; 131:1085-1089.
27. Almuneef M, Memish ZA, Abbas ME, et al. Screening health care workers for varicella zoster virus. Infect. Control Hosp. Epidemiol. 2004; 25(7) :595-598.
28. Rivest P, Bedard L, Mills E, et al. Severe complications associated with varicella in Province of Quebec, April 1994 to March 1996. Can.J. Infect. Dis. 2001; 12(1): 21-26.
29. Welgama U, Wickramasinghe C, Perera J. Varicella-zoster virus infection in the Infectious Diseases Hospital, Sri Lanka. The Cylon Medical Journal. 2003; 48(4):119-121.