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For original articles, the abstract should contain no more than 200 words and should have a structured format consisting of the objective, methodology, results and conclusion. For case reports, the abstract should be from 50 to 75 words and need not be structured. At least 3 keywords, preferably using terms from the Medical Subject Headings (MeSH) list of Index Medicus, should be listed horizontally under the abstract for cross-indexing of the article.

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The PCMC Journal

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EDITORIAL STAFF

This is the second issue of The PCMC Journal for 2017, consisting, as before, of the best papers submitted by our fellows and residents in training, as attested by their having presented in the hospital's annual research forum.

The topics in this issue are as varied as anemia, breast milk, asthma, sepsis, and Kawasaki disease, all disorders which are among the most common or most important of the diseases encountered at the PCMC, hence of great interest to all health workers of the largest pediatric specialty hospital in the country.

More importantly, this keeps us on track toward achieving our goal of coming out with at least 2 issues of the journal yearly, to qualify being indexed and searched in local and international databases such as HERDIN of the DOST and the Western Pacific Region Index Medicus (WPRIM) of the WHO.

The editorial staff enjoins everyone, trainees and consultants alike, medical and paramedical personnel, to continue producing high quality and high impact researches which we can showcase to other centers, in keeping with our original mission of being a research oriented health institution.

The Editor

The PCMC Journal

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PREDICTORS OF INTRAVENOUS IMMUNOGLOBULIN RESISTANCE IN KAWASAKI DISEASE IN A TERTIARY CHILDREN'S HOSPITAL

CHRYS KRISTOFFERSON P. AGUILERA, MD AND LEAH PATRICIA ARCEO-PLUCENA, MD

ABSTRACT

BACKGROUND AND OBJECTIVE: Kawasaki Disease (KD) is the leading cause of acquired heart disease in children in developed countries. We aimed to determine the predictors of intravenous immunoglobulin (IVIG) resistance based on clinical manifestations and laboratory parameters.

METHODOLOGY: This was a retrospective cohort study of classic KD patients.

RESULTS: Two hundred and ten patients were included in the study. The mean age was 2.0 $\pm$ 1.8 years old with slight female predominance at 51.4%. Seven (3.3%) cases were found to be IVIG resistant. There was no significant difference in age, clinical manifestations or fever duration. Univariate analysis revealed that IVIG resistant group had a heavier weight with mean of 16.4 kg $\pm$ 12.2 compared to the IVIG responder group 12.2kg $\pm$ 4.8. The IVIG resistant group had a higher white blood cell count of 23.9 $\pm$ 7.8 compared to the responder group of 17.9 $\pm$ 6.5.

CONCLUSION ANDRECOMMENDATIONS: There is an IVIG resistance rate of 3.3% among classic KD patients. A high white blood cell count and weight are probable predictors for IVIG resistant KD. We recommend a larger sample size of resistant cases and a case-control multicenter study.

KEYWORDS: Kawasaki Disease, Intravenous Immunoglobulin, IVIG resistant Kawasaki

INTRODUCTION

Kawasaki Disease (KD), also known as mucocutaneous lymph node syndrome, is the leading cause of acquired heart disease in children in developed countries.[1]It is a febrile illness and a probable infectious cause was postulated, but its etiologic cause has not yet been elucidated. It generates vasculitis, with predilection to the coronary arteries, inducing ectasia and aneurysms. A genetic role has been implicated, and there is a higher propensity for the Asian population.The disease is self-limiting, however the long-term complications of KD involve the coronary arteries, making this illness a source of further research for its prevention and treatment.

Standard treatment for KD is intravenous immunoglobulin (IVIG) and aspirin, ideally started within 10 days from disease onset. Although the exact action of IVIG in KD is still poorly understood, it has been used as the first line treatment regimen. This approach is effective in symptom resolution in up to 90% of cases, bringing down the risk for coronary artery abnormalities to 2-4% compared to 20-25% with aspirin alone.[1] However, according to studies, there is a 10-20% chance that a patient may not respond to IVIG, and may need additional IVIG or some other form of treatment such as methyprednisolone and infliximab.[2,3,5]This subset of individuals is at a greater risk of acquiring coronary artery abnormalities. Therefore, delays in recognition and treatment may be detrimental.

Increased detection of KD has revealed increasing incidence of IVIG resistant cases.[2]A number of recent studies from Asia tried to elicit possible predictors of IVIG resistance by comparing responders to nonresponders.[2,4,5,7,9] They have identified demographic and laboratory parameters, including age, day of

illness, hemoglobin, platelet count, neutrophil count, serum total bilirubin level, serum albumin level, alanine aminotransferase, lactate dehydrogenase, C-reactive protein and erythrocyte sedimentation rate as predictors of IVIG resistance.[3,4]However these studies have conflicting results, hence this local study has been proposed.

We aimed to determine the variables which predict IVIG resistance in patients with Kawasaki disease.Identifying probable IVIG resistant cases will help physicians give a more accurate prognosis and modify treatment plan. Timely and adequate treatment will decrease the risk of coronary artery abnormalities, which may cause significant morbidity and mortality. A number of laboratory tests are initially requested to support the diagnosis; hence this study may help point to the most cost-effective tests. Local policy makers will be guided in allocating and prioritizing funds to the most crucial tests needed to predict IVIG resistant KD.

The incidence of Kawasaki disease in South Korea is 86.4/100,000 population, compared to that of Japan which is 134.2/100,100.[5]Tremoulet, A et al reported increasing IVIG resistant KD in their center since 2006.[3]This is a vast number of children at risk for developing coronary artery problems. Such coronary artery abnormalities may become stenotic, develop thrombi, ischemia and cause sudden death.Kawasaki disease without IVIG treatment results in about 20-25% occurrence of coronary artery aneurysm. Although this is significantly decreased to about less than 5% with treatment, there is significant percentage, 10-20%, who will not respond to initial IVIG. These non responders are at increased risk of coronary artery complications, and thus need additional treatment either with another cycle of IVIG infusion or alternative medications (e.g. methylprednisolone).[6]Fever in Kawasaki disease is correlated to the development of complications. It is postulated to be the effect of ongoing vascular inflammation due to continued release of inflammatory cytokines. Hence, IVIG resistance is based on this clinical finding. Failure to respond is defined as persistent or recrudescent fever equal to or more than 36 hours after the initial IVIG infusion. However, many practitioners re-treat based on fever alone, which may be caused by other factors such as a recent infection.[7] Hence, there is a need for more objective parameters to determine the indication for IVIG re-treatment.

Recent studies have focused on parameters thatpredict IVIG resistance. This would allow proper prognostication, adept anticipation, and may help develop new hypotheses as to pathogenesis and treatment. Higher bilirubin, AST, percent segmenters (PMN) and decreased platelets are independent predictors of persistent or recrudescent fever in KD in Korea.[5] In a retrospective study by Lee, et al among 91 Korean children with KD, it was pointed out that fractional change CRP might be an important value for predicting IVIG resistance.[8] This was supported with a study by Hyun Cho, et al, when they reviewed 234 complete and 77 incomplete Kawasaki cases. Moreover, they added that elevated percent of segmenters as well as NT-proBNP predicted early IVIG resistance.[9]These validated a prospective study done on 129 KD patients in the same population by Kwon KH, et al, which demonstrated that CRP, NT-proBNP, and percent neutrophils were independent parameters of retreatment. There were no differences between the groups in age, gender distribution, and duration of fever prior to IVIG in this study population. Furthermore, this study proposed the following treatment recommendations: 1) Retreat patients who remain febrile despite IVIG therapy and have high values of CRP, NT-proBNP and/or percent neutrophils; 2) When patients become febrile but with normal laboratories, observe for 1-2 days for defervescence; 3) When patient is afebrile and has abnormal laboratories, do not re-treat. However, conducting larger scale prospective studies on these guidelinesare recommended.[7]Meanwhile, Young, JHM et al concluded that a low serum albumin level and a high neutrophil percentage are independent predictors of IVIG resistance in Chinese children.[4]

A scoring system for predicting IVIG resistant KD was previously formulated by

Egami, et alobtained from Japanese children and was applied by TremouletA, et alin a retrospective study conducted in San Diego County from October 1998 to September 2006, to determine its predictive value in an ethnically diverse population. However, the system missed over 60% IVIG resistant cases. The same scoring system was also applied to the Asian population and yielded specificity of 89.3% and sensitivity of 33.3%.[3]Sleeper, et al utilized the risk scoring systems for IVIG resistance developed in Japan (Egami, Kobayashi and Sano) and revealed<45% sensitivity and87% specificity when applied North American children.[10] Genetic variations as to race play a major role in KD that may be affecting disease severity and outcome.[3]

We aimed to determine the predictors of IVIG resistance in Kawasaki Disease in a tertiary children's hospital. Specifically, we wished to determine the predictors of resistance to IVIG of patients diagnosed with KawasakiDisease as to initial clinical manifestations and laboratory parameters

METHODOLOGY

This is a retrospective cohort study of Kawasaki Disease patients admitted at a tertiary children's hospital from 2004-2014.All patients admitted from January 2004 to December 2014 who fulfilled the American Heart Association Diagnostic Guidelines for complete or classic Kawasaki Disease were included in the study. Classic KD patients who were not given IVIG and incomplete or atypical Kawasaki Disease patients were excluded.

Responders were defined as patients with defervescence 48 hours after completion of IVIG infusion. Non responders were those with persistent or recrudescent fever 48 hours after completion of IVIG infusion. Using Epi Info version 7, the minimum sample size requirement was estimated to be at 156 based on the 20% incidence of IVIG resistance as reported by Cha, Yoon et al with 95% confidence level and 5% margin of error.[5]

All medical records of patients with classic KD from 2004 to 2014 were retrieved. A data collection form was used to record all pertinent data. The form included the demographic data, initial clinical manifestations and initial laboratory results of every patient with KD. Demographic data included age, sex and weight. Clinical manifestations such as day of fever, changes in extremities and oral cavity, rash, conjunctivitis, and cervical lymphadenopathy were noted. The initial laboratory results of complete blood count (white blood cell count, neutrophils and platelet count), liver function tests (alanine aminotransferase, aspartate aminotransferase, total bilirubin and albumin), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and presence or absence of 2-dimensional echocardiogram (2D echo) findings were recorded.Data analysis was done using Stata SE version 13. Quantitative variables were summarized as mean and standard deviation, while qualitative variables were tabulated as frequency and percentage. Comparison of characteristics between responders and resistant were done using independent t-test for quantitative variables, and Fisher's exact test for qualitative variables. The study was reviewed and approved by the Ethics committee prior to commencement.

RESULTS

Two hundred and ten patients with classic KD admitted and treated with IVIG from January 2004 to December 2014 were included. Baseline clinical characteristics are shown in Table 1, and lab parameters in Table 2.

Table 1. Baseline Demographic and Clinical Characteristics of Classic Kawasaki Disease Patients Admitted from January 2004 to December 2014

Characteristics (N = 210)	$\overline{x} \pm SD$ or n(%)
Age (years)	2.0 $\pm$ 1.8
Sex: Male Female	 102 (48.6%) 108 (51.4%)
Weight (Kg)	12.3 $\pm$ 5.2
Rash	197 (93.8%)
Cervical lymphadenopathy	179 (85.2%)
Hand manifestations	168 (80%)
Oral manifestations	196 (93.3%)
Conjunctivitis	186 (88.6%)
Fever duration before treatment (days)	8.9 $\pm$ 3.3

Table 2. Baseline Laboratory Parameters of Classic Kawasaki Disease Patients admitted from January 2004 to December 2014

Laboratory Parameters (N = 210)	$\overline{x} \pm SD$ or n(%)
WBC	18.2 $\pm$ 6.6
Neutrophils	0.63 $\pm$ 0.16
Platelet count	504.6 $\pm$ 191.4
Bilirubin	33.8 $\pm$ 46.6
Albumin	32.2 $\pm$ 6.1
ALT	119.2 $\pm$ 79.9
AST	80.8 $\pm$ 46.5
CRP	93.4 $\pm$ 97.8
ESR	91.4 $\pm$ 30.3
2D echo findings: Positive Negative	 90 (55.9%) 71 (44.1%)

The study included 7 (3.3%) IVIG resistant cases.The demographic, clinical and laboratory parameters of IVIG responder and resistant Kawasaki patients were compared (Table 3). There were no significant differences noted in age, clinical manifestations, fever duration and in mostinitial laboratory parameters.There were significant differences identified in terms of weight and initial WBC count. Univariate analysis using two-sample t test revealed that IVIG resistant group had a heavier weight with mean of 16.4 kg $\pm$ 12.2 compared to the IVIG responder group 12.2kg $\pm$ 4.8. Also, the IVIG resistant group revealed to have a higher WBC count of 23.9 $\pm$ 7.8 compared to the responder group of 17.9 $\pm$ 6.5.

Table 3. Comparison of Characteristics between IVIG Responders and Resistant KD

Variables	IVIG Responders (n = 203)	IVIG Resistant (n = 7)	P value
Age (years)	2.0 ± 1.8	2.7 ± 2.8	0.36
Sex: Male Female	 96 (47.3) 107 (52.7)	 6 (85.7) 1 (14.3)	0.059
Weight (Kg)	12.2 ± 4.8	16.4 ± 12.2	0.035
Rash	190 (93.6)	7 (100.0)	1.00
Cervical lymphadenopathy	174 (85.7)	5 (71.4)	0.28
Hand manifestations	163 (80.3)	5 (71.4)	0.63
Oral manifestations	189 (93.1)	7 (100.0)	1.00
Conjunctivitis	180 (88.7)	6 (85.7)	0.58
Fever duration before treatment	8.9 ± 3.1	10.4 ± 6.4	0.24
WBC	17.9 ± 6.5	23.9 ± 7.8	0.02
Neutrophils	0.63 ± 0.15	0.65 ± 0.24	0.82
Platelet count	503.3 ± 185.6	541.6 ± 334.5	0.60
Bilirubin	-	-	-
Albumin	32.4 ± 6.3	31.6 ± 6.5	0.86
ALT	118.4 ± 84.4	125.5 ± 38.9	0.91
AST	-	-	-
CRP	94.7 ± 99.5	62.4 ± 32.2	0.47
ESR	92.2 ± 29.8	70.5 ± 39.6	0.09
2D echo	85 (55.2)	5 (71.4)	0.47

DISCUSSION

In this retrospective cohort study, there was only 3.3% IVIG resistance rate observed within the 10-year period. This figure was low compared to the reports from other countries, which ranged from 7.5% to 38%.[4]We aimed to determine clinical and biochemical predictors to IVIG resistance that may help to prevent fatal complications and to pave way for additional or innovative anti-inflammatory therapies. Several studies have reported distinct predictors of IVIG resistance and many have developed a scoring system. Egami et al analyzed 320 Japanese children and derived a bedside score that designated one point for each of the following: (1) infants less than 6 months old; (2) diagnosed before four days of illness;(3) platelet count ≤300 x 109/L; and (4) CRP ≥80 mg/L, Lastly, the score also designated two points for ALT ≥80 IU/L. Using a cut-off point of three, the prediction score was shown to single out IVIG resistance with 78% sensitivity in this study group.[4]However, in our study, the resistant cases were not comparable based on age (2.7 years ± 2.8), duration of fever (10.4 days ± 6.4), platelet count (541.6 ± 334.5), CRP (62.4 ± 32.2) and ALT (125.5 ± 38.9) levels.

A study of Young et al proposed a high neutrophil percentage as an independent predictor of IVIG resistance. Aninhibited neutrophil apoptosis in the acute phase of Kawasaki results in an increase in number of circulating neutrophils.Consequently, the prolonged neutrophil life span may contribute to the pathogenesis of vasculitis with autotoxic mediators released by neutrophil into thecirculation, thus worsening the inflammation.[4] However, this was not observed in our study with only 0.65 ± 0.24 neutrophil count and is not significant compared to IVIG responders instead our study revealed significant difference between responders (17.9 ± 6.5) and resistant cases (23.9 ± 7.8) with the WBC while Young reported it was not significant.

No study has proposed sex and weight as independent predictors, however our study showed significance between the weight of IVIG responders (12.2 kg ± 4.8) and IVIG non responders (16.4 kg ± 12.2). There was a

predominance of male gender in the IVIG resistant group 85.7% (6 out of the 7 cases); however, it showed no significant difference compared to the responder group.

CONCLUSION

In conclusion, our study reported an IVIG resistance rate of 3.3% among admitted classic KD patients for a period of 10 years. They all fulfilled the diagnosis of classic Kawasaki Disease and received IVIG. Our study showed that high WBC and weightas probable predictors for IVIG resistant Kawasaki disease.

The limitation of this study is the small sample size of IVIG resistant cases. Further analysis to determine other probable predictors was impossible with the 7 resistant cases. A case-control study design could had been done to compare IVIG responders and resistant cases using multivariate analysis; and possibly strengthen high white cell count and weight as probable predictors for IVIG resistance. A territory-wide and multi-centered collaboration is essential to further investigate for possible predictors and help develop a locally applicable scoring system with more significant clinical or biochemical predictors.

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"EFFECT OF PHILHEALTH ENROLMENT IN THE DELAY IN DISCHARGE OF PEDIATRIC PATIENTS WITH ACUTE ILLNESS IN A GOVERNMENT TERTIARY HOSPITAL"

JAN KAMILLE R. CORONEL, MD AND SORAYA ALVARADO, MD

ABSTRACT

Background: Philhealth, the national health insurance program, aims to reduce out-of-pocket expenditure to eradicate blockage to healthcare access by subsidizing expenses during admission. A possible determinant of its success is the timeliness of management and prevention of discharge delay.

Objective: To determine the association of Philhealth enrollment with the timeliness of patient discharge in a government tertiary hospital.

Methods: A retrospective cohort study involving 98 patients (49 Philhealth members and 49 non-Philhealth members) was done. Data including age, sex, admitting diagnosis, social service classification, and type of Philhealth membership were collated from the admitting section. Reasons for delay in discharge and number of days delayed was obtained from discharge clearance list of the social service unit. Association between PhilHealth coverage and hospital delay was analyzed using logistic regression analysis.

Results: Discharge delay is 3 times more likely to occur among non-Philhealth members as compared to the Philhealth group. Other variables were not significantly associated with discharge delay.

Conclusions: Discharge delay is significantly associated with non-membership to Philhealth.Further investigation through focus group discussions with patients' families may be done to uncover other possible causes for discharge delay.

INTRODUCTION

Hospital length of stay has been used as an indicator of efficiency of hospital resource utilization [4, 14]. Although it is largely affected by disease severity and several other non-patient factors, it reflectshow proficiently hospital processes are carried out from the time of admission to discharge.Any delay in such processes ultimately leads to prolonged hospital stay.

Financial barrier is only one of the factors identified to contribute to extension of patients' hospital stay. In the Philippine setting, it may be deemed as the major considerationfor delays in discharge. The "fear of incurring a significant financial burden for health care or falling into a 'medical poverty trap' can cause ill individuals to either delay or refrain from seeking essential medical services " [11]. The same rationale may explain to some extent, the delays encountered in performing necessary procedures and administering essential medications in the in-patient setting. Patients who delay consult for any condition are more likely to present with more severe manifestations and complications as compared to those who seek healthcare early into the disease.

The National Health Insurance Program embodied by the Philippine Health Insurance Corporation (Philhealth), is the government's effort to have a universal healthcare coverage for all Filipinos in order to eliminate finances as a stumbling block for health utlization. Since Philhealthcan cover the expenses for laboratory work-up and medications, utilization of its benefits reduces the financial burden of patients. In so doing, it also speeds up the diagnostic and therapeutic management of admitted patients leading to faster recovery and timely discharge. However, since majority of the health

expenditure still comes from out-of-pocket resources, the effect of having Philhealth coverage on the health outcomes is yet to be established. Therefore, this study aims to determine the association of Philhealth benefit availmentwith the timeliness of discharge of patients admitted for acute conditions.

The results of this study can help determine whether enrolment of patients to Philhealth contribute to maximization of the hospitals resources, particularly bed turnover. Also, this study may be able to elucidate other bottlenecks in the discharge planning process,

We aimed to determine the effect of Philhealth enrollment in the timeliness of discharge of patients with acute illness in our institution. Our specific objectives were:

1) To present the demographic profile of patients who have utilized Philhealth benefits during their admission
2) To demonstrate how many patients stayed more than 1 day after being given a send home order
3) To determine how many patients who have Philhealth accounts overstayed
4) To identify other reasons for delay in discharge of patients with acute illness
5) To illustrate the current prevalence of Philhealth coverage in the Philippines and compare it to that in our institution

METHODOLOGY

A retrospective cohort study was done to determine which among those children admitted for acute illness were enrolled and availed of Philhealth benefits and were discharged timely.The sample population was randomly selected from the database of admitted patients from January 2016 to June 2016. All private patients were excluded. Those patients who have chronic illness as co-morbidities (i.e. all forms of malignancy, cardiac conditions, neurologic conditions) as well as neonates were also excluded. However, those patients admitted for acute exacerbations of a chronic illness are included (i.e. bronchial asthma).

Using NCSS-PASS 2013, the minimum sample size requirement is at least 98 based on the percentage of uninsured patients with hospital delay = 32.2% [15] and odds ratio= 4.51 [12] with level of significance= 5% and power= 80%.

The service patients were classified as to having Philhealth membership or not. From the list of patients with Philhealth, 49 were randomly selected. The same number was randomly selected from the list of non-Philhealth patients. All patients who are enrolled at the beginning of the study, except those who expired, were analyzed at the end of the study regardless of outcome (i.e. acute illness which complicated during admission).

The demographic profile (age, sex), the acute condition for which the patient was admitted, the social service classification and the Philhealth membership type were taken from the patient database from the Admitting section. The discharge delay form was duly accomplished based on such data.

A list of discharge clearances during the time of the study was obtained from the Social Services Unit. From such list, each patient included in the study was cross-referenced to determine delay in discharge. If the patient was not in the list, the patient was considered able to go home on the day the discharge order was made. On the other hand, if the patient's name is on the discharge clearance list, the number of discharge clearances was noted. This was noted in the discharge delay form. The number of discharge clearances was equivalent to the number of days that the patient's discharge was delayed from the time the order for discharge was made. The reason for the delay is also noted in the discharge clearance list provided by the Social Service Unit. Moreover, fund sources (PCSO, DSWD, etc.) for settling the hospital bill of patients who were unable to go home at the discharge point were recorded in the said document.

The collected data were tabulated in Microsoft Excel. The characteristics of the sample population like age, sex, social service

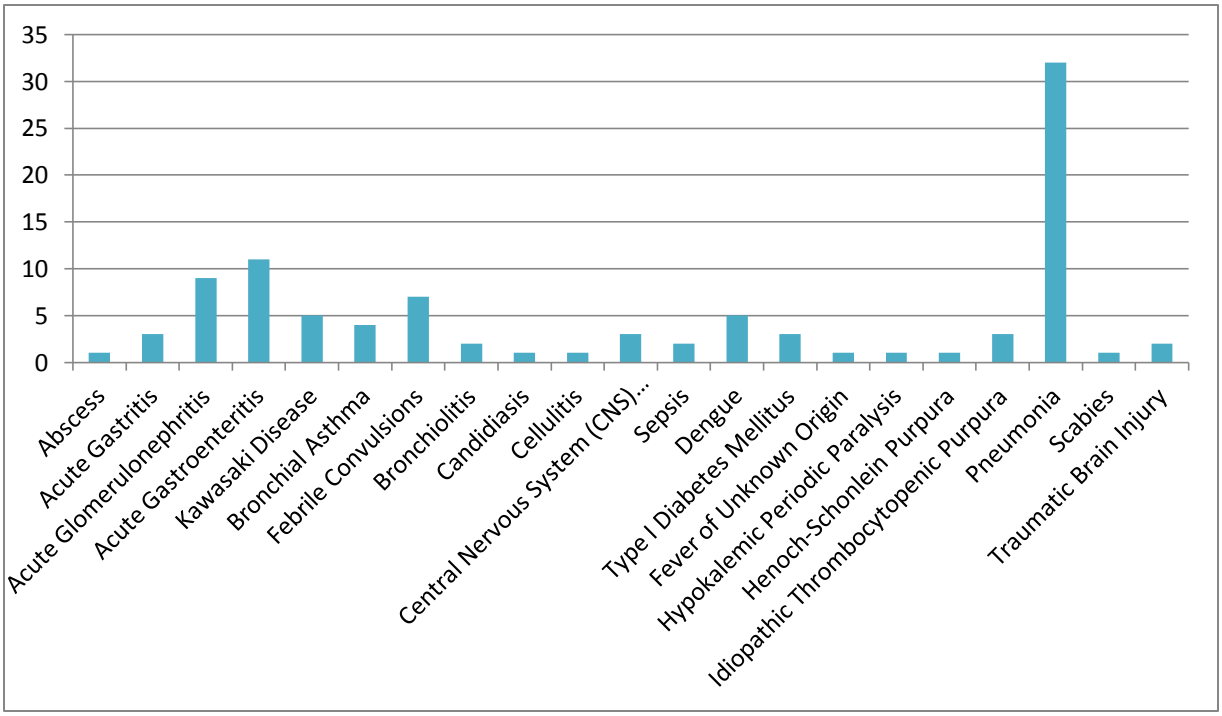
classification (C1, C2 and C3), diagnosis and type of Philhealth membership (for the Philhealth group) were analyzed against the timeliness of discharge. The association between variables was determined using the chi-square test.

Data analysis was performed in Stata SE version 13. Quantitative variables were summarized as mean and standard deviation, while qualitative variables were tabulated as frequency and percentage. Comparison of baseline characteristics was analyzed using independent t-test for quantitative variables, and Fisher's exact test for qualitative variables. Association between PhilHealth coverage and hospital delay was analyzed using logistic regression analysis. The level of significance was set at 5%.

RESULTS

There are 3,302 patients who were admitted at the service wards from January – June 2016. Of these patients, 2,845 or 86% were enrolled to Philhealth, while the remaining 457 or 14% were non-Philhealth members. There was a total of 98 patients included in the study, 49 are enrolled in Philhealth while the remaining are non-Philhealth members. Approximately 45% of the patients belong to the 1 – 4 years old age group. Majority of the patients included in the study are females, comprising 62%. The most common conditions of patients included in the study are pneumonia (32%), acute gastroenteritis (11%) and acute glomerulonephritis (9%) (Figure 1). This finding is reflective of the hospital-wide data, which also shows these conditions to be the most prevalent diagnoses of admitted patients.

Figure 1. Acute Conditions of Patients in the Study.



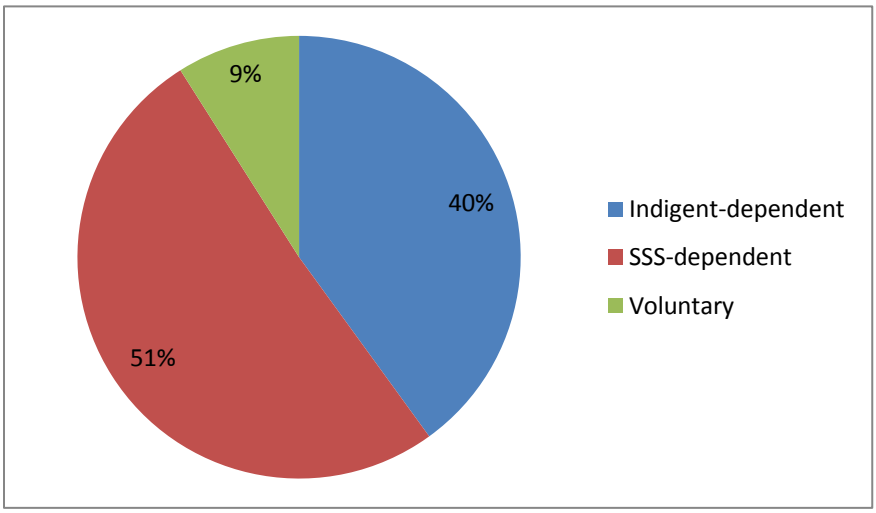
Based on the social service classification, most of the patients (75%) are classified as C3, indicating that a considerable number of patients belong to the lower income bracket (Table 1).

Table 1. Demographic Profile of Subjects with Philhealth and Non-Philhealth

	With Philhealth (n = 49)	Without Philhealth (n = 49)	p-value
Age			
1 mo – 11 mos	13 (26.53%)	15 (30.61%)	0.332
1 – 4 years old	26 (53.06%)	18 (36.73%)	
5 – 9 years old	4 (8.16%)	10 (20.41%)	
10 – 13 years old	3 (6.12%)	4 (8.16%)	
14 – 18 years old	3 (6.12%)	2 (4.08%)	
Sex			
Male	19 38.78%)	18 (36.73%)	1.00
Female	30 (61.22%)	31 (63.27%)	
Social Service Classification			
C1	0 (0%)	1 (2.04%)	0.152
C2	15 (30.61%)	8 (16.33%)	
C3	34 (69.39%)	40 (81.63%)	

However, it is of note that there are more patients in the C3 group who do not have Philhealth. This contrasts with the other social service classification group (C2), which shows that there are more patients who are enrolled to Philhealth than those who are non-members. Further classification of the Philhealth group based on the membership type showed that the most common membership type is the SSS-dependent type comprising 51% of the said group (Figure 2), while indigent-dependent members and those who have voluntary contributions consist 40% and 9%, respectively.

Figure 2. Distribution of Patients with Philhealth based on MembershipType



The characteristics of enrolled patients such as age, sex and acute illness for which they are admitted were analyzed against discharge delay to identify other contributory factors, but none of them demonstrated significant association. However, analyzing Philhealth enrolment against delay in discharge showed that non-membership to Philhealth is a risk factor for delay in discharge, in that those without Philhealth are 3 - 4 times more likely to experience delay in discharge compared to those with Philhealth (Table 2). On the average, those who experience delay in discharge stay in the hospital for 1-2 days after being given the discharge order.

Table 2. Discharge Delay among Study Patients.

	With delay (n = 16)	Without Delay (n = 82)	P-value
Age			
1 mo – 11 mos	3 (18.75%)	25 (30.5%)	
1 – 4 years old	9 (56.3%)	35 (42.7%)	
5 – 9 years old	0 (0%)	14 (17.1%)	0.08
10 – 13 years old	3 (18.75%)	4 (4.88%)	
14 – 18 years old	1 (6.25%)	4 (4.88%)	
Sex			
Male	7 (43.7%)	30 (36.6%)	0.58
Female	9 (56.3%)	52 (63.4%)	
Acute Illness			
Abscess	0 (0%)	1 (1.22%)	
Acute Gastritis	0 (0%)	3 (3.66%)	
Acute Glomerulonephritis	0 (0%)	9 (10.98%)	
Acute Gastroenteritis	2 (12.5%)	9 (10.98%)	
Kawasaki Disease	0 (0%)	5 (6.10%)	
Bronchial Asthma	0 (0%)	4 (4.88%)	
Febrile Convulsions	3 (18.75%)	4 (4.88%)	
Bronchiolitis	0 (0%)	2 (2.44%)	
Candidiasis	0 (0%)	1 (1.22%)	
Cellulitis	0 (0%)	1 (1.22%)	
Central Nervous System (CNS) Infection	0 (0%)	3 (3.66%)	
Sepsis	1 (6.25%)	1 (1.22%)	0.59
Dengue	1 (6.25%)	4 (4.88%)	
Type I Diabetes Mellitus	1 (6.25%)	2 (2.44%)	
Fever of Unknown Origin	0 (0%)	1 (1.22%)	
Hypokalemic Periodic Paralysis	1 (6.25%)	0 (0%)	
Henoch-Schonlein Purpura			
Idiopathic Thrombocytopenic Purpura	0 (0%)	1 (1.22%)	
Pneumonia	0 (0%)	3 (3.66%)	
Scabies			
Traumatic Brain Injury			
	7 (43.75%)	25 (30.49%)	
	0 (0%)	1 (1.22%)	
	0 (0%)	2 (2.44%)	
Philhealth Membership			
With Philhealth	4 (25%)	45 (54.9%)	0.03
Without Philhealth	12 (75%)	37 (45.1%)	

All patients who experienced delay in discharge identified financial constraint as the reason for delay. The patients' discharges were facilitated by securing funds out-of-pocket and from government agencies such as the Philippine Charity Sweepstakes Office (PCSO) and Department of Social Welfare and Development (DSWD). For the Philhealth group, all patients who experienced delay utilized out-of-pocket resources to fund their hospital bill. Similar findings were seen in the non-Philhealth group - 83% of those who had delay in discharge used out-of-pocket resources to pay for their hospital bill. Other sources of funding identified in this study were PCSO and DSWD (Table 3).

Table 3. Non-Philhealth Sources of Funding Among Patients with Delay

Source of Funding	With Philhealth (n = 4)	Without Philhealth (n = 12)
Out-of-pocket	4 (100%)	10 (83%)
PCSO	0 (0%)	1 (8.3%)
DSWD	0 (0%)	1 (8.3%)

DISCUSSION

Delay in discharge contributes to the cascade of administrative concerns of hospitals, especially in the setting of resource scarcity. Discharge delays have been shown to increase the cost of treatment and even aggravate the patients' outcome (15). In the study by Gaughan et al., the cost of delay in discharge is identified as both financial and clinical. The hospital tends to unnecessarily spend resources for a patient who is deemed clinically fit for discharge by allotting a bed which could have been occupied by another patient who has a greater need for medical service. Its clinical implication is also note-worthy as patients who experience delay in discharge may acquire hospital-associated infections, leading to a prolonged hospital stay than expected.

Studies have been made to elucidate the factorscontributing to discharge delaysto adequately address them. Most researchers conclude that majority of discharges are delayed due to medical causes (awaiting laboratory results, managing complications, awaiting consultants' opinion). However, little is known regarding non-medical causes of discharge delay, moreso in the Philippine setting.

Financial capacity to access healthcare has been identified in several studies to cause delay in seeking treatment (11, 16). Social health insurance health programs have been developed and encouraged to alleviate the financial burden that has been an impediment to healthcare access, especially by the poor. In the study by Fowler et al., being uninsured was identified as a risk factor for discharge delay presumably because these patients arrive in the hospital in a more critical state, needing more intensive management due to delay in initial medical consult. However, the direct effect of health insurance status and its utilization to health outcomes has not been thoroughly considered, especially in the local setting.

An indicator of a successful national health insurance program is a decreased percentage of out-of-pocket expenditure (16). Therefore, reinforcing the national health insurance program through Philhealth benefit utilization is one strategy to alleviate the financial concerns of the patients and expedite the diagnosis, management and ultimately the discharge of these patients.

During the study period, 86% of admitted patients are Philhealth members while the remaining are non-Philhealth members. This is comparable to the statistics released by Philhealth where approximately 9 out 10 Filipinos are covered by Philheatlh.By increasing their coverage,Philhealth comes close to achieving its aimto for universal healthcare by easing the financial burden imposed by healthcare costs. Aside from voluntary and compulsory contributions, the government also subsidizes funding of the said program especially for those belonging to the lowest economic quintiles through the Indigent Program (IP). Despite these efforts, there are still many Filipinos who remain non-members or become members but do not utilize the benefits of Philhealth to avail healthcare services. In the 2015 Philhealth statistics, only 12% of total eligible Philhealth members utilize its benefits. In this research, however, it is assumed that Philhealth membership is equivalent to utilization of its benefits during the admission. Philhealth members who did not avail of Philhealth benefits were not identified in this paper.

The reasons for non-enrolment to Philhealth are beyond the scope of this study.In a study by Faraon et al., sex, income and type of Philhealth membership were identified variables to be significantly associated with underutilization of Philhealth. The outcome of this study had a similar result. Based on the patients' social service classification, vis-à-vis their income status, most of non-Philhealth members belong to C3 or the lowest income group. Such finding may be supposed to be also linked to the educational attainment and subsequently, the knowledge and awareness of the families of these patients regarding the Philhealth benefits and how to avail them. Other reasons for underutilization of Philhealth are as follows:

- Lack of knowledge on benefits
- Lack of knowledge on filing claims
- Lack of cooperation from doctors in getting required documents
- Cumbersome or unmanageable process
- High transaction costs compared to benefits
- Ineligible claim

Although the association between the social service classification and Philhealth membership was not found to be significant in this study, it may be prudent to consider such pattern in succeeding investigations.

In this study, most patients are subscribed to Philhealth by compulsory contributions through the employer, also known as SSS-dependent members, comprising 51% of the sample population. However, Latest Philhealth data demonstrates that majority of covered members are indigents, comprising 49%. This discrepancy may be explained by the fact that some patients are initially admitted as non-Philhealth members and are only enrolled to Philhealth during admission, through the assistance of the social service unit. These patients are usually indigents who are granted a no balance billing status (NBB) during their stay. Such enrolment mechanism is noted in the records of social service and the admitting section. However, none of the patients who are included in this study experienced said mechanism of enrolment to Philhealth. All non-Philhealth members remained unenrolled to Philhealth until discharge.

Those patients who experienced delay in discharge reported financial setback as the reason for the delay. Data from the social service unit showed that majority of these patients still resorted to out-of-pocket resources to settle their hospital bill, regardless of their Philhealth membership. However, there are 2 patients who sought financial assistance from PCSO and DSWD. This agrees with the finding that household budget for healthcare is still mostly out-of-pocket (16). This reliance on out-of-pocket sources for healthcare has detrimental effects. In the study by Ulep and Dela Cruz, high out-of-pocket expenditure for health needs can:

- redistribute income "in the wrong direction" (i.e., from chronically ill to healthy individuals and, typically, from the relatively poor to the relatively affluent groups [Plumper and Neumayer 2012])
- lead people to make tough choices concerning their health such as not complying with prescribed drug use due to high costs, forgoing necessities, or borrowing money to pay for prescriptions;
- affect women and minorities who may forgo critical prevention screenings and skimp on medications due to high costs; and
- increase the financial burden on those with valid insurance (Aji et al. 2013)

CONCLUSION

In summary, this study demonstrated that Philhealth enrolment and its subsequent utilization is associated with timeliness of discharge of pediatric patients admitted for acute illnesses. Non-membership to the national health insurance program increases the risk of discharge delay three-fold. Other patient factors such as age, sex, social service classification and the acute illness for which the patients are admitted did not show any significant association with discharge delay.

Further investigations can be made to elucidate on other factors affecting discharge delay. This study is limited to review of patients' records from the admitting section and social service unit. It is recommended that a prospective study be done to identify the reasons for delay in discharge as will be reported by patients' families, which may not be solely financial in nature. Focus group discussions among the patients' parents may be done to fully understand the reasons for delay. Such method of study can also give light to the reasons for non-Philhealth membership. Recognizing other variable affecting discharge delay may be helpful for the hospital administration and even the national government, to draft policies and systems to improve the health insurance program so as to achieve universal healthcare for all.

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POST-TRANSFUSION HEMOGLOBIN ESTIMATION IN FILIPINO CHILDREN AND ADOLESCENTS USING AN ALTERNATIVE RED CELL VOLUME TRANSFUSION FORMULA

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ABSTRACT

BACKGROUND AND OBJECTIVE: Conventional red cell transfusion formulae used in clinical practice has shown underestimation of the actual post-transfusion hemoglobin level. To address this problem, we aimed to determine if there is an agreement between computed and actual post-transfusion hemoglobin levels using an alternative red cell transfusion formula.

METHODOLOGY: This was a prospective, cross-sectional study. Using Morris' formula, the red cell volume requirements of the participants were computed and post-transfusion hemoglobin levels were obtained for comparison.

RESULT: Majority of the 116participants belongs to age between 2 to 5 years (39.5%) and female (54.3%). Most common indication was hemoglobin level < 7 g/dL with manifestations of anemia (56%). The computed and actual post-transfusion hemoglobin were in agreement. The increase in hemoglobin had direct relationship to the volume of blood transfused and inverse relationship to the age and weight of the patients.

CONCLUSION: Using Morris' formula, the computed and actual post-transfusion hemoglobin values were in agreement. The volume of transfused red cells, age, and weight are predictors of the increase in post-transfusion hemoglobin. This formula can be adopted for Filipino pediatric patients and can obviate the need for hemoglobin determination after transfusion.

KEYWORDS: post-transfusion hemoglobin, red cell volume transfusion formula, blood transfusion formula, pediatrics, packed red blood cells

INTRODUCTION

Anemia may occur because of blood loss, hemolysis, or impaired production of red blood cells (RBC).Blood transfusion therapy is vital to supportive management of anemia. In the Philippines where there is high healthcare cost and limited medical resources, it is essential to evaluate existing clinical practice and determine guidelines to optimize the use of blood products.

Once a physician decides to transfuse packed RBC, a reliable formula is vital to compute for the volume of blood needed. Pediatric transfusion formulae take account of the child's weight, estimated circulating blood volume, and the hemoglobin (Hgb) concentration or hematocrit (Hct) of the blood to be transfused [1-5]. It has been shown that existing formulae used in clinical practice result in a lower than expected actual post-transfusion Hgb level [6]. Hence, the patient may need another transfusion resulting in exposure to further transfusion risks and additional costs. Moreover, since packed RBC transfusion in children requires only a fraction of a unit of blood and the remainder of the unit discarded, it is prudent to maximize the use of every blood units for resource-limited settings. Therefore, aside from the medical aspect of care, the caring physician should consider the impact of transfusion on the financial capacity of the

patient's family and the utilization of limited blood products like packed RBC. Thus, the adoption of an alternative red cell transfusion formula that is valid to all pediatric age and diagnostic groups that will result in a close estimation of the actual post-transfusion Hgb level may provide substantial health and economic benefits in the local setting.

To date, there is no standard formula for calculation of RBC transfusion volumes in pediatrics. In our institution, the two commonly used formulae are: volume of packed RBC = [desired Hct (%) – actual Hct (%)] x weight (kg) x 1; and volume of packed RBC = [desired Hgb (g/dL) – actual Hgb (g/dL)] x weight (kg) x 3. Our experience with the above formulae showed that the actual post-transfusion Hgb values usually fall below the computed Hgb values. In 2014, our records showed that 95% of patients who were transfused with packed RBC had actual post-transfusion Hgb levels that were>1g/dL lower than the computed Hgb level. This means that the desired Hgb level is not attained.

Hence, to avoid the clinical consequences of having suboptimal Hgb level, the adoption of an alternative blood transfusion formula that will provide a higher level of actual post-transfusion Hgb than the conventional formulae are essential. The formula must be valid for all pediatric age groups and should give close estimation of the actual post-transfusion Hgb value.

Calculation of RBC transfusion volumes varies among pediatricians and various institutions. Many pediatric unit protocols use a calculation of: weight of the patient (kg) x difference in Hgb(g/dL) x a transfusion factor [8]. Transfusion factors of 3 and 4 is recommended by The British Committee for Standards in Haematology guidelines [9], and by McIntosh et al.[10], respectively. As stated above, the formula used in our institution showed that the actual post-transfusion Hgb values were> 1g/dL lower than the computed Hgb level. Similarly, a study in 2005showedthat our conventional formulae performed poorly in achieving the desired post-transfusion Hgb level among pediatric patients in the United Kingdom (U.K.) [6]. Locally, no published studies exist that propose blood transfusion formula for children and adolescents.

The advantage of using the formula of Morris et al. [6] over the other red cell volume transfusion formulae like that proposed by Davies et al. [8]is the ability to compute the volume of red cell transfusion even without the Hct level of the packed RBC unit. Since the Hct level of the packed RBC unit is not readily available in actual clinical practice, it is more convenient to use the formula of Morris et al. in our setting.

We aimed to determine if there is an agreement between computed and actual post-transfusion Hgb levels using an alternative red cell volume transfusion formula in calculating for red cell transfusion requirement among Filipino children and adolescents who require RBC transfusion. We also aimed to establish the relationship between increase in Hgb count to the volume of RBC transfused, in terms of age, sex, and weight of the patients since it has not been clearly extrapolated among Filipino children.

METHODOLOGY

This was a prospective, cross-sectional study conducted from March 2015 to September 2015 wherein approximately 15-20 patients are transfused with packed RBC per day with an average of 450 packed RBC transfusions per month.

Patients more than 28 days old and less than 19 years old who required transfusion with packed RBC during admission or outpatient visit, and whose indication for transfusion was included in the institution's guideline. The following were excluded from the study: patients with (1) active bleeding; (2) autoimmune and alloimmune antibodies;(3) hypersplenism (defined as abnormally large spleen causing premature destruction of RBC and platelets); (4) plasma leakage; (5) disseminated intravascular coagulation;(6) acute leukemia with myelo suppression as a result of the disease and / or treatment;(7) severe anemia of $\leq$ 5 g/dL; (8) transfusion requirement of < 5 cc/kg/hour or > 10 cc/kg/hour;(9) prior

transfusion of other blood products (e.g. platelet concentrates, fresh frozen plasma and cryoprecipitate), administration of colloid solutions (e.g. albumin) and plasma exchange that was administered 6 hours before or after packed RBC transfusion;(10) transfusion reaction to packed RBC; and (11) surgical procedure of any forms in the preceding 72 hours of packed RBC transfusion.

A minimum of 86 patients were required for this study based on an 80% power to detect a standardized mean difference of 0.4. Descriptive analysis was used to summarize the clinical profile of the patients who received packed RBC transfusion. To determine agreement between computed and actual post-transfusion Hgb levels, a Bland-Altman analysis was conducted. Multiple logistic regression was utilized to determine factors predictive of the increase in Hgb after transfusion. STATA 12 was used in data processing and analysis.

After eligible participants were identified and consents obtained, the RBC transfusion formula of Morris et al. was used to compute for the RBC transfusion requirement. After transfusion, the remaining RBC volume in the intravenous tubing was pushed with isotonic saline to deliver the complete volume of packed RBC to the recipient. Post-transfusion blood extraction was done via venipuncture after 6 hours. The Hgb level was determined using Unicel DHX 800 Beckman Coulter and Coulter HMX Hematology Analyzer at the hospital laboratory. Pertinent information was recorded using the Data Collection Sheet. Only one transfusion for each patient, even for those who had previous and subsequent transfusions, was recorded in the study.

The primary outcome is the statistical agreement between the computed and actual post-transfusion Hgb levels after using the RBC transfusion formula of Morris et al. Secondary outcome measure is the assessment of the relationship between the increases in Hgb count to the volume of packed RBC transfused with consideration of predictive factors such as age, sex, and weight of the patient.

This study was conducted in accordance to the ethical principles based on the Declaration of Helsinki and the National Guidelines for Biomedical Research of the National Ethics Committee of the Philippines and approved by the Institutional Review Board – Ethics Committee. All observations were preceded by a written documentation of informed consent / assent. Participation in the study was purely voluntary and without financial compensation. The interventions and data were recorded only in writing, and were not recorded via video or audio. An alphanumeric code was assigned for each subject to maintain confidentiality of patient information and results.

RESULTS

A total of 116 patients referred for packed RBC transfusion were included in the study [Table 1]with a median age of 4 years (5 months to 18 years old) and majority are females (54.3%).By etiology[Table 2], anemia secondary to a chronic illness / infection accounts for half the cases with the following breakdown: anemia of chronic illness (47%), iron deficiency anemia (36%), anemia secondary to infection (10%), and anemia due to multifactorial etiology (7%). The rest belongs to anemia due to a primary hematologic disease (49.1%) that was composedof: acute lymphoblastic leukemia (65%), acute myeloid leukemia (21%), and thalassemia (14%). There was a single case (0.9%) of 5-month old male who presented with Hgb level of 8 g/dL and had packed RBC transfusion prior to excision of a choledochal cyst. The most common indication for transfusion was Hgb < 7 g/dL innormovolemic patients with clinical manifestations of anemia(56.9%). The second most common indication (40.5%) were cases of patients who had Hgb level of >7 g/dL with clinical manifestations of anemia; or asymptomatic patients with concomitant illness / condition (e.g. leukemia)that warranted supportive transfusion. The average pre-transfusion Hgb was 7.025 (± 0.93) g/dL, with an overall mean packed RBC volume of 274.5 (± 183.95) mL transfused and a mean actual post-transfusion Hgb of 10.75 (± 1.4) g/dL.

Table 1. General characteristics of study participants (n = 116)

	Frequency (%); Mean ± SD
Age	
Below 2 years old	22 (19.0)
Two to below 5 years old	46 (39.7)
5 to 12 years old	35 (30.2)
Above 12 years old	13 (11.2)
Gender	
Male	53 (45.7)
Female	63 (54.3)
Weight	
≤ 15 Kg	58 (50)
>15 kg	58 (50)
Diagnostic category	
Primary hematologic disorder	57 (49.1)
Anemia secondary to a chronic illness / infection	58 (50)
Others	1 (0.9)
Indication for packed RBC transfusion	
Hemoglobin level less than 7 g/dL in normovolemic patients with clinical manifestations of anemia	65 (56.0)
Hypertransfusion for chronic hemolytic anemias (e.g. thalassemia)	2 (1.7)
Candidates for major surgery and hematocrit less than 30%	1 (0.9)
Hypovolemia from acute blood loss with signs of shock or anticipated blood loss of >10%	0
Hemoglobin less than 13 mg/dL / hematocrit less than 40% in patients with severe pulmonary disease, with assisted ventilation, cyanotic heart disease	0
Standby for operation use	0
Others	47 (40.5)
Transfusion variables	
Pre-transfusion hemoglobin (g/dL)	7.025 ± 0.93
Actual post-transfusion hemoglobin (g/dL)	10.75 ± 1.4
Volume of transfused packed RBC (mL)	274.5 ± 183.95

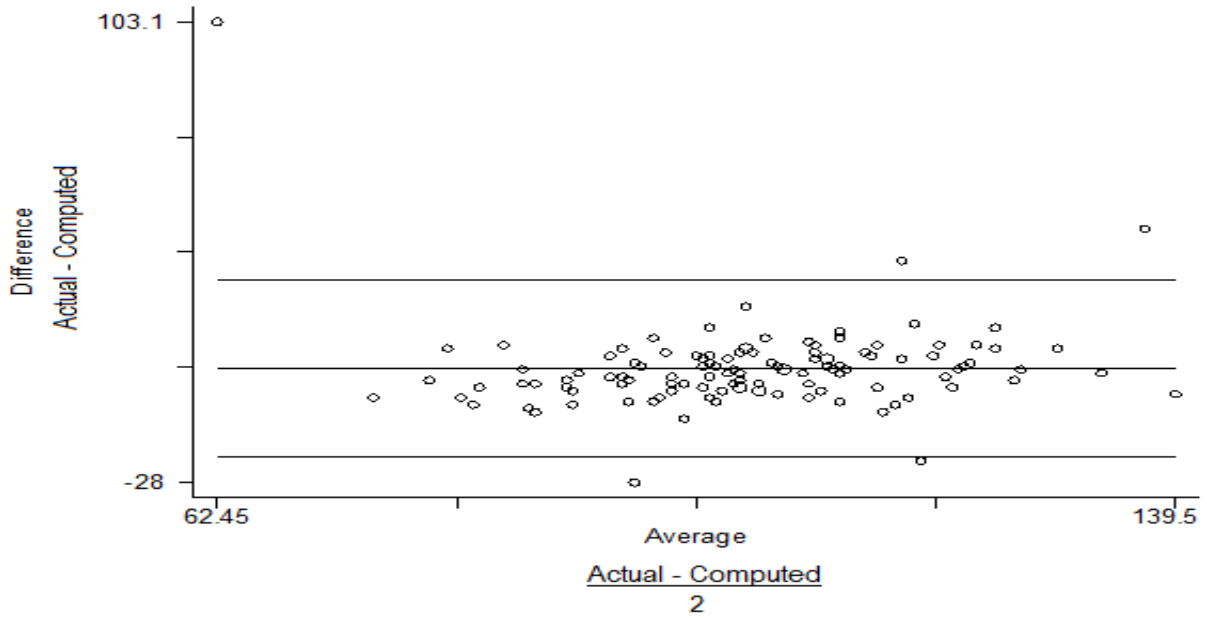
Table 2. Breakdown of cases in terms of diagnostic category (n = 116)

Diagnostic category	Frequency (%)
Anemia secondary to a chronic illness / infection	58 (50)
Anemia of chronic illness	27 (47)
Iron deficiency anemia	21 (36)
Anemia secondary to infection	6 (10)
Anemia due to multifactorial etiology	4 (7)
Primary hematologic disease	57 (49.1)
Acute lymphoblastic leukemia	37 (65)
Thalassemia	12 (21)
Acute myeloid leukemia	8 (14)
Others	1 (0.9)
Choledochal cyst *	1 (100)

* *transfused prior to excision*

Our results showed that the computed and actual post-transfusion Hgb were in agreement, with values falling within a 95% confidence interval (CI -20.8 to +29.8). The mean difference between computed and actual Hgb was at 4.441 g/dL (95% CI 2.12 to 6.76). These parameters of agreement are applicable for actual post-transfusion Hgb values from 6.245 g/dL to 13.95 g/dL. Furthermore, there was no significant difference between computed and actual post-transfusion Hgb values. The figure shows the Bland-Altman plot illustrating the agreement between the computed and actual Hgb values.

Figure 1. Bland-Altman plot depicting agreement between computed and actual post-transfusion Hgb using the transfusion formula provided by Morris et al.



For every unit increase in transfused packed RBC, Hgb increased by approximately 0.085 g/dL provided that other factors were constant. Age and weight on the other hand showed an inverse relationship, wherein for every unit increase in age (years) and weight (kg), the difference in pre- and post- transfusion Hgb decreased by 1.04 and 0.778 g/dL, respectively. Multiple logistic regression analysis conducted revealed the following factors to be significant in the model: volume of transfused RBC, age, and weight. The model had an R-square of 52.81%, which means that these factors explain 52.81% of the variation of the difference between pre-transfusion and actual post-transfusion Hgb.

DISCUSSION

Our study showed the agreement between the computed and actual post-transfusion Hgb values using the formula of Morris et al. The noted predictors of increase in the Hgb level include volume of transfused packed RBC, age, and weight of the recipient.

The most common indication of packed RBC transfusion for these patients was a hemoglobin level less than 7 g/dL in normovolemic patients with clinical manifestations of anemia. Although no standard optimal Hgb threshold for RBC transfusion is established in pediatrics, most of the practicing physicians in our institution follow the guideline set by the BTC. In 2007, a trial was conducted among stable, critically ill children to find out the optimal Hgb threshold for RBC transfusions. Their conclusion was that Hgb threshold of 7 g/dL for RBC transfusion could decrease the requirement for transfusion without increasing adverse outcomes [11].

The formula proposed by Morris et al. was shown to be valid among all pediatric age groups in U.K. [6]. Locally, we wanted to test whether this formula was applicable to the Filipino child's hematologic profile. To further elucidate the hematologic variation between different races, Dallman et al. studied the hemoglobin concentration among children of black, white and Oriental ancestry [12]. They found that the median Hgb concentration values were consistently lower in black children. Additionally, the median values are almost identical among whites and Orientals. Thus, the formula of Morris et al. appears reliable and applicable to be used among Filipino children provided that they have the same clinical conditions as those included in this study.

Clinically, this agreement between the computed and actual post-transfusion Hgb values may imply that a post-transfusion CBC may be obviated. For some institution such as ours, wherein most physicians request post-transfusion CBC, the economic implication of this finding is remarkable. The cost of additional laboratory like CBC adds burden to financially-challenged patients. Another advantage of obviating post-transfusion CBC is minimizing the exposure of patients to painful blood extraction procedure. Several studies have stated that many hospitalized children are experiencing acute procedural pain related to procedures that may potentially cause immediate and long-term consequences [13-14]. Furthermore, our results revealed that there was insufficient evidence to demonstrate a significant difference between computed and actual post-transfusion Hgb levels, hence the probability of under and over transfusion becomes very minimal.

Our results showed that the increase in Hgb had a direct relationship with the volume of packed RBC, and an inverse relationship to age and weight. This means that an increase in Hgb is more attainable with a higher volume of transfused packed RBC, younger age of the patient, and lower weight of the recipient. In contrast, the study of Davies et al. found no association between the transfusion factor and the other external variables such as weight, starting Hgb level, and length of transfusion [8]. However, similar to their study, we also found no relationship between the increase in Hgb and the gender of the patient.

The technical aspect of packed RBC preparation and transfusion is also vital to achieve the optimum rise in Hgb. RBC viability drops progressively once taken out of the body leading to a decline in survival [15]. The post-transfusion RBC survival index fall to<70% if transfusion is done longer than the shelf life of the blood unit[16]. In our study, all packed RBC units were transfused within its shelf life with Hct levels ranging from 0.65 to 0.75. Another important aspect is the transfusion of the exact volume of blood. This was carried out in the study by pushing intravenously the remaining RBC in the tubing to the recipient using isotonic saline solution.

The scope of the limits of agreement that we demonstrated is appropriate only to a certain range of Hgb level (6.245 g/dL to 13.95 g/dL)that was included in this study. We cannot conclude for Hgb values outside of this range, i.e. those who are severely anemic and polycythemic patients.

CONCLUSION / RECOMMENDATION

Using the red cell transfusion formula of Morris et al., the computed and actual post-transfusion Hgb values were in agreement. The volume of transfused packed RBC, age, and weight were predictors of the increase in Hgb after transfusion. The use of the above formula in calculating the red cell transfusion volume provided a more accurate estimation of actual post-transfusion Hgb level. It is applicable among Filipino children and adolescents. Therefore, the practice of performing post-transfusion CBC to check the Hgb level may be obviated.

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TIDAL BREATHING ANALYSIS AS A TOOL FOR ASTHMA DIAGNOSIS IN CHILDREN AGED SIX MONTHS TO FIVE YEARS

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ABSTRACT

BACKGROUND: The diagnosis of asthma is difficult to establish using spirometry in children below 5 years old. Tidal breathing analysis (TBA) can provide useful information about lung function in infants and young children, as it is effort-independent.

OBJECTIVES: To determine if baseline and post-bronchodilator ratios of the time and volume until peak expiratory flow to the total expiratory time and volume, (t_{PEF}/t_E and V_{PEF}/V_E) can distinguish asthmatics from normal children.

METHODS: This is a cross-sectional study wherein 146 children ages 6 months to 5 years old completed TBA before and 15 minutes after administration of 250µg of salbutamol via nebulization. Children 3 years old and below who did not cooperate were given sedation with oral diphenhydramine (1mg/kg/dose). The t_{PEF}/t_E and V_{PEF}/V_E were compared between the controls and asthmatics.

RESULTS: In children below 2 years old, the baseline t_{PEF}/t_E of asthmatics and non-asthmatics were 29.6 ± 13.8and 22.0 ± 6.6. The area under the curve (AUC) was 0.649 at$t_{PEF}/t_E$of 32.250, with a sensitivity and specificity of 50% and 97%. The baseline V_{PEF}/V_E of asthmatics and non-asthmatics were 32.7 ±12.4 and 26.0 ± 4.9.AUC was 0.661 atV_{PEF}/V_Eof34.500, with a sensitivity and specificity of 50% and 97%.In subjects 2 to 5 years old, the baseline t_{PEF}/t_E of asthmatics and non-asthmatics were 35.3 ± 14.7 and 35.0 ± 13.1. The baseline V_{PEF}/V_E were 37.0 ± 12.3 and 36.7 ± 10.7. After salbutamol nebulization, the t_{PEF}/t_E of asthmatics and non-asthmatics in all ages were 30.9±13.7 and 27.9± 10.8. The V_{PEF}/V_E were 34.1± 11.4 and 30.9± 9.0.

CONCLUSION: Baseline t_{PEF}/t_E and V_{PEF}/V_E can distinguish asthmatics from non-asthmatics in children below 2 years old. However, baseline t_{PEF}/t_E and V_{PEF}/V_E in children 2 to 5 years old and post-bronchodilator t_{PEF}/t_E and V_{PEF}/V_E in all ages could not distinguish asthmatics from non-asthmatics after nebulization with 250µg of salbutamol.

KEYWORDS: Tidal breathing analysis (TBA), ratio of time to peak tidal expiratory flow and expiratory time (t_{PEF}/t_E), ratio of volume at peak tidal expiratory flow and expiratory volume (V_{PEF}/V_E), tidal breathing flow volume (TBFV), bronchodilator challenge

INTRODUCTION

Asthma diagnosis is usually made clinically. In infants, clinical diagnosis is sometimes difficult, as they do not necessarily follow the classic symptoms that older children have.[1]Measurements of lung function are important to provide an assessment of the severity of airflow limitation and its reversibility, to provide confirmation of the diagnosis of asthma.[2] It is known that asthma is characterized by variable expiratory airflow limitation(i.e. expiratory lung function varies over time and in magnitude).[3] Lung function may vary between completely normal and severely obstructed in the same patient. Thus, bronchodilator challenge testing is recommended aside from studies of airflow limitation due to the possibility that asthmatics may have normal baseline lung function but still demonstrate reversibility. [4]

The common techniques in measuring pulmonary function are frequently used in older children and adolescents. In infants and young children, it is often difficult to measure pulmonary function because of lack of patient cooperation, short attention span and inability to follow instructions. [5]

Spirometry is the most frequently used method for measuring lung function. [6] This test requires cooperation from subjects, hence, cannot be used for infants and most preschoolers. Forced oscillation technique (FOT) or impulse oscillometry (IOS) requires passive patient cooperation.[7]On the other hand, body plethysmography and tidal breathing analysis (TBA) have the potential to provide useful information about lung function in infants and young children as it only needs quiet respiration, hence suitable for this age group.[8]

TBA is a non-invasive pulmonary function test that measures changes in the flow and volume at the airway opening or from body surface measurements. It has been used in several foreign and local studies in newborns, infants and pre-school children to establish reference values for tidal breathing.[9-14]It was used in several studies for assessing airway obstruction with varying results[15-18].

Comparison of the baseline t_{PEF}/t_E and V_{PEF}/V_E between asthmatic and non-asthmatic children below 5 years old were only seen in foreign literature, but local studies on the normative values of t_{PEF}/t_E and V_{PEF}/V_E showed that Filipinos had lower t_{PEF}/t_E and V_{PEF}/V_E when compared with the corresponding reference data.[13, 14]A local study by Corpuz et al[20] assessed the reversibility of t_{PEF}/t_E and V_{PEF}/V_E between asthmatics and non-asthmatics using 15% change from baseline by computing for the sensitivity and specificity. The study neither determined the difference in the baseline t_{PEF}/t_E and V_{PEF}/V_E between the two groups nor computed for the specific percentage change that will distinguish the asthmatics from the non-asthmatics.

We aimed to determine the utility of tidal breathing analysis in diagnosing children with asthma. Specifically, we wished to determine the sensitivity and specificity of baseline t_{PEF}/t_E and V_{PEF}/V_E, and if reversibility of t_{PEF}/t_E and V_{PEF}/V_E to salbutamol respiratory solution could distinguish asthmatics from normal children.

METHODOLOGY

This was a cross-sectional study. We coordinated with the Quezon City Health Office to notify randomly selected heads of day care centers, pre-schools, and barangay health centers. Prospective subjects were sent to the Pulmonary Laboratory at Philippine Children's Medical Center. Demographic data and medical history were taken and recorded in a questionnaire evaluation form after purposive sampling of study participants. Physical examination was performed on each subject, and anthropometric measurements such as weight and height/length were recorded. Subjects who fulfilled the inclusion criteria were enrolled into the study after thorough explanation of the procedures to the parents and guardians, and after getting their informed consent.

Subjects aged 6 months to 5 years old were included if both parents were Filipino, asymptomatic at the time of enrollment, and had normal physical examination findings at the time of enrollment. Subjects with history of respiratory tract infection for the past 2 months, congenital malformation of the respiratory tract or chest wall or diaphragm, chronic lung disease, prematurity, cardiopulmonary or other systemic illness such as collagen diseases, nephropathies, any malignancy, neuromuscular disease such as Guillain-Barré Syndrome, myasthenia gravis and muscular dystrophies, thoracic and chest wall deformities, thoracic or abdominal surgery within the past 3 months, intake of any medication except vitamins for the past 4 weeks, exposure to tobacco smoke at home , and malnutrition (Weight for Length / Height Z score below -2 or above 3) were excluded from the study.

Physician-diagnosed asthmatics (re-evaluated by a pediatric pulmonologist) who were classified as intermittent asthmatic based

on the Philippine Consensus for the Management of Childhood Asthma (2002)[21] as reference standard were included. Other inclusion criteria for the asthmatic group were:1) any one of either parental asthma, physician diagnosis of atopic dermatitis, or evidence of sensitization to allergens in the air; and 2) any two of physician-diagnosed allergic rhinitis, wheezing unrelated to colds, and blood eosinophils >4%.

Subjects who used inhaled ß$_2$-agonists, ipratropium bromide or any other bronchodilating drugs, inhaled corticosteroids or disodium cromoglycate within12 hours before measurement, systemic ß$_2$-agonists or theophylline/aminophylline within 24 hours before measurement, and asthma controller medications (inhaled corticosteroids alone or in combination with long-acting ß$_2$-agonists, leukotriene receptor antagonists, herbal medications) in the past 4 weeks were excluded from the asthma group.

The performance of TBA followed the recommendations of the American Thoracic Society/European Respiratory Society statement on pulmonary function testing in pre-school children (2006).[6] Subjects rested for at least 10 minutes prior to the procedure**.** Children less than 3 years of age who did not sit quietly were sedated using oral diphenhydramine (1 mg/kg/dose, maximum: 50 mg) 30 minutes prior to the procedure. Those who vomited the said medication within 30 minutes after ingestion of the drug were given another dose. Patients not sedated after an hour from administration of the medicine were also given another dose, provided that the maximum dose was not yet reached.

The study consisted of measurements of tidal breathing flow volume (TBFV) loops: 1) at baseline and 2) 15 minutes after inhalation of salbutamol respiratory solution. A senior respiratory therapist measured the TBFV loops using MasterscreenPaed Jaeger Pediatric (Version 4.67 2012) in baseline condition and after inhalation of salbutamol respiratory solution. The respiratory therapist ensured that the respiratory pattern was stable and regular before starting data recording. A minimum of 30 seconds of tidal breathing was recorded to obtain a stable epoch of 20 tidal breaths per trial for a total of three consecutive trials. The computer calculated the final value, and the mean value was reported. Breaths were not included for analysis if they were obviously different in shape or size from surrounding breaths (e.g., sighs), if there were doubtful points of zero flow (e.g., pause between inspiration and expiration), or if there was more than one peak of expiratory flow. Indices obtained were calculated for 20 consecutive individual breaths per trial.

The nebulized solution was salbutamol (*Ventolin Respiratory Solution*), 250 µg placed in *DevilbissPulmo-Aide* compressor/nebulizer with a flow of 9L/min, attached to a face mask. The output of the nebulizer was 0.15mL/min with particles having a diameter below 5µm. The senior respiratory therapist measured the TBFV loops again, as previously described. Gathered data were recorded in a data sheet by the principal investigator.

The measurements were performed between 0800h and 1200h at the Pulmonary Laboratory of the Philippine Children's Medical Center in a cool and quiet environment. The average duration of each measurement of lung function was 10 minutes, and the entire sequence of lung function measurements before and after nebulization of salbutamol, including nebulization time took approximately 45 minutes.

A total sample size of 144 subjects achieved 82% power to detect a difference of -6 in t_{PEF}/t_E and V_{PEF}/V_E if the mean ratio of t_{PEF}/t_E and V_{PEF}/V_E for the control group was 31.77 with a standard deviation of 12.38. This calculation was based on a significance level of 0.05 using a 2-sided two-sample T-test based on a previous study done by Carlsen KH et al. [19]

Descriptive statistics was used to summarize the clinical characteristics of the patients. Frequency and proportion was utilized for nominal variables, and mean and SD for interval/ratio variables. Independent sample T-test was used to determine the significant difference between respondents with and

without asthma on interval/ratio scale type of data, while Chi-square analysis for the frequency of two groups. All valid data were included in the analysis. Missing variables were neither replaced nor estimated. Null hypotheses were rejected at 0.05 α-level of significance. Statistical analysis (SSPS Statistics 15.0) was used for data analysis.

RESULTS

There were one hundred and ninety-three (193) children recruited from health centers and day care centers in the Quezon City area, as well as in the private and out-patient clinics of Philippine Children's Medical Center. Participating barangay health centers were drawn at random from a list provided by the city health office. Six subjects were excluded due to malnutrition. TBA was attempted in all subjects, but twenty were entirely not subjected to the procedure due to the following reasons: infants unable to sleep despite sedation, and preschoolers who refused to do the test. Among those who completed the baseline measurements, two refused to undergo the post-bronchodilator determination and nineteen infants awakened during and/or after nebulization. The failure rate was 11.2%.

A total of 146 patients were included, 38 of whom were asthmatics. The patients without asthma were significantly younger, and subsequently had lower weight and height. Gender distribution was similar between groups (Table 1).

Table 1. Demographic Profile of Patients

Age Group	n	Demographic Characteristics	Control n (sd)	Asthmatic n (sd)	p-value
0 to < 2 years old	35	Female (14)/Male (21)	13 (44.8)/16 (55.2)	1 (16.7)/5 (83.3)	0.366 (NS)[a]
		Age (years)	0.83 (0.21)	1.00 (0.00)	0.000**[d]
		Weight (kg)	8.52 (1.60)	10.22 (1.55)	0.023*[c]
		Height (cm)	72.97 (7.87)	79.83 (5.27)	0.050 (NS)[c]
2 to 5 years old	111	Female (61)/Male (50)	44 (55.7)/35 (44.3)	17 (53.1)/15 (46.9)	0.805 (NS)[b]
		Age (years)	3.62 (1.14)	4.19 (0.93)	0.008**[d]
		Weight (kg)	14.27 (2.90)	15.38 (2.60)	0.064 (NS)[c]
		Height (cm)	96.63 (9.77)	101.47 (7.95)	0.015*[c]
All ages	146	Female (75)/Male (71)	57 (52.8)/ 51 (47.2)	18 (47.4)/ 20 (52.6)	0.566 (NS)[b]
		Age (years)	2.87 (1.58)	3.68 (1.45)	0.006**[c]
		Weight (kg)	12.72 (3.66)	14.56 (3.10)	0.006**[c]
		Height (cm)	90.28 (14.03)	98.05 (10.98)	0.002**[c]

a using Fisher's Exact test
b using Pearson Chi-square test
c using ANOVA F test
d using t-test for equality of means
* significant at the 0.05 level of significance
** significant at the 0.01 level of significance
(NS) not significant

To check whether the baseline tidal breathing analysis parameters were different between asthmatics and non-asthmatics, ANOVA F-Test was used. Table 2 compares the average baseline tidal flow parameters between patients with and without asthma. Only children less than 2 years old had statistically significant difference when baseline tidal flow parameters were compared.

Table 2. Comparison of Baseline Tidal Flow Parameters in the Controls and Asthmatics

Tidal Flow Parameters	n	Control (n = 108)	Asthmatics (n = 38)	p-value
t_{PEF}/t_E				
0 to < 2 years old	35	22.0 ± 2.4	29.6 ± 11.0	0.046*
2 to 5 years old	111	35.0 ± 2.9	35.3 ± 5.1	0.903 (NS)
V_{PEF}/V_E				
0 to < 2 years old	35	26.0 ± 1.8	32.7 ± 9.9	0.031*
2 to 5 years old	111	36.7 ± 2.4	37.0 ± 4.3	0.911 (NS)

* significant at the 0.05 level of significance
(NS) not significant

Receiver operating characteristic (ROC) curves were drawn for diagnosing asthma in children below 2 years old based on baseline TBA. At t_{PEF}/t_E, the AUC was 0.649 (95% CI: 0.305 to 0.993). Using the cutoff point of 13.300, the sensitivity and specificity of the t_{PEF}/t_E to diagnose asthma were 83% and 3%, respectively. Using the cutoff point of 23.550, the sensitivity and specificity of the t_{PEF}/t_E to diagnose asthma were 67% and 62%, respectively. Using the cutoff point of 32.250, the sensitivity and specificity of the t_{PEF}/t_E to diagnose asthma were 50% and 97%, respectively (Figure 2a). At V_{PEF}/V_E, the AUC was 0.661 (95% CI: 0.302 – 1.000). Using the cutoff point of 19.250, the sensitivity and specificity of the V_{PEF}/V_E to diagnose asthma were 83% and 3%, respectively. Using the cut off point of 28.050, the sensitivity and specificity of the pre-bronchodilator at V_{PEF}/V_E to diagnose asthma were 67% and 66%, respectively. Using the cutoff point of 34.500, the sensitivity and specificity of the V_{PEF}/V_E to diagnose asthma were 50% and 97%, respectively (Figure 2b).

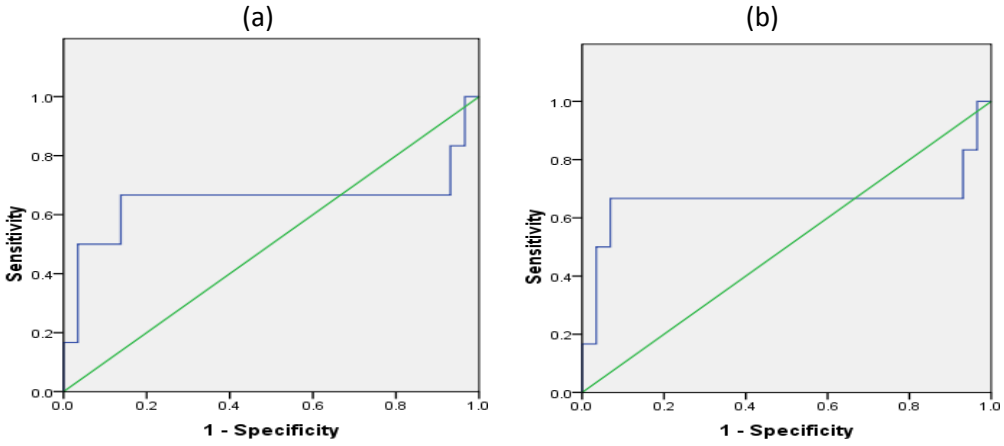

Figure 1. ROC Curve for children less than 2 years old at baseline (a)t_{PEF}/t_E [AUC 0.649 (95% CI: 0.305 to 0.993)] **and (b)V_{PEF}/V_E**[AUC 0.661 (95% CI: 0.302 – 1.000)]

Table 3 shows the comparison in reversibility of t_{PEF}/t_E and V_{PEF}/V_E between patients with and without asthma. Results showed that there was a significant difference between the baseline and post-bronchodilator T_{PEF}/T_E in the older age group, but not in the younger patients of both asthmatics and non-asthmatics. Results for V_{PEF}/V_E showed that there is no difference in all subjects except for the 2 to 5 years old non-asthmatics.

Table 3. Comparison of Reversibility of t_{PEF}/t_E and V_{PEF}/V_E in Controls and Asthmatics

Tidal Flow Parameters	n	Controls (n = 108)		p-value	Asthmatics (n = 38)		p-value
T_{PEF}/T_E		Baseline	Repeat		Baseline	Repeat	
0 to < 2 years old	35	22.0 ± 2.4	20.0 ± 5.8	0.064(NS)	29.6 ± 11.0	24.1 ± 15.8	0.266 (NS)
2 to 5 years old	111	35.0 ± 2.9	30.8 ± 10.8	0.001**	35.3 ± 5.1	32.2 ± 13.1	0.023*
V_{PEF}/V_E							
0 to < 2 years old	35	26.0 ± 1.8	24.4 ± 4.7	0.065(NS)	32.7 ± 9.9	28.1 ± 13.8	0.196 (NS)
2 to 5 years old	111	36.7 ± 2.4	33.2 ± 9.1	0.001**	37.0 ± 4.3	35.2 ± 10.8	0.136 (NS)

Statistical Analysis used: Paired T-test
* significant at the 0.05 level of significance
** significant at the 0.01 level of significance
(NS) not significant

The use of percent change after bronchodilation measured the reversibility of t_{PEF}/t_E and V_{PEF}/V_E in each individual. Table 4 shows that neither t_{PEF}/t_E nor V_{PEF}/V_E was significantly different between children with and without asthma.

Table 4. Comparison of means of tidal flow parameters between the asthmatics and controls using percent change of reversibility

Tidal Flow Parameter	n	Change from baseline in controls (%, sd)	Change from baseline in asthmatics (%, sd)	p-value
T_{PEF}/T_E				
0 to < 2 years old	35	-6.3 (23.1)	-13.8 (34.7)	0.511(NS)
2 to 5 years old	111	-6.1 (34.0)	-6.3 (19.3)	0.984(NS)
All ages	146	-6.2 (31.4)	-7.5 (22.0)	0.817(NS)
V_{PEF}/V_E				
0 to < 2 years old	35	-4.8 (16.3)	-12.8 (21.8)	0.312(NS)
2 to 5 years old	111	-6.1 (24.6)	-2.8 (16.3)	0.478(NS)
All ages	146	-5.8 (22.6)	-4.4 (17.4)	0.722(NS)

Statistical test used: independent T-test
* significant at the 0.05 level of significance
** significant at the 0.01 level of significance
(NS) not significant

DISCUSSION

TBA is a non-invasive pulmonary function test that measures changes in the flow and volume at the airway opening or from body surface measurements. The breathing pattern obtained during tidal breathing contains significant physiological information pertaining to a number of processes related to respiratory control and pulmonary mechanical function.[8] Most published data measured the flow and volume, and related this to timing of peak tidal expiratory flow namely: (*1*) Time to peak tidal expiratory flow (t_{PEF}) (s), (*2*) total expiratory time (t_E) (s), (*3*) the ratio of these (t_{PEF}/t_E), (*4*) volume at peak tidal expiratory flow (V_{PEF}) (ml), (*5*) expired tidal volume (V_E) (ml), and (*6*) the ratio of these (V_{PEF}/V_E). The peak tidal expiratory flow measures, t_{PEF}/t_E and V_{PEF}/V_E are commonly used in the assessment of obstructive pulmonary diseases.[6] t_{PEF}/t_E and V_{PEF}/V_E were also correlated to other parameters of airway obstruction including FEV1[22] hence, the present study used these parameters.

t_{PEF}/t_E measures the delay between the initiation of expiratory flow and peak expiratory flow during tidal breathing. In healthy patients, peak flow occurs at approximately one-third of the expiratory phase.[23] With airway obstruction, the round tidal loop takes on a more triangular appearance as the peak expiratory flow occurs closer to the beginning of expiration.[23]

Both the t_{PEF} and t_E influence the final value of the ratio t_{PEF}/t_E. Among patients with symptomatic bronchiolitis, slow exhalation secondary to an increased expiration time constant will increase t_E. In addition, active laryngeal breaking is diminished, leading to decreased $t_{PEF.}$[15] These patients with airflow obstruction are therefore expected to have decreased t_{PEF}/t_E. Behavior of the parameters t_{PEF} and t_E were comparable with that of V_{PEF} and V_E.[18]

There is only one foreign study and one local study in the literature comparing the baseline t_{PEF}/t_E and V_{PEF}/V_E between asthmatics and non-asthmatics less than 2 years old.[19, 21] Both showed that the baseline t_{PEF}/t_E and V_{PEF}/V_E of asthmatics were lower than those of non-asthmatics, which is in contrast with that of the present study. Clarke and associates showed that in healthy infants t_{PEF}/t_E values were significantly higher at 1 month than at 6 months and did not alter between 6 and 12 months.[25] This change seems to be entirely due to an increase in t_E, whereas the t_{PEF} remains constant during the 12 months.[26] The increase in t_E is caused by a gradual decrease in respiratory rate during the first year of life.[27] Thereafter, t_{PEF}/t_E values in infants gradually become similar to those obtained in older children and adults.[28] These physiologic changes could explain why t_{PEF}/t_E and V_{PEF}/V_E values in the present study differ from that of previous studies. None of the asthmatics were below 12 months old, and majority of the non-asthmatics were less than 1 year old.

ROC curves were used to determine baseline cutoff values of t_{PEF}/t_E and V_{PEF}/V_E to discriminate asthmatics from non-asthmatics. Three values were presented each for t_{PEF}/t_E and V_{PEF}/V_E. Using a cutoff value of 23.550 for t_{PEF}/t_E and 28.050 for V_{PEF}/V_E may not discriminate asthmatics from non-asthmatics. Using a cutoff value of 13.300 for t_{PEF}/t_E and 19.250 for V_{PEF}/V_E may miss out asthmatics in 17% of cases. Using a cutoff value of 32.250 for t_{PEF}/t_E and 34.500 for V_{PEF}/V_E will diagnose asthmatics correctly in 97% of cases, but may misdiagnose 3% of non-asthmatics as having asthma. Only for this purpose, to rule in asthma, use a higher t_{PEF}/t_E and V_{PEF}/V_E cutoff value.

There are two studies assessing reversibility of t_{PEF}/t_E and V_{PEF}/V_E after administration of salbutamol respiratory solution to children less than 2 years old.[19,21] Both studies show that there is a significant difference in the t_{PEF}/t_E and V_{PEF}/V_E at baseline and after bronchodilation between asthmatics and non-asthmatics. This is also in contrast with the result of the present study. The differences in ethnicity, inclusion/exclusion criteria, state of arousal, and TBA software used could have been sources of differences in the results of the present study from the previous studies. In the study by Carlsen and colleagues, participants

were all Caucasians and asymptomatic. Asthmatic children were in different grades of severity, some on controller medications. They were measured awake (in sitting position), using Sensor Medics 2600 system. The nebulized solution was 500 μg of salbutamol respiratory solution. In the study by Corpuz et al, participants were symptomatic Filipino children with recent use of systemic corticosteroids, sedated at the time of measurement using an older version of MasterscreenPaed Jaeger Pediatric, and were nebulized with 250 μg of salbutamol respiratory solution. Investigators of the present study did not follow the inclusion/exclusion criteria of the previous studies because presence of symptoms at the time of enrollment and use of controller medications could affect the results of the TBA. Participants of the present study were asymptomatic; asthmatic subjects were only those who were classified as intermittent asthmatics, without recent use of systemic corticosteroids. All subjects less than 2 years old were sedated hence measured in the supine position, the rest were awake and seated. The software used was MasterscreenPaed Jaeger Pediatric (Version 4.67 2012).

Three previous studies in children above 2 years old have shown differences in the baseline t_{PEF}/t_E and V_{PEF}/V_E and their reversibility among asthmatics and non-asthmatics.[19,21,24] Most of these studies were done in subjects who presented with signs of airflow obstruction. Only 2 of these studies were definite with the technique in performing TBA, the software used, dose of the bronchodilating agent and the state of arousal of participating subjects. All 3 studies showed that the baseline t_{PEF}/t_E and V_{PEF}/V_E of asthmatics were lower compared to controls, and that there was significant reversibility after bronchodilation in asthmatic subjects. Again, these were in contrast with that of the present study, wherein controls had lower t_{PEF}/t_E and V_{PEF}/V_E values and that there was no significant change in reversibility between asthmatics and non-asthmatics. Theoretically, sedation may reduce t_{PEF}/t_E by diminishing active laryngeal breaking.[15] This theory was in contrast to the study of Stocks and coworkers, that although there were no statistically significant differences in t_{PEF}/t_E or t_E between sedated and awake children, sedated children had higher tidal volume, higher PTEF, and a higher breathing frequency.[27] Higher t_{PEF}/t_E and V_{PEF}/V_E may be explained by arousal state, as awake infants seem to have higher tidal flows than sleeping infants, as shown in previous studies.[8] It must be noted that in the present study, most of the asthmatic population belong to the older age group. Only those 3 years old and below who did not cooperate were given sedation. This may explain the lack of significant differences in t_{PEF}/t_E and V_{PEF}/V_E between the asthmatic and control groups. A study by Cutrera and associates found out that under baseline conditions, V_{PEF}/V_E did not differentiate between asymptomatic asthmatic children and control subjects.[29]

The present study used 250 μg of salbutamol to assess reversibility based on the Global Initiative for Asthma Global Strategy for Asthma Management and Prevention 2012.[2] There was evidence of reversibility in subjects greater than 2 years old, both in asthmatics and non-asthmatics (Table 4). But when post-bronchodilator t_{PEF}/t_E and V_{PEF}/V_E between asthmatics and non-asthmatics were compared, the difference was not significant. Although a standard method of aerosol delivery and standard dose of bronchodilator were used, altered respiratory mechanics due to airflow obstruction in asthmatics may have lead to a decrease in the delivered dose of bronchodilator resulting in a lack of significant difference in the reversibility between asthmatics and non-asthmatics in the present study. A comparison of lung function methods for assessing dose-response effects of salbutamol was done by Houghton et al.[29] in adult subjects, showing that with 100 μg the asthmatic group already showed improvement in pulmonary function.

This study had the following limitations: only asymptomatic subjects were included since presence of symptoms could affect the tidal breathing parameters. This could be one reason why the t_{PEF}/t_E and V_{PEF}/V_Ecould not distinguish asthmatics from the non-asthmatics in the older age group, where the greater bulk of asthmatics subjects belonged. Another limitation of the

study was the use of only one dose of salbutamol respiratory solution. An earlier study that made use of 500 µg of salbutamol respiratory solution was able to distinguish asthmatics from non-asthmatics.[19] The authors made use of 250 µg of salbutamol respiratory solution since the local study done by Corpuz et al was also able to distinguish between the two groups by having greater than 15% reversibility post-bronchodilator in the asthmatic group.[20]Although the dose of bronchodilating agent was within the recommended dose based on the Global Initiative for Asthma Global Strategy for Asthma Management and Prevention 2012[2], physiologic changes in the infant airways may contribute unpredictability of the response to bronchodilating agents. Since the post-bronchodilation values of t_{PEF}/t_E and V_{PEF}/V_E were not statistically significant between the two groups, studying dose-response curve to salbutamol respiratory solution in this age group could help determine the appropriate amount that must be administered.

CONCLUSIONS

Baseline t_{PEF}/t_E and V_{PEF}/V_E could distinguish asthmatics from non-asthmatics in children less than 2 years old, but not in older children. The cutoff point of 32.250 for $t_{PEF}/t_E$and 34.500 for V_{PEF}/V_E may be used to rule in asthma, both with a specificity of 97%. Post-bronchodilator t_{PEF}/t_E and V_{PEF}/V_E in children 6 months to 5 years old could not distinguish asthmatics from non-asthmatics after nebulization with 250 µg of salbutamol.

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THE ROLE OF HEPARIN – BINDING PROTEIN IN THE DIAGNOSIS AND PROGNOSIS OF SEPSIS SYNDROME IN PEDIATRIC PATIENTS AT THE PHILIPPINE CHILDREN'S MEDICAL CENTER

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ABSTRACT

BACKGROUND: The burden of sepsis is global despite measures to improve its prompt recognition. However, there is no single reliable parameter for its early detection. Heparin-binding protein (HBP) is a new and promising biomarker for sepsis. Presently, there are no published reports in children apart from a limited study on UTI.

OBJECTIVE: To evaluate the role of HBP as a diagnostic tool and prognostic marker of sepsis syndrome among pediatric patients.

METHODS: This prospective cohort study enrolled pediatric patients who were categorized as SIRS or sepsis syndrome. HBP assay was determined on Day1. Likewise, blood culture was taken. A 7-day observation period using PELOD scoring was done. Final category as SIRS or sepsis syndrome was done on Day7. Statistical analysis was done to know relationship of HBP level to SIRS and sepsis.

RESULTS: 106 patients were included in this study. There was statistical significance in the correlation of HBP assay with presence of growth in blood culture and toxic granulations, length of ventilator support, and development of complications including mortality. The cutoff point was >125ng/mL. Sensitivity and specificity for HBP in sepsis syndrome were 98.31% and 97.87% respectively. Positive predictive value was 98.3%. Negative predictive value was 97.9%. Positive likelihood ratio was 46.2. Negative likelihood ratio was 0.017. Risk ratio was 47.6. Subjects with HBP level of >125 ng/mL had 47.6 times the risk of having sepsis syndrome as compared to those with level <125 ng/ml.

CONCLUSION & RECOMMENDATIONS: Elevated HBP level is a useful diagnostic and prognostic marker for childhood sepsis syndrome. Determination of HBP levels at different time intervals within a longer observation period may give a more accurate description of subject's clinical improvement or progression to MODS or mortality.

KEYWORDS: Heparin-binding protein, HBP, Pediatric Sepsis, Sepsis, SIRS

INTRODUCTION

Sepsis is the body's systemic inflammatory response to infection and can progress to severe sepsis, septic shock and ultimately multiple organ dysfunction syndrome.[1] The burden of sepsis is felt globally in both developed and developing countries with an estimate of 20 to 30 million patients afflicted every year. It is a significant cause of morbidity and mortality not only in adults but also in children. In the developing world, sepsis accounts for 60-80% of lost lives in childhood, with more than 6 million neonates and children affected annually[2]. In the Pediatric Critical Care Unit of Philippine Children's Medical Center, sepsis, septic shock and MODS are consistent entries in top 10 morbidity and mortality lists. As the early presentation of childhood sepsis is often difficult to distinguish from less serious viral illnesses, numerous studies have attempted to identify parameters to distinguish children at risk from sepsis.

Several biomarkers have been used to assess the risk of sepsis. These include white blood cell count (WBC), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Likewise, several clinical scales have also been tried to assess the risk of sepsis. However, these have not shown to be adequately sensitive nor specific tools in determining sepsis in the pediatric population. Numerous published reports mostly in adults have identified novel biomarkers as promising laboratory parameters for predicting the risk of sepsis. These include lactate, prolactin and interleukin. Unfortunately, there is no single reliable parameter to assess the risk of sepsis in children.

More recently, a few studies have shown the usefulness of another biomarker in predicting sepsis and mortality. Heparin Binding Protein is a new marker that has demonstrated utility in identifying patients at risk of developing severe sepsis. Heparin-Binding Protein (HBP), also known as CAP37 and azurocidin, is synthesized in neutrophils.[4] Once released from activated neutrophils, it induces a rearrangement of the endothelial cell cytoskeleton, resulting in increased permeability of the endothelium resulting to vascular leakage. At the site of infection, HBP is responsible for the recruitment and activation of monocytes and other inflammatory mediators. It is also internalized by monocytes to prolong survival and enhance cytokine production.[5] HBP therefore directly contributes to the maintenance and progression of inflammation.[6]

In a comprehensive review of the utility of biological mediators in the diagnosis of sepsis, HBP is considered one of the emerging biomarkers. The non-specific physiologic criteria of sepsis syndrome do not adequately identify patients who might benefit from either conventional anti-infective therapies or from novel therapies that target specific mediators of sepsis. The utility of a biomarker is a function of the degree to which it adds value to the available clinical information in the domains of screening, diagnosis, risk stratification and monitoring of the response to therapy. Validated biomarkers of sepsis may improve diagnosis and therapeutic decision making for these high risk patients, but will require an unprecedented degree of systematic investigation and collaboration.[8]

In a prospective study of 233 adult febrile patients with suspected sepsis, high plasma levels of HBP helped to identify patients with an imminent risk of developing sepsis with circulatory failure. HBP was shown to be the best predictor of severe sepsis with Receiver-operating characteristic (ROC) plot showing an Area Under Curve (AUC) of 0.95, exceeding that of Procalcitonin, IL-6 and Lactate. A plasma HBP level $\geq$ 15 ng/mL was a better indicator of severe sepsis (with or without septic shock) than any other laboratory parameter investigated. Thirty-two of the 70 patients with severe sepsis were sampled up to 12 hours before signs of circulatory failure appeared and in 29 of these patients, HBP plasma concentrations were already elevated.[9]

Another prospective study was conducted of two patient cohorts totaling 179 patients treated in the ICU at Karolinska University Hospital Huddinge in Sweden in 2012. Plasma HBP levels were significantly higher in patients with severe sepsis or septic shock compared to patients with a non-septic illness in the ICU. HBP was associated with severity of disease and an elevated HBP at admission was associated with an increased risk of death. HBP that rises over time may identify patients with a deteriorating prognosis.[10]

Recently, Lertdumrongluk et al. investigated heparin-binding protein as a diagnostic tool in acute pyelonephritis (APN). Urine HBP (UHBP) levels were measured at baseline and 1 month after antimicrobial therapy in children suspected with APN vs. controls. Results showed levels of 47.0 $\pm$ 8.4 and 16.6 $\pm$ 3.8 vs. 15.0 $\pm$ 2.9 ng/mL respectively. Test performance characteristics were calculated against a gold standard of positive urine cultures and compared with leukocyte esterase (LE) and nitrite measured by dipsticks and pyuria by microscopy. The sensitivity and specificity for UHBP levels $\geq$ 34 ng/mL were 100% and 100%.[13]

Despite the growing interest in HBP as a diagnostic tool in severe infections and inflammatory conditions, most studies are done in adults. The limited data on children were focused on urinary tract infection. At present, there are no published reports on the usefulness of HBP in childhood sepsis.

The mortality associated with sepsis is still substantial despite the increasing awareness of the diagnosis and recent advancements in treatment strategies. An important task for the clinician is to recognize sepsis before it progresses into a more severe state with signs of circulatory failure. Thus, a reliable molecular tool identifying patients who are at risk of developing severe sepsis among patients presenting with fever and signs of systemic inflammatory response would decrease the critical span to adequate treatment and be of considerable clinical value.[9]

At the Pediatric Critical Care Unit of the Philippine Children's Medical Center, there are approximately 40-50 admissions and referrals each month. Around 30-50% of them are due to sepsis syndrome.

We aimed **t**o evaluate the role of heparin-binding protein as a diagnostic tool and prognostic marker of sepsis syndrome among pediatric patients less than 19 years old admitted at the Philippine Children's Medical Center. Specifically, we wished to ascertain the relationship of heparin-binding protein and the following sepsis categories:

a) SIRS
b) Sepsis Syndrome**;**
to compute for the overall sensitivity, specificity, predictive value and likelihood ratio of heparin-binding protein in detecting the following:

a) SIRS
b) Sepsis Syndrome**;**
and to determine the cutoff value of heparin binding protein for

a) SIRS
b) Sepsis Syndrome

METHODOLOGY

This is a prospective cohort study. Pediatric patients less than 19 years old and fulfilling the SIRS and sepsis syndrome based on the International Consensus Conference on Pediatric Sepsis were included in the study.[16] Those with febrile neutropenia and obvious viral infection were excluded from this study.

Each subject was initially categorized as to SIRS or Sepsis Syndrome. After extraction of blood samples, a 7-day observation period ensued. Subjects were then reclassified as to SIRS or Sepsis Syndrome as final category. Subjects under the Sepsis Syndrome were further subcategorized as Sepsis, Severe Sepsis, Septic Shock or MODS.

Demographic profile was tabulated including name, age and sex. Laboratory profile was tabulated including white blood cell count, actual neutrophilic count, presence of toxic granules, actual platelet count, blood culture and other cultures taken, biomarkers lactate and procalcitonin when available and the HBP assay in ng/ml. Clinical profile was tabulated including initial category, number of hospital days, use of ventilatory support, outcome (no complications, with complications, mortality or readmission) and final category after the 7-day observation period.

A daily PELOD scoring was done for seven days from extraction of HBP level to monitor possible development or progression of condition to a sepsis spectrum like MODS. After seven days from enrolment to the study, a diagnosis with the worst sepsis syndrome category was labeled as the final category. This final category was compared to the actual HBP level. Clinical and Laboratory Outcome measurements such as the length of hospital stay and ventilator days, outcome, WBC count, blood culture result, presence or absence of toxic granules were also correlated with the HBP levels.

Data were described using means, standard deviations, percentages and frequency counts. T-test for independent samples was used to determine significant difference between

means of two groups, ANOVA one way to compare three or more groups. Chi-square and Fischer's exact test were used to determine significant difference in the distribution of categorical variables. Odds ratio was computed where feasible. Minitab Ver 17 was used as statistical software. ROC Analysis using MedCalc software was done to determine optimum cutoff points, after which accuracy parameters were determined such as sensitivity, specificity, predictive values and likelihood ratios. For all tests, a 95% confidence level was considered significant ($p<0.05$).

RESULTS

One hundred and nine (109) subjects were enrolled in the study. However, only 106 fulfilled the inclusion criteria. All 106 subjects were categorized as to SIRS vs. Sepsis Syndrome twice. Initially, upon enrolment to the study and prior to the HBP assay which was based on history, clinical parameters and basic laboratory exams including blood counts. Finally, after the 7-day observation period when the HBP assay was run which was based on further clinical findings as well as other diagnostic parameters including blood culture. Final category that was correlated with the HBP assay was the post-7-day observation period.

Table 1 shows the demographic profile of the subject population. Mean age was more than 3.5 to less than 4.5 years which did not show statistical significance on both categories. Gender likewise did not show statistical significance with male predominance on both categories. Of the 106 subjects, 44% (N = 47) was classified as SIRS while 56% (N = 59) was classified as Sepsis Syndrome.

TABLE 1. DEMOGRAPHIC PROFILE

	SIRS (N = 47)	**Sepsis Syndrome** (N = 59)	**P Value**
Age			0.461
Mean+/SD	*4.45+/5.13*	*3.72+/4.91*	
Sex			0.845
Male	*28 (60%)*	*34 (57.6%)*	
Female	*19 (40%)*	*25 (42.4%)*	

The SIRS category included the following diseases: 61.7% (29 of 47) was pneumonia; 6.4% (3 of 47) was acute gastroenteritis; 4.3% (2 of 47) each for systemic lupus erythematosus in flare, rheumatic heart disease in failure and enterocolitis; and 2.1% (1 of 47) each for typhoid fever, shunt malfunction, cor pulmonale, lymphoma, cellulitis, rhabdomyosarcoma, ear trauma with hemophilia B, fatal arrhythmia with electrolyte imbalance and thalassemia and abscess.

The Sepsis Syndrome Category is broken down into the following subcategories: 23.7% (14 of 59) was sepsis; 20.3% (12 of 59) was severe sepsis; 47.5% (28 of 50) was septic shock; and 8.5% (5 of 59) was MODS.

Table 2 shows the clinical profile for each category. All parameters including the hospital stay, ventilator days and outcome were statistically significant. For purposes of this study, "complications" included worsening of clinical symptoms or development of conditions that can affect prognosis like pneumothorax, effusions; change of current antibiotic due to worsening condition or non-response; increase in oxygen requirement, or need for ventilatory support.

There were more subjects with $\geq$ 7 days hospital stay in sepsis syndrome at 73% compared to SIRS at 27%. Comparatively, more subjects had shorter hospital stay of < 7days in SIRS at 47%. Expectedly, fewer subjects required ventilator support in SIRS at 85.1% compared to sepsis syndrome at 35.6%. On subject outcome, majority of those under the SIRS category showed no complications at 72.3%. In contrast, 62.7% showed complications, 33.9% expired and 1.7% was readmitted under the sepsis syndrome category.

TABLE 2. CLINICAL PROFILE

	SIRS (N = 47)	**Sepsis Syndrome** (N = 59)	**P Value**
Hospital Stay			0.043
< 7 days	*22 (47%)*	*16 (27%)*	
≥ 7 days	*25 (53%)*	*43 (73%)*	
Ventilator Days			**< 0.0001**
<7 days	*3 (6.4%)*	*20 (33.9%)*	
≥ 7 days	*4 (8.5%)*	*18 (30.5%)*	
Not ventilated	*40 (85.1%)*	*21 (35.6%)*	
Outcome			**< 0.0001**
No complications	*34 (72.3%)*	*1 (1.7%)*	
With complications	*9 (19.2%)*	*37 (62.7%)*	
Expired	*4 (8.5%)*	*20 (33.9%)*	
Readmitted	*0 (0%)*	*1 (1.7%)*	

Table 3 shows the laboratory profile of the subjects. White blood cell counts under SIRS are slightly lower than in the sepsis syndrome with mean values of 14.8 and 18.1 respectively. While there was leukocytosis in both categories, there was no statistical significance. Likewise, platelet counts in both categories did not show statistical significance. Blood cultures and appearance of toxic granules, however, showed statistical significance. Blood cultures showed positive growth on 30.5% of subjects under sepsis syndrome and 0% growth on SIRS. Toxic granules appeared on 13.6% of subjects under sepsis syndrome and none under SIRS.

TABLE 3. LABORATORY PROFILE

	SIRS (N = 47)	**Sepsis Syndrome** (N = 59)	**P Value**
WBC			0.075
Mean +/SD	*14.84+/8.48*	*18.07+/9.66*	
Platelet Count			0.249
Mean +/SD	*357.34+/209.85*	*309.95+/207.18*	
ANC			0.210
Mean+/SD	*9,935.85+/7054.56*	*11,743.42+/ 7571.10*	
Culture			**0.00003**
Positive	*0 (0%)*	*18 (30.5%)*	
Negative	*47 (100%)*	*41 (69.5%)*	
Toxic granules			**0.0084**
Positive	*0 (0%)*	*8 (13.6%)*	
Negative	*47 (100%)*	*51 (86.4%)*	

Table 4 shows the relationship of clinical and laboratory parameters with HBP levels. Although there was an increase in the mean HBP level with hospital stay ≥ 7 days, this was not statistically significant. The length of ventilator days was significantly associated with HBP levels. Mean HBP level of subjects without ventilator support was significantly lower than those with ventilatory support. However, mean HBP level of subjects with ventilator use of < 7 days and ≥ 7 days use was not statistically significant.

Outcome was also significantly associated with HBP levels. Subjects without complications had significantly lower mean HBP level compared to those with complications and those who expired. However, the mean HBP level of the group with complications did not differ significantly from that of the group with subjects who died. The outcome variable "readmitted" was not included in the analysis since it only had one subject, thus, mean and standard deviation could not be computed.

The mean HBP level of subjects found with toxic granules was significantly higher than the subjects without toxic granules. Similarly, the mean HBP level of subjects with growth on blood culture was significantly higher than those without growth. There was increase in the mean HBP level for age group 1 month to 1 year with WBC > 17.5 as well as for age group 6 to 12 years with WBC > 13.5, In contrast, the mean HBP level was close for age group of 2 to 5 years and for age group 13 to 19 years with varying WBC counts. Despite these findings, there was no statistical significance across all age groups.

TABLE 4. RELATIONSHIP OF CLINICAL AND LABORATORY PARAMETERS WITH HBP LEVELS

	HBP (ng/ml)
Hospital Stay (days)	
< 7 days (N = 38)	125.6+/91.0
≥ 7 days (N = 68)	150.5 +/73.4
P value	*0.126*
Ventilator Days	
No ventilator (Group 1: N = 61)	110.7 +/75.7
< 7 days (Group 2: N = 23)	201.3 +/64.7
≥ 7 days (Group 3: N = 22)	164.8 +/67.3
P value	***< 0.0001***
Post-hoc T-test after a significant ANOVA	*Group 1 vs. Group 2 = SIG* *Group 1 vs. Group 3 = SIG* *Group 2 vs. Group 3 = NS*
Outcome*	
No complications (Group 1: N = 35)	57.8 +/39.3
With complications (Group 2: N = 46)	177.2 +/57.1
Mortality (Group 3: N = 24)	194.3 +/74.4
P value	***< 0.0001***
Post-hoc T-test after a significant ANOVA	*Group 1 vs. Group 2 = SIG* *Group 1 vs. Group 3 = SIG* *Group 2 vs. Group 3 = NS*
Toxic Granules	
With (N = 8)	202.8+/28.8
Without (N = 98)	136.6 +/81.5
P value	***< 0.0001***
Blood Culture	
No growth (N = 88)	127.5 +/79.3
With growth (N = 18)	210.3+/45.0
P value	***< 0.0001***
WBC	
For age group 1 month - 1 year	
> 17.5 or < 5 (N = 26)	163.3 +/61.2
5 - 17.5 (N = 29)	131.1+/82.4
P value	*0.115*
For age group 2 years - 5 years	
> 15.5 or < 6 (N = 13)	131.8+/75.6
6 - 15.5 (N = 8)	131.7+ 91.6
P value	*1.00*
For age group 6 - 12 years	
> 13.5 or < 4.5 (N = 12)	154.7+/88.6
4.5 - 13.5 (N = 8)	113.7 +/94.9
P value	*0.335*
For age group 13 – 19 years	
>11 or < 4.5 (N = 5)	143.6+/100.7
4.5-11 (N = 5)	142.4+/104.4
P value	*0.984*

**One subject readmitted, not included in outcome.*

Table 5 shows the mean HBP levels of SIRS and Sepsis Syndrome and the sub-categories of Sepsis Syndrome. Statistical significance was seen between the values in SIRS vs. Sepsis Syndrome with mean HBP level of 62.7 ng/mL and 204.5 ng/mL respectively. Statistical significance was likewise seen in SIRS vs. the different sub-categories of Sepsis Syndrome. Among the different sub-categories, the statistical difference of Category 2 (Sepsis) vs. Categories 3 (Severe Sepsis), 4 (Septic Shock) and Category 5 (MODS) was significant. The statistical differences among Categories 3, 4 and 5 were not significant.

TABLE 5. MEAN HBP LEVEL OF SIRS AND SEPSIS SYDROME AND SUB-CATEGORIES OF SEPSIS SYNDROME

	Mean	**SD**
HBP Determination Post-7 Days Observation Period		
Sepsis Syndrome	204.5	42.0
SIRS	62.7	34.4
P value	***< 0.0001***	
***Sub-Categories of Sepsis Syndrome**		
Category 1: SIRS (N = 47)	62.7	34.4
Category 2: Sepsis (N = 14)	161.4	26.4
Category 3: Severe Sepsis (N = 12)	210.6	33.5
Category 4: Septic Shock (N = 28)	219.6	37.6
Category 5: MODS (N = 5)	226.1	43.9
P value	***< 0.0001***	

**Significant Pairs: Category 1 vs. 2; Category 1 vs. Category 3; Category 1 vs. Category 4; Category 1 vs. Category 5; Category 2 vs. Category 3; Category 2 vs. Category 4; Category 2 vs. Category 5.*
**Category 3 vs. Category 4 and Category 5, and Category 4 vs. Category 5 not significantly different.*

The line diagram in Figure 2 illustrates the diagnostic role of HBP assay in determining Sepsis Syndrome. The HBP cutoff point for this study was determined by receiver operating characteristic (ROC) analysis.

FIGURE 2.

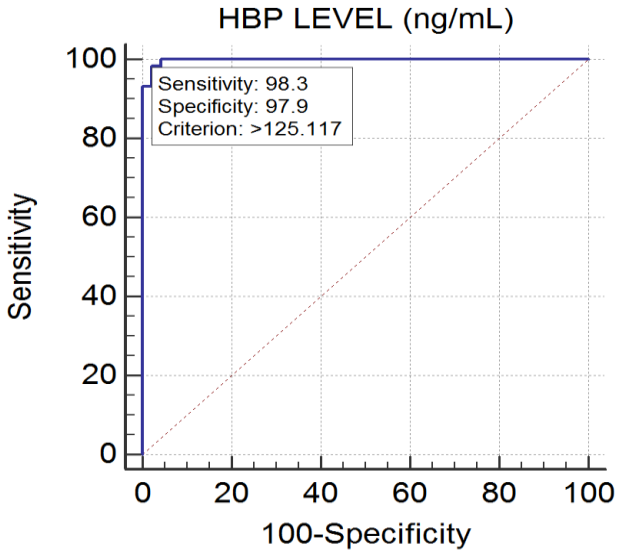

Table 6-A shows that for Day 1, HBP level for low risk subjects based on PELOD score was significantly lower than those categorized as medium to high risk with computed difference of 89.6 ng/ml. For Day 7, HBP level for low risk was likewise significantly lower than those categorized as medium to high risk with computed difference of 94.2 ng/ml.

TABLE 6-A. ASSOCIATION OF HBP WITH PELOD SCORES AT DAY 1 AND DAY 7

PELOD SCORE DAY 1				
	LOW RISK < 10 (N=80)	MEDIUM TO HIGH RISK * ≥ 10 (N=26)	P VALUE	Mean A – Mean B and 95% CI of the difference
Mean HBP ± SD	119.6 ± 77.3	209.2 ± 46.8	< 0.0001	- 89.6 (± 24.96)
PELOD SCORE DAY 7				
	LOW RISK <10 (N=60)	MEDIUM TO HIGH RISK * ≥10 (N=46)	P VALUE	Mean A – Mean B and 95% CI of the difference
Mean HBP ± SD	100.7 ± 67.4	194.9 ± 63.9	< 0.0002	- 94.2 (± 25.5)

**Only 2 subjects with Medium Risk for Day 1 and 3 subjects for Day 7, hence, pooled with High Risk category. T-test for independent samples used.*

In Table 6-B, the correlation coefficient between values of HBP and PELOD scores was high and significant, with R higher at Day 7.

TABLE 6-B. CORRELATION OF HBP LEVELS WITH PELOD SCORES (HBP AS DEPENDENT VARIABLE) AT DAY 1 AND DAY 7

	PEARSON'S R OR CORRELATION COEFFICIENT	CRITICAL VALUE FOR SIGNIFICANCE	P VALUE
DAY 1	0.440	0.196	< 0.0001
Day 7	0.527	0.196	< 0.0001

Categories based on pre-HBP determination were significantly associated with PELOD score at Day 1. That is, with a higher proportion of subjects categorized as medium to high risk associated with having sepsis syndrome (85%) than those categorized as low risk (40%). The computed odds ratio was 8.25 indicating that the odds or chance of a subject categorized as medium to high risk for progression to MODS was 8.25x higher among those with sepsis syndrome than those with SIRS. At Day 7, the association was also highly significant between the two variables, with computed odds ratio of 15.2 indicating that those with sepsis syndrome have 15x greater odds or chance to progress to MODS than those with SIRS.

SIRS is significantly associated with PELOD score at day 7, with SIRS 5.2% less likely to occur with a PELOD Score of ≥ 10 or medium to high risk for progression to MODS. Sepsis and severe sepsis, on the other hand did not show significant association with PELOD. Septic shock was significantly associated and is 15.3x more likely to occur with a PELOD Score of ≥ 10. MODS was also significantly associated with PELOD but the odds ratio could not be computed in the presence of a zero value. However, an estimate of RR showed that subjects with MODS were 2.5x more likely to get a PELOD Score of ≥ 10.

DISCUSSION

The diagnosis of sepsis and its progression to MODS remain elusive despite several published guidelines and the introduction of novel biomarkers. It is for this reason that studies have continually been conducted to find more reliable parameters.

The data and findings of this study showed that clinical parameters alone for sepsis might not be sufficient in its early stages. Likewise, even standard laboratory parameters including blood culture are not totally reliable. Hence, HBP as an early diagnostic marker for sepsis is very useful and potentially lifesaving.

Upon initial enrolment to the study, the diagnosis of SIRS was 49% (52 of 106) of the subject population while Sepsis Syndrome stood at 51% (54 of 106). In the 7-day observation period, 27% (14 of 52) of patients with initial diagnosis of SIRS progressed to Sepsis Syndrome. Significantly, all of the 14 subjects had an HBP level of > 125 ng/ml with values ranging from 142.92 – 275.49 ng/ml. In the same manner, 17% (9 of 54) of Sepsis Syndrome turned out to be SIRS. And all of the 9 subjects had an HBP level < 125.11 ng/ml with values ranging from 15.74 – 89.03 ng/ml. These findings highlight the limitation of clinical findings alone in the diagnosis of sepsis syndrome. Out of the 14 subjects with initial SIRS, 6 turned out to be Sepsis (42.8%) with HBP levels ranging from 143.22 – 209.58 ng/ml; 2 progressed to Severe Sepsis (14.3%) with HBP levels ranging from 208.716 – 275.49 ng/ml; and 6 progressed to Septic Shock (42.8%) with HBP levels ranging from 142.92 – 228.132 ng/ml.

Out of the 45 subjects with initial and post-7 days observation period diagnosis of Sepsis Syndrome, 8 turned out to be Sepsis (17.8%) with HBP levels ranging from 117.32 – 197.827 ng/ml; 10 progressed to Severe Sepsis (22.2%) with HBP levels ranging from 173.4 – 264.87 ng/ml; 22 progressed to Septic Shock (48.9%) with HBP levels ranging from 168.06 – 283.96 ng/ml; and 5 progressed to MODS (11.1%) with HBP levels ranging from 170.58 – 289.62 ng/ml. Although there was no statistical significance among the subcategories of Sepsis Syndrome, it is interesting to note that there was progressive increase of HBP levels as the severity of Sepsis Syndrome increases. In this group, the Sepsis subcategory had the lowest HBP level at 117.32 ng/ml while the MODS subcategory had the highest HBP level at 289.62 ng/ml.

There were 26 mortalities in the study population giving a mortality rate of 24.5%. Out of these, 84.6% (22 of 26) belonged to the category of Sepsis Syndrome. Sepsis accounted for 1 mortality (3.8%) with HBP level of 181.309 ng/ml. Severe Sepsis also accounted for 1 mortality (3.8%) with HBP level of 173.4 ng/ml. Likewise, MODS accounted for 1 mortality (3.8%) with HBP level of 242.226 ng/ml. Septic Shock accounted for 19 mortalities (86.4%) with HBP levels ranging from 168.06 – 283. 96 ng/ml. Interestingly, the highest HBP level of 283.96 ng/ml in this study belonged to the MODS subcategory who expired on the 3rd day of observation period.

There were 4 mortalities in the SIRS category with HBP levels ranging from 19.45 – 70.88 ng/ml. While these 4 subjects only fulfilled the SIRS criteria and had HBP levels below the cutoff value established in this study, their mortality outcome was surprising. Within the 7-day observation period, their progression of illness was confirmed as due to non-septic causes. One mortality was diagnosed with Rhabdomyosarcoma and expired from Stage 4 Disease. Another mortality was diagnosed with Thalassemia and died of fatal arrhythmia secondary to hypokalemia. Another mortality was diagnosed with Rheumatic Heart Disease, Mitral Stenosis Severe who died of low cardiac output syndrome. While the last mortality was diagnosed with Prader Willi Syndrome, Obstructive Sleep Apnea who expired from complications of Cor Pulmonale. These findings add credibility to the usefulness of HBP assay in ruling out sepsis as an initial diagnosis especially since the above HBP levels fall way below the cutoff level of > 125.11 ng/ml established in this study. Moreover, it helps the physicians to consider causes other than sepsis given the limitation of clinical findings. This

may prove timely and lifesaving in the management of other illnesses mimicking or overlapping with sepsis syndrome. In addition the cost of sepsis burden is minimized when the diagnosis is early and management is prompt.

There were 35 subjects out of the total population (33%) who did not develop complications with HBP levels ranging from 5.9 – 140.319 ng/ml. Thirty-four out of 35 (97.1%) belonged to the SIRS category while only 1 (2.9%) belonged to the Sepsis Syndrome category. Out of the 34 SIRS, 19 subjects (55.88%) did not require ventilator support and were discharged in less than seven days with HBP levels ranging from 5.9 – 125.117 ng/ml. Out of the 34 SIRS 13 subjects (38.23%) did not require ventilator support but stayed in the hospital for more than seven days for varying reasons with HBP levels ranging from 8.08 – 106.27 ng/ml. Only 2 subjects out of the 34 SIRS (5.88%) required ventilatory support and stayed in the hospital for more than seven days with HBP levels ranging from 67.79 – 76.54 ng/ml. Of the 2 SIRS requiring ventilator support, one subject was diagnosed with Hemophilia A Severe with Intracranial Bleed and developed healthcare-associated infection while the other subject was diagnosed with Congestive Heart Failure. These diagnoses could explain the low HBP levels noticeably falling below the cutoff value of > 125.11 ng/ml established in this study. The lone subject belonging to the Sepsis Syndrome Category stayed in the hospital for more than seven days but did not require ventilatory support had an HBP level of 140.319 ng/ml which was the highest for the group that did not develop complications.

These findings confirmed the utility of HBP as a credible biomarker for sepsis syndrome and its vast potential for predicting progression to MODS. The disparity between published HBP cutoff point for adults in the diagnosis of sepsis with highest cutoff level > 50 ng/ml and this study with cutoff level >125 ng/ml might be due not only to age difference but also to ethnicity and geographic location.

CONCLUSION

This study showed the usefulness of heparin binding protein in the diagnosis of sepsis syndrome. The HBP cutoff level of > 125.11 ng/ml gave a sensitivity of 98.3% with specificity of 97.87%, PPV of 98.3 with NPV of 97.9 and LR+ of 46.2 with LR- of 0.017. The computed risk ratio revealed 47.6 times the risk of having sepsis syndrome at the same cutoff level. While this study did not establish a statistically significant cutoff point for progression to MODS, the mean HBP levels progressively rose to > 200 ng/ml with progression to MODS.

This study was limited to a one-time determination of HBP level. Determining the HBP levels at different time intervals within a longer observation period may give a more accurate description of the subject's clinical improvement or progression to MODS.

The population distribution as to the different sepsis syndrome categories was also limited in number. It may be helpful to do a study focusing on the different sepsis syndrome subcategories each with a larger population size to produce a more reliable comparison and analysis.

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18. Axis – Shield Heparin Binding Protein EIA Assay Pack Insert

COMPARISON OF TOTAL AEROBIC COUNT BEFORE AND AFTER APPLICATION OF DIFFERENT ANTISEPTIC SOLUTIONS PRIOR TO VENIPUNCTURE AND ITS CORRELATION TO DEVELOPING PHLEBITIS, IN CHILDREN ADMITTED AT A TERTIARY HOSPITAL IN QUEZON CITY

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ABSTRACT

OBJECTIVE: To compare the effect on Total Aerobic Count (TAC) of application of Isopropyl alcohol, Chlorhexidine, and povidone iodine plus alcohol prior to venipuncture and development of phlebitis in children admitted at a tertiary hospital.

METHODS: A prospective randomized clinical trial in a tertiary hospital in Quezon City. A total of 129 patients one-year old and above with physician orders for IV insertion and extractions. Pre and post swabbing of the venipuncture site was done and placed on a blood agar plate. The three antiseptic solutions were applied over the venipuncture site and swabbed and placed on the agar plate. The primary outcome measure was the TAC in each blood agar of the tested antiseptic solution and correlation to developing phlebitis.

RESULTS: The CFU/mL after disinfection was significantly different between groups, with the lowest CFU/mL observed among patients disinfected with Chlorohexidine. Phlebitis was only noted in the alcohol group. No reactions were observed from patients who were disinfected with povidone iodine and chlorohexidine.

CONCLUSIONS: Single application of Chlorhexidine is the optimal method to be used as antisepsis prior to procedures like venipuncture. However, the use of povidone iodine plus 70% isopropyl alcohol also has comparable effect to Chlorhexidine.

KEYWORD: Venipuncture, Total aerobic count, antisepsis

INTRODUCTION

The daily use of devices for venipuncture is associated with increase risk and complications that has impact on the clinical status and outcome of a patient. [3] Apart from sepsis and pain from infiltration and phlebitis, peripheral intravenous cannulation, can also cause increased rates of morbidity and mortality, length of hospitalization, staff workload and financial burden on the patient and his family.[3]

For centuries skin antisepsis has been a part of medical practice. Aseptic techniques are set of specific practices and procedures performed under carefully controlled conditions with the goal of minimizing contamination by pathogens. To minimize the risk of contamination to a device, thorough disinfection is needed. [6]Antiseptic solutions are used to prevent contamination of venipuncture areas. The most commonly used substance for skin antisepsis are alcohol, chlorhexidine and povidone iodine. Among these, alcohol is the only solution that is microbiologically active without residual activity. Chlorhexidine gluconate (CHG) and povidone iodine (PVI) on the other hand are as effective but is noted to have residual activity on the skin.[5]Several studies have been made on different skin antiseptic solutions over venipuncture sites. However, it is still unclear which antiseptic solution should be used effectively to minimize contamination after intravascular access. Some studies have conflicting results but most were designed to detect organisms commonly

contaminating blood culture tests.

Several studies have already investigated the antiseptic efficacy of different solutions including, alcohol [6], chlorhexidine[11,12], and combination of povidone iodine [6,11,13,14,15] and alcohol or Chlorhexidine with alcohol.[14,15] The effects of these antiseptic solutions in decreasing contamination of blood cultures and preventing surgical site infections were also topics of concern for some researchers. However, no local studies were done comparing these antiseptic solutions and their effect on the total colony counts on a patients' skin prior to venipuncture.

With the increasing number of cases of hospitalized patients developing CRBSI the question of what is the most effective antiseptic solution to prevent CRBSI poses this query. Furthermore, can alcohol alone be more effective than the standard Chlorhexidine or povidone iodine with alcohol in skin antisepsis?

Although several guidelines and preventive measures have been proposed to address HCAIlike CRBSI in the pediatric age group there is still much to learn about the antiseptic solutions used for venipuncture. Several studies have been made on the use of alcohol, Chlorhexidine and Povidone Iodine, for antisepsis prior to venipuncture, the search for the most effective antiseptic solution still poses a question.

We wanted to determine and compare the effect on Total Aerobic Count (TAC) before and after application of Isopropyl alcohol, Chlorhexidine, and povidone iodine plus alcohol prior to venipuncture and its correlation to developing phlebitis in children admitted at a tertiary hospital.

In 2004, Barenfanger J., Drake C., et al, did a comparison of Chlorhexidine and Tincture of Iodine for skin antisepsis in preparation for blood sample collection. This study was held in a hospital in Springfield and made use of 11, 738 blood cultures over two periods of time (January to June 2002, and August 2002 to February 2003). This study compared the rates of blood culture contamination when iodine tincture was used to prepare the skin versus those obtained when chlorhexidine was used. The contamination rate for iodine tincture was 2.7%; and for chlorhexidine was 3.1%, which was not significant.[15]

In 2007, Mimoz, O., Villeminey S., Ragot S., et al, made a randomized study on the use of Chlorhexidine-based antiseptic solution versus alcohol based povidone iodine for disinfection of catheter insertion sites and their ability to reduce central catheter related infection. The clinical trial was conducted from May 14, 2004 through June 29, 2006 at a surgical ICU of a University affiliated hospital. A total of 538 central venous catheter inserted into jugular or subclavian veins were randomly assigned to be disinfected with 5% povidone iodine in 70% ethanol or with a combination of 0.25% chlorhexidine gluconate, 0.025% benzalkonium chloride, and 4% benzylic alcohol. Compared with povidone iodine, the chlorhexidine-based solution was associated with a 50% decrease in the incidence of catheter colonization 11.6% vs 22.2%; with a trend towards lower rates of catheter-related bloodstream infection. The use of a chlorhexidine-based solution rather than povidone iodine is likely to result in decreased catheter colonization, hence should be considered as a replacement for povidone iodine or combination of alcohol and povidone iodine. [14]

Reichel M., Heisig P., et al, made a prospective, randomized, uni-center, double-blind study and determined the most effective antiseptic solution in reducing the populations of aerobic skin flora in 180 volunteer subjects. In all subjects, ethanol, *n*-propanol, and isopropanol were tested in parallel at a specific concentration and for a specific application time. An application time of 2 minutes was significantly less effective than 3 minutes and 4 minutes on the forehead and the upper back, regardless of the type of alcohol or its concentration. This study made use of different concentration and type of alcohol as skin antisepsis over different skin sites, using the standardized swab sampling method. In their

study, n-propanol at an 89.5% concentration was the most effective alcohol in reducing aerobic skin flora but a higher concentration or longer duration of application of ethanol, or isopropyl alcohol is needed to achieve the same reduction. Its combination with Chlorhexidine is appropriate whenever re-colonization of the skin must be limited. Recommendations of the study included further studies to determine the most effective concentration of Chlorhexidine in *n*-propanol to provide the best protection against re-colonization of the skin. [17]

In 2009, Kiyoyama, T., Tokuda, Y., et al, conducted a prospective, nonrandomized partially blinded study in a community teaching hospital in Okinawa Japan. The study participants were children more than 15 years old who were suspected of having bacteremia between October 2007 and March 2008. This study elucidated the additive efficacy of two antiseptics, 70% isopropyl alcohol only and 70% isopropyl alcohol plus povidone-iodine in reducing contamination rates of blood cultures. There was no significant difference in the contamination rates between the two groups. This study concluded that the use of a single application of 70% isopropyl alcohol is a sufficient and a more cost- and time-effective method of obtaining blood samples for culture than the use of a combination of isopropyl alcohol and povidone-iodine. [11]

The studies mentioned were randomized clinical trials testing the effectiveness of different antiseptic solutions (alcohol, povidone iodine and chlorhexidine alone or in combination) in decreasing bacterial load or contamination rate in blood cultures, or surgical sites. However, only the study done by Reichel M., Heisig P., et al, [17] made use of different types of alcohol (n-propanol and ethanol) in reducing aerobic skin flora prior to venipuncture.

This is the first study conducted locally in a tertiary pediatric hospital to compare the efficacy of three antiseptic solutions (povidone iodine and alcohol, chlorhexidine alone and isopropyl alcohol alone) as antiseptic agent/s prior to venipuncture.

MATERIALS AND METHODOLOGY

The study was a randomized clinical trial conducted at a Philippine Childrens Medical Center, a Tertiary Hospital in Quezon City. The study population included all patients one year old and above with physician orders for IV insertion and extractions, with a signed written informed consent from parents or guardians, and a written assent from patients seven years old and above. Only those patients that had initial IV insertion and extraction were included in the study.

Excluded in the study are those with known sensitivity to any component of the antiseptic solutions, those with secondary bacterial infection or active skin lesion over the area or site for venipuncture, those patients with multiple attempts of intravenous insertions and those unstable or critically ill patients. The study took place at Emergency Room (ER) and Service ward of Philippine Childrens Medical Center a Tertiary Children's Hospital. The research team headed by the primary investigator conducted the study. The team was comprised of a Medical Technologist (MT), a research assistant, a statistician and Pediatric Residents assigned at the ER and service ward during the study period.

The eligible participants were randomized into one of the three groups – (Group A) Isoprophyl Alcohol only, (Group B) Chlorhexidine group and (Group C) Povidone plus Iodine Alcohol Group. They were stratified according to intravenous cannula insertion and extraction.

The study was not observer-blinded as the solution look considerably different in terms of color, consistency and smell. The members of the research team assigned to supervise the application of the treatment have access to the unblinded data. Complete blinding of the participants will not be possible.

The agar plates used in this study were commercially prepared with sterility and labeling control, and were subjected to quality control. A registered Medical Technologist (MT) was responsible in handling and plating

the discs in a controlled setting using a biosafety cabinet or a laminar flow hood. The specimens were incubated for a total of 72 hours and were observed on the 24th, 48th and 72nd hour of incubation.

The primary investigator followed up the patients at the ward for development of phlebitis using the Jackson's Visual Infusion Phlebitis Scale Performa. This grading scale has been validated in the study of Nagpal, P., Khera G., et. al., The patients were observed for 72 hours. This was done on the 24th, 48thand 72nd hour from the time the venipuncture was done.

Three Antiseptic solutions were used in this study, 10% povidone-iodine in 70% Isopropyl alcohol, 0.25% chlorhexidine gluconate, and 70% Isopropyl alcohol. The subjects were chosen from the admitted patients at the Emergency Room and the service ward. The research assistant/primary investigator provided the Medical Technologist with the envelope containing the materials for the procedure. The resident in charge (RIC) performed proper hand washing prior to starting the procedure. The RIC determined the site of venipuncture (dorsal aspect of the hand or antecubital area of the arm). All the materials needed for the procedure were set up at bedside. A registered Medical Technologist determined the primary outcome measure of this study. All data gathered by Medical Technologist were recorded in a separate sheet and was retrieved by the primary investigator at the end of data collection for analysis. Immediate reactions noted after the application of the antiseptic solution were mostly erythema and were addressed by the research team or the primary investigator.

The primary outcome measure is the total aerobic count in each blood agar plate before and after the application of the tested antiseptic solution and its correlation with the development of phlebitis. A scoring system was used to assess the presence of phlebitis (Appendix I). Secondary outcome measures included the incidence of immediate adverse effects and delayed reactions.

Descriptive statistics was used to summarize the demographic and clinical characteristics of the patients. Frequency and proportion was used for nominal variables, median and range for ordinal variables, and mean and SD for interval/ratio variables. Kruskal-Wallis test was used to compare the median across groups while Fisher's Exact test was used to test the association of the groups to the demographic variables. Sign test was used to determine if there is an improvement in the CFU/mL after using the antiseptic for each of the treatments. All valid data were included in the analysis. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05 α-level of significance. STATA 12 was used for data analysis.This paper was presented to the Institutional Review Board, Ethics Committee of a Tertiary Children's Hospital.

RESULTS

One hundred twenty-nine patients were recruited for this study. Forty-three(43) subjects were assigned in each of the three groups designated to use three different kinds of antiseptics prior to venipuncture. Comparative analysis of the demographic profile of the patients assigned in the three groups showed no significant difference in terms of age, sex, purpose and site of venipuncture (Table 1). The mean age of the subjects was five, seven and eight years old from the Alcohol group, povidone iodine plus alcohol group and Chlorhexidine group, respectively, with a median age of 1 to 18 years old. The subjects of each group were further stratified into blood extractions, and intravenous catheter insertion. The site of venipuncture for venous extractions was all taken from the antecubital area. The site of venipuncture for intravenous insertions was done on the dorsal aspect of the hand. There were 4 subjects each from the alcohol group and betadine plus alcohol group with venipuncture in inserted on the foot.

Table 1. Demographic and Clinical Profile of Children who underwent Venipuncture (n=129)

	Alcohol (n = 43)	**Povidone Iodine plus alcohol (n = 43)**	**Chlorhexidine (n = 43)**	**P-value**
	Mean ± SD, Frequency (Expected), median (range)			
Age	7 (1-18)	5 (2-18)	8 (2-17)	0.567 ŧ
Sex				0.534 ǂ
Male	21 (20.0)	22 (20.0)	17 (20.0)	
Female	22 (23.0)	21 (23.0)	26 (23.0)	
Purpose of venipuncture				1.000 ǂ
Blood extraction	22 (21.7)	21 (21.7)	22 (21.7)	
Peripheral IV access	21 (21.3)	22 (21.3)	21 (21.3)	
Site of venipuncture				0.279 ǂ
Antecubital	22 (21.3)	21 (21.3)	22 (21.3)	
Dorsal aspect of hand	17 (18.7)	18 (18.7)	21 (18.7)	
Foot	4 (2.7)	4 (2.7)	0 (2.7)	

Table 2 shows that 71% (15), 72% (16) and 100% (21) of the patients in the alcohol group, betadine group and Chlorhexidine group utilized soluset as mode of infusion. Six subjects from the Alcohol and povidone iodine plus Alcohol group was maintained on heplock. Majority of the infusion rate was between 50-100 mL per hour (Alcohol n= 11, povidone iodine plus Alcohol n=16, and Clorhexidine n= 21). Gauge 24 needle was used in nine of the subjects in the alcohol group, 18 in the povidone-iodine plus alcohol group and 21 from the chlorhexidine group. Crystalloid was the most common intravenous fluid infused in this study. In all the groups, antibiotics were the type of intravenous drug.

Table 2. Clinical Characteristics of patients with an intravenous catheter (n=64)

	Alcohol (n = 21)	Povidone Iodine plus alcohol (n = 22)	Chlorhexidine (n = 21)	**P-value**
	Frequency (%)			
Mode of infusion				**0.027**
Continuous	0	0	0	
Intermittent	0	0	0	
Bolus	0	0	0	
Through soluset	15 (71)	16 (72)	21 (100)	
None (Heplock)	6 (29)	6 (28)	0	
Rate of infusion				**0.030**
< 50ml/hr	2 (13.33)	0	0	
50-100ml/hr	11 (73.34)	16 (100)	21 (100)	
> 100ml/hr	2 (13.33)	0	0	
Total amount of fluid infused in 72 hours				**0.004**
< 250ml	0	0	0	
> 250 to 1000ml	9 (60)	14 (87.5)	21 (100)	
>1000 ml	6 (40)	2 (12.5)	0	
Size of intravenous cannula/ needle gauge used				**0.016**
Gauge 20	0	0	0	
Gauge 22	0	0	0	
Gauge 24	14 (42.86)	18 (81.82)	21 (100)	
Gauge 26	7 (19.05)	4 (13.64)	0	
Types of intravenous fluid infused				0.155
Crystalloid	15 (100)	18 (41.86)	21 (100)	
Free water solution	0	0	0	
Colloid	0	0	0	
Potassium Chloride	0	0	0	
Bicarbonate	0	0	0	
Blood products	0	2 (4.65)	0	
Type of IV drug				0.660
Antibiotic	15 (71.43)	14 (63.64)	16 (76.19)	
Other medications	0	0	0	

Table 3 presents the incidence of phlebitis from the three groups of antiseptic solutions. No phlebitis was noted among the three groups during the first 24 hours of placement of an IV catheter or extraction. On the 48th and the 72nd hour of observation, there was note of chemical phlebitis in two subjects in the alcohol group. These were the subjects that received more than 1000ml of IV fluids within 72 hours.

Table 3. Incidence of phlebitis in the Alcohol, Betadine plus Alcohol and Chlorhexidine group at different time of observation.

	Alcohol (n = 43)	**Povidone Iodine plus alcohol (n = 43)**	**Chlorhexidine (n = 43)**
	Frequency (Expected)		
Phlebitis			
24 hours	0 (0 %)	0 (0 %)	0 (0 %)
48 hours	2 (4 %)	0 (0 %)	0 (0 %)
72 hours	2 (4 %)	0 (0 %)	0 (0 %)

The clinical pattern of phlebitis grading among the three groups were determined using the Jackson's Visual Infusion Phlebitis Scale Performance. Emphasis in Figure 1 presented the pattern of phlebitis in the Alcohol only group. One hundred percent (n=43) of the subjects in the Alcohol group had a Grade of 0 during the 24th hour of observation. It is described as an IV site that appeared healthy. On the 48th hour of observation, 2 patientshad Grade 1 phlebitis described as erythema or pain over the IV site. During the 72 hours of observation 2 patients had grade 2 phlebitis.

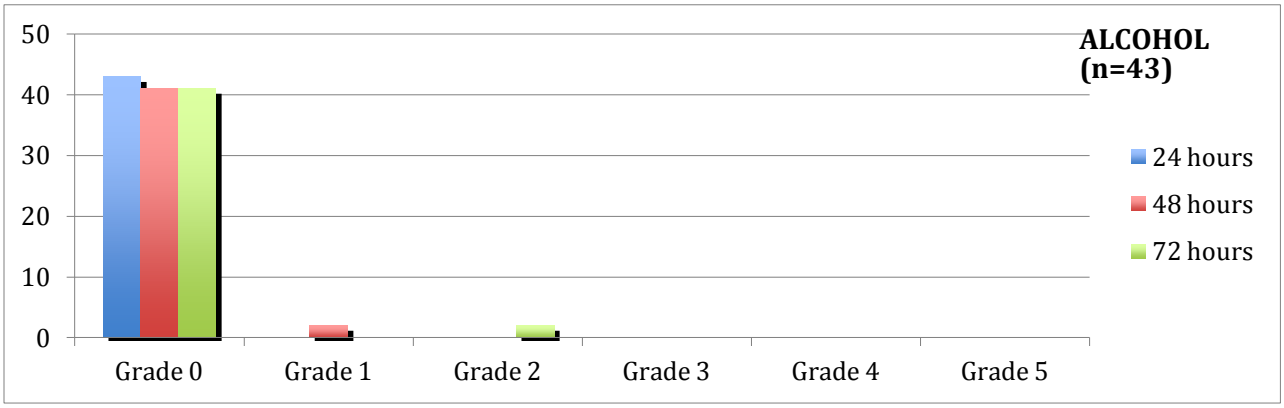

Figure 1: Clinical Pattern of phlebitis secondary to venipuncture among the Alcohol group at different hours of observation.

As shown in figure 2 none of the 43 patients in the Povidone Iodine plus Alcohol group developed phlebitis at different hours of observation.

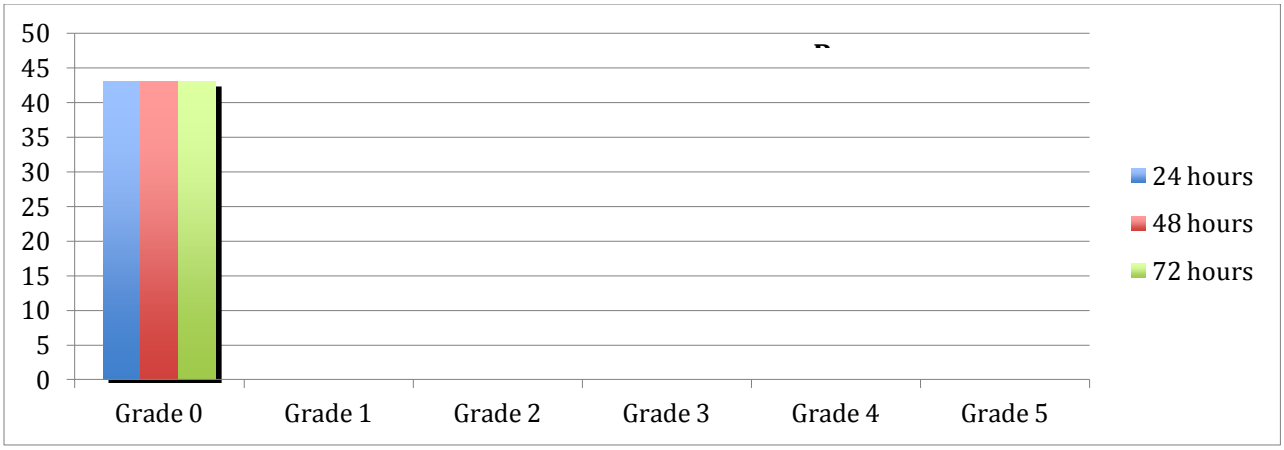

Figure 2: Clinical Pattern of phlebitis secondary to venipuncture among the Povidone Iodine plus Alcohol group at different hours of observation.

Figure 3 showed that all patients (n=43) in the Chlorhexidine group did not develop phlebitis at different hours of observation

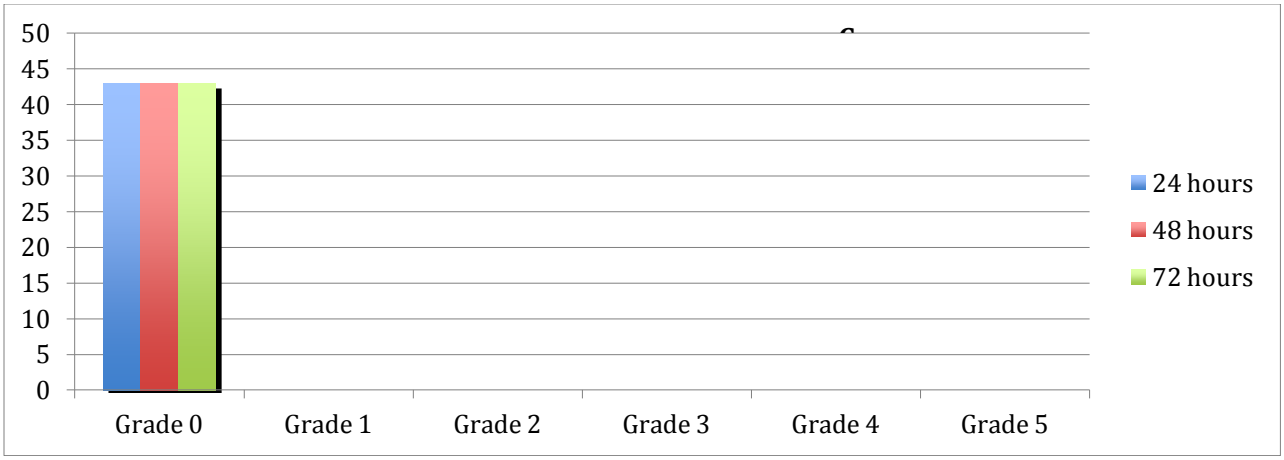

Figure 3: Clinical Pattern of phlebitis secondary to venipuncture among the Chlorhexidine group at different hours of observation.

The colony forming units per mL (CFU/mL) was computed after counting the bacterial colonies pre and post application of the three antiseptic solutions. The cotton swab was directly inoculated on the plates hence the inoculum was undiluted.The values of the CFU/mL for the three groups were compared and analyzed. The CFU/mL prior to the disinfection of the three antiseptics was not significantly different. However, the CFU/mL after disinfection was significantly different between groups, with the lowest CFU/mL observed among patients disinfected with Chlorohexidine.

Table 4. Colony Counts Pre and Post Application of Alcohol, Povidone Iodine plus Alcohol and Chlorhexidine solution

	Alcohol (n=43)	**Betadine (n=43)**	**Chlorhexidine (n=43)**	**P-value ǂ**
	Median (Range)			
Total Aerobic Count on patients' skin prior to venipuncture (CFU/mL)				
Before	530 (40-6000)	650 (30-9000)	1200 (130-7000)	0.2169
After	10 (0-760)	0 (0-90)	0 (0-0)	**0.0001**
Percentage Difference (%)	96 (17-100)	100 (75-100)	100 (100-100)	**0.0001**
P-value ǂ	**0.000**	**0.000**	**0.000**	

Statistical tests used: ǂ Kruskall Wallis test; ǂ Sign test

The site of venipuncturewas observed for immediate and delayed reactions after using the three antiseptics. Only erythema was observed in two patients who were under the alcohol group. No reactions were observed from patients who were disinfected with povidone iodine plus alcohol and chlorohexidine.

Table 5. Adverse reactions observed pre and post application of Alcohol, Povidone Iodine plus Alcohol and Chlorhexidine solution (n=129)

	Alcohol (n = 43)	Povidone Iodine plus Alcohol (n = 43)	Chlorhexidine (n = 43)	P-value
	Frequency (%)			
Intermediate adverse reactions				0.328
Erythema	2 (4.65)	0	0	
Itchiness	0	0	0	
Blanching	0	0	0	
Edema	0	0	0	
Others	0	0	0	
Delayed adverse reactions				0.328
Erythema	2 (4.65)	0	0	
Itchiness	0	0	0	
Blanching	0	0	0	
Edema	0	0	0	
Others	0	0	0	

Statistical test used: Fisher's Exact test

DISCUSSION

We wished to determine the effect on the total aerobic count before and after application of different antiseptic solutions prior and post venipuncture. The results of this study showed that gender and age is an independent variable and cannot affect the incidence of phlebitis in children.

The results of the study showed that only 2% of the subjects developed Grade 1-2 phlebitis based on the Jackson's visual infusion. Compared to the study of Nagpal, this study showed that majority of children developed Grade 2 phlebitis. A hospital in Portugal showed that these findings are consistent with the study conducted by Pedro, et al., which showed that among the 317 patients admitted at a central hospital in Portugal there was higher incidence of Grade 2 phlebitis at 44.5% from peripherally intravenous catheters.

Clinical characteristics of patients with intravenous access were noted in this study. These factors, included mode of infusion, rate of infusion total amount of infusion, site of IV cannula, and IV fluids infused. Compared to the study of Nagpal et al, there was a higher percentage of children receiving free water solutions who developed phlebitis, However, in this study, most of the patients received crystalloids. Analysis showed that this was not a factor in the development of phlebitis.

The present study further revealed that the incidence of phlebitis was noted on the 48th and 72nd hours of observation in the Alcohol group. However, the study of Nagpal, et al said otherwise. In their study the incidence of phlebitis started to increase 3.7% on the day of insertion to 21.2 % after 24 hours and by 27.5% after 48 hours and was maximum on the third day. These findings of the study were consistent with the findings of study by Annamaria, which concluded that onset of signs of phlebitis increase from 12% to 34% after first 24 hours and by 65% after 48 hours.

The present study showed thatthe use of the different antiseptic solutions was sufficient in reducing CRBSI. The results of this study showed that alcohol, povidone iodine plus alcohol and chlorhexidine, had a statistical difference in the bacterial growth pre and post application. A single application of alcohol may be effective in preventing CRBI. However, it is not effective in eradicating the microbial growth as compared to the Chlorhexidine and povidone iodine plus Alcohol group. The review of literature showed evidence that Alcohol provides a rapid bactericidal effect against most gram-positive and gram-negative bacteria, *Mycobacterium tuberculosis*, and certain enveloped viruses as a result of protein coagulation and denaturation.

Alcohol is the most common antiseptic solution used in the hospital setting for skin

antisepsis prior to any procedure including venipuncture. The Centers for Disease Control and Prevention (CDC) recommend both ethanol and isopropanol as an active agent to prevent sursigal site infection. The U.S. Food and Drug Administration (FDA) assessed aqueous ethanol at 60% to 95% and isopropanol at 70% to 91.3% (vol/vol) as safe and effective for patient preoperative skin preparations. The study by Reichel et al, revealed that n-propanol is as effective however, it is not approved by the FDA to be safe and effective for skin antisepsis. Hence, the use of isopropanol and ethanol at a higher concentration and longer duration is needed to achieve the same effect. No systematic studies have compared the efficacy of these alcohols in skin antisepsis, although similar findings have been reported for hand hygiene. In this study, the alcohol preparation used was 70% isoprophyl alcohol that was applied over the venipuncture site prior to the procedure for 30 seconds. Results showed that the use of alcohol showed significant decrease in the TAC post application, but did not provide complete eradication of bacterial growth.

The use of povidine iodine (PVI) and iodine povidine alcohol (IPA) was more clinically feasible to reduce contamination rates because none of the group developed phlebitis and adverse reaction. The use of PVI results in the slow and continuous release of free iodine that is effective against almost the same spectrum of microorganisms as alcohol, but can be neutralized by proteins on the skin surface. Venipuncture of a patient's arm does not need such a persistent antimicrobial effect, and no data about the different depths to which the antiseptics permeate the patient's skin. Therefore, if alcohol permeates the skin surface as well as PVI does, the application of PVI theoretically cannot have an additional antiseptic effect. However in this study, it was noted that compared to the alcohol alone group, there was note of significant eradication of bacterial growth with the PVI plus IPA group that was comparable with the standard which is chlorhexidine.

The results of the current study supportthe study reported by Calfee and Farr. The study compared the efficacies of four skin antiseptics, including 10% PVI, 70% IPA, tincture of iodine, and PVI with 70% ethyl alcohol to prevent blood culture contamination. They recommend that IPA was the optimal antiseptic because there were no significant differences in the blood culture contamination rates when these four antiseptics were used. However, the reason for the significantly lower positive culture rate for the group of patients for whom the IPA disinfectant was used is not clear.

This study only showed grade 1-2 score of phlebitis described as erythema or pain over the site of venipuncture based on the Jackson's Infusion Scale Performa. The adverse reactions however were possible inevitable occurrence that cannot be purely attributed to these agents.

CONCLUSION

Our study showed that both Chlorhexidine and Povidone iodine plus alcohol were effective in eliminating bacterial colonies as both showed 0 TAC after treatment. Alcohol also decreased TAC (96% decrease) but to a lesser extent. The difference among the 3 treatments was statistically significant pre and post disinfection. The use of chlorhexidine and povidone iodine plus alcohol did not result in phlebitis while 2 patients randomized to the alcohol alone group developed phlebitis.

In conclusion, a single application of Chlorhexidine is the optimal method to be used as antisepsis prior to procedures like venipuncture. The use of povidone iodine plus 70% isopropyl alcohol also has comparable effect to Chlorhexidine, as it can also decrease total aerobic count if not eliminate microbial growth. Furthermore, 70% isopropyl alcohol can significantly decrease the total bacterial count by 10 times. However, it is not as effective as the Povidone Iodine plus alcohol and Chlorhexidine in total elimination of the growth of bacteria. Also, all three solutions could equally prevent the development of phlebitis if the procedures of venipuncture and proper aseptic technique are observed. The most common adverse event observed was erythema noted in 4.65% of subjects in the alcohol only group. No adverse

effects occurred in the chlorhexidine and povidone iodine plus alcohol group.

Since Povidone Iodine plus alcohol has comparable effect in reducing total aerobic count to chlorhexidine, it can be used as the standard antiseptic solution not only for blood culture extractions but also for intravenous extractions and other blood extractions.

Some limitations should be acknowledged. The assessment of the insertion site was limited only for three days. It is therefore recommended that a longer time of assessment be done or the previous IV insertion site or extraction site should be observed even after the IV catheter was removed. The subjects were limited to children ages 1 until 18 years old, and were only taken from the patients admitted at the emergency room and the service ward. It is recommended that a study on the safety and efficacy of these antiseptic solutions in the younger age group, like neonates conducted in the future. It is also recommended that a larger sample size be used to establish an association between a total aerobic count and phlebitis.

The study was also limited in identifying the total aerobic count before and after venipuncture. It is therefore recommended that future studies be made on the identification of the specific microorganism that can cause phlebitis in patients who had venipuncture. The primary investigator recommends future researches that will assess the use of different concentrations of alcohol in changing (increase or decrease) the bacterial count prior to venipuncture.

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