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EDITORIAL

RACE TO THREE

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We are on with our 3rd issue since we took on this task 3 years ago.

The number three spells mystery and magic. In the Biblical tradition, it symbolized wholeness, completeness, perfection. In the Chinese culture, it spelled luck. It is the first number to which "everything" was ascribed. It is the number representing the whole - it has a beginning, middle, and an end.

This is what this 3rd release is all about. There is no unifying theme to speak of, just a cacophony of medical literature drafted to perfection.

This issue takes pride in studies on vaccination programs – A Prospective Cross-Sectional Study on the Prevalence and Factors Associated with Seroprotection after Primary Series of Hepatitis B Vaccination and Compliance to the National Immunization Program of Grade 1 Students in a Public School in Manila.

Sepsis updates are in with A Meta-Analysis on the role of GCSF in Improving Outcomes of Neonatal Sepsis and Diagnostic Accuracy of Renal Angina Index in Predicting Acute Kidney Injury in Pediatric Patients with Sepsis.

Dengue is not to be missed with a study looking into The Relationship between Immature Platelet Fraction and Platelet Count among Pediatric Patients with Dengue Fever.

Lastly, in this era of multi-drug resistant organisms, we give you A Study on the Outcome of Children with Extensively Drug Resistant Gram-Negative Infection Treated with Colistin vs Other Antimicrobials.

May these writings, diverse they may be, contribute to your wholeness - enrich your mind, calm your body, and liven up your spirit -- just as organized noise turns into music and soothes the soul.

NOTE: There has been an erratum in the uploading of the paper entitled Serum Concentration of Pyrazinamide Suspension in Children with Tuberculosis: A Therapeutic Drug Monitoring published in the PIDSP Journal Vol. 9, No. 2, July-Dec 2005. Please find the correct link to the article as follows http://www.pidsphil.org/home/2005-journals/.

ORIGINAL ARTICLE

A PROSPECTIVE CROSS-SECTIONAL STUDY ON THE PREVALENCE AND FACTORS ASSOCIATED WITH SEROPROTECTION AFTER PRIMARY SERIES OF HEPATITIS B VACCINATION

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

OBJECTIVES: To determine the prevalence and factors associated with seroprotection among children 3 months to 18 years old with primary Hepatitis B vaccination series.

METHODOLOGY: This is a prospective cross-sectional study done among children 3 months to 18 years old with complete primary series of Hepatitis B vaccination. Demographic, social and clinical data were correlated with reactivity to antibody to Hepatitis B surface antigen (anti-HBs) ($\geq$10 IU/L), total antibody to Hepatitis B core antigen (total anti-HBc) and Hepatitis B surface antigen (HBsAg) serologic tests.

RESULTS: Among 110 subjects from different age groups, 52% had seroprotective anti-HBs levels, with the highest noted among infants (3 months-2 years) at 82%, followed by 41% from the childhood group (3-9 years) and 26% from adolescent group (10-18 years). Seventy-four percent of subjects with <5 years interval from vaccination were seroprotected, 26% in subjects after 5-10 years, and 38% at more than 10 years after vaccination with significant difference on multi-logistic regression (p value 0.000/0.020). None of the other factors including gender, geographic area, age at first dose, vaccination schedule, type and place of vaccination were significantly associated with seroprotection.

CONCLUSION: Fifty-two percent of patients among different age groups were seroprotected. Seroprotection was significantly associated with the interval year after vaccination demonstrated at < 50% 5 years and beyond post-vaccination.

KEYWORDS: *Hepatitis B seroprotection, Hepatitis B immunization, anti- HBs*

INTRODUCTION

The burden of Hepatitis B infection cannot be underestimated. The disease accounts for 30% of liver cirrhosis and 53% of hepatocellular carcinoma (HCC) cases worldwide. In young children, most Hepatitis B infections are asymptomatic and unrecognized until complications develop after decades. This disease though is a vaccine-preventable one since the introduction of the hepatitis B vaccine.

Information on the prevalence of seroprotection among children who completed Hepatitis B vaccine in the country is still limited. Factors affecting such response to Hepatitis B vaccine have been shown in some studies [1]. Furthermore, among those who initially responded to a primary three-dose vaccination series, around 15–50% demonstrate low or undetectable antibody to Hepatitis B surface antigen (anti-HBs) levels 5–10 years after primary vaccination [2]. Since the infection and its sequelae are not essentially an issue for childhood survival, Hepatitis B immunization primarily targeting young children remains to be under prioritized [3].

While the universal infant Hepatitis B vaccination was introduced into the National Immunization Program in 1992, because of insufficient funds, the program was never fully implemented in our country until January 2007[4]. And despite an 88% estimated coverage with three doses of Hepatitis B-containing vaccine after a birth dose, we know that seroprotection is more important as this translates to prevention of the disease rather than immunization coverage.

This paper aims to determine the prevalence of seroprotection among children given primary series of Hepatitis B vaccine and look onto the factors that may affect response. Data on these can help identify certain areas that need improvement in our national immunization program including vaccination schedule recommendation to fully combat the scourge of Hepatitis B infection and address factors that affect seroprotection after primary immunization with hepatitis B vaccine.

MATERIALS AND METHODS

This prospective cross-sectional study was done among children ages 3 months to 18 years old seen at outpatient department with records showing complete immunization of primary series of Hepatitis B vaccine with details on the vaccination schedule used, number of doses, place of vaccination, type of vaccine and the site of injection. Children without any record or with incomplete record of immunization history, in immunocompromised state, those who received the last dose of vaccine within four weeks from the time of conduct of the study and those who received booster doses of hepatitis B vaccine were excluded.

Study Procedure

Subject recruitment of children ages 3 months to 18 years old among patients at the pay and charity outpatient department with records of immunization of primary series of Hepatitis B vaccine was done. The principal investigator screened for potential subjects eligible for the study. Informed consent/assent was obtained, and history and physical examination were done. Laboratory requests were given to the parents for the child's serologic examination where ~ 5 ml of blood was obtained. Determination of Hepatitis B surface antigen (HBsAg), antibody to Hepatitis B surface antigen (anti-HBs) and total antibody to Hepatitis B core antigen (total anti-HBc) using enzyme-linked immunosorbent assay (ELISA) method were done in our medical center's laboratory. Parents/guardians were notified in person or via phone communication of the results of serologic tests and medical advice was given accordingly.

Based on statistics that children aged 1 to 5 years old with presenting symptoms of HBV infection would have probability of presenting with acute hepatitis at a rate of 5% to 15% [5], then, using the 5% rate of disease to develop with +/-5% margin

of error estimated at CI 95%, the sample size needed in this study was 100 cases as representative of patients in the general and adolescent pediatrics who have immunization records showing the status of hepatitis B vaccine during their infancy. The sample size was computed with projected dropout rate, the equation 100/(1-0.10) was used to arrive at a final sample size of 111 subjects.

Sample size [100] = {[1.96]^2 x [0.15] x [1-0.07]} / [0.07]^2

Ethics and IRB approval

This study adhered to the ethical principles set out in relevant guidelines, including the Declaration of Helsinki, WHO guidelines, International Conference on Harmonization-Good Clinical Practice, and National Ethics Guidelines for Health Research. This paper has been reviewed by the Institutional Review Board-Ethics Committee of the Philippine Children's Medical Center and was funded by the same institution, as well as by the Philippine Pediatric Society.

Operational Definition of Terms:

1. Seroprotection- the point in time when the amount of antibody in the blood is high enough to confer protection from the antigen that induced its production[6]. Seroprotection against HBV infection is having an anti-HBs level ≥10 IU/L when measured at least 1-3 months after having received a complete immunization schedule[7] using enzyme linked immunosorbent assay (ELISA) method.
2. Primary series of Hepatitis B vaccine-

a. Three-dose series of Hepatitis B vaccine using the schedule 0-1-2, 0-1-6, or other schedule, given at a minimum of 4-week interval, with or without a fourth dose (in cases when the third dose was given at < 24 weeks of age, or when the first dose was given to infants weighing < 2 kgs, OR

b. Three or four-dose Hepatitis B vaccine series as part of the National Immunization Program given by the Department of Health (0-6-10-14)

3. Booster – refers to a vaccination given some time after a primary vaccination series and with the aim of providing rapid protective immunity against a significant breakthrough infection[8]
4. HBsAg nonreactive, anti-HBs reactive, total anti-HBc nonreactive – there is seroprotection against Hepatitis B infection as a result of previous vaccine
5. HBsAg nonreactive, anti-HBs nonreactive, total anti-HBc nonreactive – low or no protective antibodies against Hepatitis B infection despite vaccination which may either be due to waning levels of antibody, or nonresponse to the vaccine
6. HBsAg reactive, anti-HBs nonreactive, total anti-HBc nonreactive – the patient is either in an early acute state of Hepatitis B infection, or in a carrier state with low or no protective antibody
7. HBsAg nonreactive, anti-HBs reactive, total anti-HBc reactive – there are protective antibodies as a result of natural infection with Hepatitis B
8. Place of vaccination – where vaccination was administered either from private clinic, local health center or both
9. Site of vaccination – part of the body where the vaccine was injected either in the thigh, deltoid, gluteal area, etc.
10. Type of vaccine - either monovalent vaccine alone was used or combination (two or more vaccines given as single shot) with or without monovalent dose
11. Age at the first dose of vaccine - vaccination given within 24 hours, > 24 hours-7 days, or > 7 days
12. Schedule of vaccine – schedule followed in giving of vaccine either 0-1-2, 0-1-6, 0-6-10-14, others
13. Interval years from last dose of vaccine to the conduct of this study – either 0-4 years, 5-10 years, > 10 years

Data Collection and Outcome

The following data were gathered from the subjects: age, gender, weight, length/height, weight-for-length / weight-for-height / BMI ; presence of jaundice, icteric sclera, organomegaly and other physical findings that may pertain to liver disease; parental educational attainment (elementary, high school, college, others); parents' occupation, vital status of each parent (alive or

deceased); socioeconomic status based on the income bracket, geographical area or the patient's place of residence based on the regions of the country where the child spent more than half of his lifetime. For analysis against seropositivity to HBsAg, the following data were also obtained: objective evidence of maternal HBsAg status (reactive, nonreactive, unknown) during prenatal check-up, history of transfusion of any blood product ≥ 1 week prior to conduct of study, mode of delivery (vaginal or caesarean section); history of sexual contact, and/or illicit drug use for subjects 10-18 years old. Patients' immunization records were evaluated and the following information were also collected to be analyzed against seroprotection: the site of vaccine injection, age at the first dose of vaccine, age at succeeding doses to identify the schedule used, interval in years from last dose of hepatitis B vaccine to the conduct of this study, place of vaccine administration and the type of vaccine given.

Seropositivity to anti-HBs, total anti-HBc and HBsAg, were the primary outcomes of interest in this study. Determination of patients' demographic profile and analysis of factors possibly affecting seropositivity were the secondary outcome measures. The association between the previously mentioned variables and the serologic results were statistically analyzed.

Data Processing and Data Analysis

Demographics, anti-HBs, total anti-HBc positivity results and HBsAg seropositivity were expressed in frequency and percentages. In testing associations among patients' profiles and anti-HBs, total anti-HBc or HBsAg seropositivity results, chi square test of independence with 2x2 Fischer Exact test adjustment were performed. Analysis in predicting seroprotection was done using multi-logistic regression modelling as well as estimating the odds ratio CI 95%. Any associated p-value less than 0.05 alpha was considered significant. STRATA ver.14 was the statistical software used in processing the data.

RESULTS

Among the 202 children screened with immunization records, only 163 subjects had complete primary series of Hepatitis B immunization. Upon exclusion using the criteria, 111 parents signed the informed consent, however, one parent refused blood re-extraction for validation of result hence was considered a drop out. A total of 110 subjects were left as study participants where fifty subjects (45%) were male and sixty subjects (55%) were female. The male to female ratio was 1:1.2. All subjects completed their vaccination within 1 year of age. Majority belonged to the age group 3-9 years old (42%) with a mean age in years of 5.23 +/- 4.34 SD. Eighty percent came from the National Capital Region (NCR) belonging to the lower middle income socioeconomic status (49%) but most parents were able to attain tertiary level of education (61%). Eighty-seven subjects (79%) had normal nutritional status (Table 1).

Among 110 subjects, 8 (7%) had nonreactive maternal HBsAg while only 1 (1%) had reactive results. The remaining 101 subjects (92%) had unknown maternal HBsAg status. Five percent had history of blood transfusion, while 93% of patients were delivered via normal spontaneous delivery. Among subjects 10-18 years old, none had history of sexual contact nor illicit drug use.

Table 1. Distribution of Subjects According to Sociodemographic Factors

SOCIODEMOGRAPHIC VARIABLES	TOTAL (n/%)
Age	
3 months-2 years old	41 (38%)
3-9 years old	46 (42%)
10-18 years old	23 (20%)
Mean +/- SD	5.23 +/- 4.34
Gender	
Male	50 (45%)
Female	60 (55%)
Geographic area	
Region I-V	22 (20%)
NCR	88 (80%)
Region VI-VIII	0 (0%)
Region IX-XIII	0 (0%)
Socioeconomic status	
Pay (Upper class)	20 (18%)
C1 (Upper middle class)	13 (12%)
C2 (Lower middle class)	54 (49%)
C3 (Upper lower class)	23 (21%)
Indigent (Lower class)	0 (0%)
Parents' Educational attainment	
Elementary	3 (3%)
High School	37 (33%)
College	67 (61%)
Others - Postgraduate	3 (3%)
Nutritional status	
Normal	87 (79%)
Overweight	13 (12%)
Obese	10 (9%)

Seropositivity to anti-HBs, total anti-HBc, HBsAg

Reactivity to anti-HBs (≥10 IU/L) was noted in 54% (59/110) of the study population (Figure 1). Among them, 52% (57/110) were seroprotected after Hepatitis B immunization having concomitant nonreactive results to total anti-HBc (Table 2) with nonreactive result to HBsAg. Three out of 110 participants (3%) were found to be reactive to total anti-HBc, while 1 subject (1%) had reactive results to HBsAg (Figure 1).

Figure 1: Seropositivity to anti-HBs, total anti-HBc and HBsAg

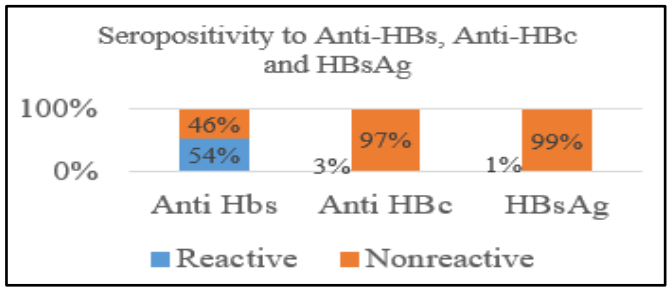

Table 2: Anti-HBs and total anti-HBc Results

	Anti-HBs	
	Reactive (≥ 10 IU/L)	Nonreactive (< 10 IU/L)
Total		
Anti HBc		
Reactive	2 (2%)	1 (1%)
Nonreactive	57 (52%)	50 (45%)

Factors affecting response after primary series of Hepatitis B Vaccine

Among the 57 seroprotected patients from hepatitis B vaccine, the highest seroprotection rate was noted at 82% (32/39) among the youngest age group of 3 months to 2 years, followed by 41% (19/46) among the 3 years to 9 years old group, and only 26% (6/23) from the adolescents 10-18 years old. Fourteen percent of the non-seroprotected subjects (7/51) were as young as 1-2 years old who all received vaccination from the local health center within NCR using combination vaccine with or without monovalent dose.

Majority from the seroprotected group at 56% (32/57) were female (Table 3). However, gender was not significantly associated with seroprotection (p 0.74).

Table 3. Factors Affecting Response to Primary Series of Hepatitis B Vaccine

VARIABLES	SEROPROTECTED (N/%) N= 57	NON-SEROPROTECTED (N/%) N=51	TOTAL N=108	P VALUE
Gender				
Male	25 (51%)	24 (49%)	49 (45%)	0.74
Female	32 (54%)	27 (46%)	59 (55%)	
Geographic Area				
Region I	1 (100%)	0 (0%)	1 (1%)	
Region II	0 (0%)	1 (100%)	1 (1%)	0.15
Region III	2 (29%)	5 (71%)	7 (6%)	
Region IV-A	10 (77%)	3 (23%)	13 (12%)	
NCR	44 (52%)	42 (48%)	86 (80%)	
Place of vaccine				
Private	8 (53%)	7 (47%)	15 (14%)	
Local health center	41 (50%)	41 (50%)	82 (76%)	0.37
Combination	8 (73%)	3 (27%)	11 (10%)	
Age at first dose of vaccine				
Within 24 hrs old	29 (55%)	24 (45%)	53 (49%)	0.79
>24 hours–7 days old	8 (57%)	6 (43%)	14 (13%)	
>7 days	20 (49%)	21 (51%)	41 (38%)	
Schedule of vaccine				
0-1-2	9 (32%)	19 (68%)	28 (26%)	
0-1-6	3 (100%)	0 (0%)	3 (3%)	
0-6w-10w-14w	15 (68%)	7 (32%)	22 (20%)	0.05
Others	30 (55%)	25 (45%)	55 (51%)	
Type of vaccine				
Monovalent alone	22 (41%)	32 (59%)	54 (50%)	
Combination with or without monovalent	35 (65%)	19 (35%)	54 (50%)	**0.01**
Interval years from the last dose of vaccine				
0-4 years	42 (74%)	15 (26%)	57 (53%)	
5-10years	10 (26%)	28 (74%)	38 (35%)	**0.0000**
>11 years	5 (38%)	8 (62%)	13 (12%)	

Seventy seven percent (44/57) from the seroprotected group came from NCR (National Capital Region). All patients were injected in the thigh. Considering the place of vaccination, the number of seroprotected and nonseroprotected subjects were almost equally distributed among those from the private clinic and from the local health center. Although 72% (41/57) of the seroprotected group received their vaccines from local health center, this was not statistically significant (p 0.37), similar to geographic area when analyzed with seroprotection (p 0.15). Only 49% (53/110) of our study population received their

initial dose within 24 hours as recommended. Fifty one percent (29/57) of the seroprotected group received their first dose within 24 hours of birth, while 35% (20/57) were given beyond 1 week of age. Data also revealed that 53% of the seroprotected group (30/57) followed non-specific schedules with minimum of one-month interval between doses. None of these parameters (age at first dose and schedule followed) showed significant association with seroprotection, having p values of 0.79 and 0.05 respectively (Table 3).

The type of vaccine showed a significant difference when analyzed against seroprotection rate. Thirty-five patients (61%) from the seroprotected group were given combination vaccine, with a significant p value of 0.01. Among those who received monovalent type only, 59% (32/54) were nonseroprotected. Meanwhile, majority of those given combination vaccine were seroprotected at 65% (35/54). Protective anti-HBs levels were maintained in 74% (42/57) of subjects less than 5 years after vaccination, 26% (10/38) in cases after 5-10 years, and 38% in cases after 10 years from the last vaccine. This interval year from the last vaccination was analyzed and was also noted to have a significant p value of 0.0000.

Multilogistic regression analysis done on statistically significant variables (type of vaccine and interval years from last vaccination) revealed that only the interval time after vaccination was significant (Table 4). Children are 7 (1/0.13) times less likely to be seroprotected 5-10 years after the last vaccination and children are 4 (1/0.22) times less likely to be seroprotected more than 10 years after vaccination compared to children less than 5 years after vaccination.

Table 4. Multilogistic Regression Analysis of Interval Years as a Factor Affecting Seroprotection

	Odds Ratio	P>|z|	[95% Conf. Interval]	
Interval years from last vaccine dose				
0-4 years				
5-10 years	0.1275510	0.000	0.0502177	0.3239747
>10 years	0.2232143	0.020	0.0631043	0.7895593

Seropositivity to HBsAg and total anti-HBc

Out of 110 subjects, the only patient found to be reactive to HBsAg had concomitant reactive result to total anti-HBc and nonreactive result to anti-HBs (Table 2). The subject is a 6-year-old female, born to an HBsAg positive mother via normal spontaneous delivery (NSD), with no history of blood transfusion, and who received a birth dose of Hepatitis B vaccine together with Hepatitis B immunoglobulin within 12 hours of birth from a private hospital. Patient was asymptomatic at the time of serological survey.

Two of the study participants tested reactive to both total anti-HBc and anti-HBs, with nonreactive HBsAg. One was a 4-month-old male born to a mother with an unknown HBsAg status, delivered by NSD, received his first dose of vaccine at 1 week of life from the local health center. The other one was a 4-year-old female also born to a mother with an unknown HBsAg status, delivered by NSD, given the first dose of vaccine at 1 month of life from the local health center. Both were asymptomatic with no history of blood transfusion.

DISCUSSION

Hepatitis B immunization remains to be the mainstay in the prevention of Hepatitis B infection due to unavailability of specific treatment for the said virus. A complete series of Hepatitis B vaccine induces protective antibody levels in more than 95% of infants, children and young adults [9].

The prevalence rate of the anti-HBs among the pediatric Hepatitis B vaccinees after 5-11 years since primary immunization was determined to be 71.5% in 2007[10]. In an unpublished study in Cagayan de Oro in 2014, about 54% of children had anti-HBs seroprotective levels (>10mIU/ml) 5-6 years after three doses of Hepatitis B vaccine[11], while another unpublished study in Cebu City in 2015 found a seroprotection rate of 48% among children 3-6 years old[12]. In our study done among different age groups, 52% of the overall population were found to have seroprotective anti-HBs levels

after a complete Hepatitis B immunization. However, when grouped according to age, 82% were seroprotected among ages 3 months to 2 years old, 41% among 3-9 years old, and only 26% among 10-18 years old. Due to higher availability of immunization records among younger infants and children, majority of the study participants came from the said age groups and less from the adolescent group, which contributed to the non-homogenous distribution of subjects by age.

Previous studies demonstrated that anti-HBs titers decline over time[6,7]. Though according to a meta-analysis, protection provided by three or four doses of monovalent HB vaccine persists for at least two decades in the great majority of immunocompetent individuals[8], in some studies, antibodies have been demonstrated to become negative in 15-50% of the vaccine responders within 5-10 years[2, 13]A study in 2014 found that 88% seroprotection was seen in less than 5 years after vaccination, however, with less significant decrease to 78% between 5-10 years after vaccination, and 74% 10 years after vaccination[14]. It is quite alarming that, in our study, more than 50% of subjects are not seroprotected and assumed to be already at risk for Hepatitis B infection 5 years after their vaccination. Although a decreasing trend was observed, the higher seroprotection rate 11 years after vaccination compared with 5-10 years after vaccine may be due to recruitment bias that affected the distribution of subjects by age. Although Hepatitis B booster dose is not generally advised among immunocompetent persons due to vaccine-induced immune memory that persists for more than 20 years after immunization[9, 15], a three-dose booster series was recommended among nonseroprotected subjects in this study. Since nonseroprotected subjects in this study cannot be classified as to either being non-responders or secondary to waning levels of antibody because the serologic tests were done at various interval time post-vaccination, a three-dose Hepatitis B vaccination was recommended following a 0, 1, 6 months schedule. According to Su et al in 2013, 95% maintained protective anti-HBs level after a three-dose booster[16], unfortunately, repeat post vaccination testing was beyond the scope of our study.

Male gender is said to be associated with nonresponse to Hepatitis B vaccine, owing to the effect of the sex hormone testosterone that damages the production of the immunoglobulins[17]. Moreover, numerous immunological genes are also found in the X chromosome while only few ones are mapped in the Y chromosome[18]. However, similar to other findings[6, 7, 15] no gender difference was observed in this study.

Our center is a referral center in an urban setting thus most subjects came from National Capital Region with almost equal seroprotection and nonseroprotection rate based on geographical location. The youngest among the nonseroprotected group aged 1-2 years old came from NCR, specifically Quezon City. According to He et al in 2015, the possibility of a low level of or even a negative anti-HBs for children at or under age 3 should be a concern[19]. Circumstances surrounding administration of the vaccine should be investigated especially vaccine handling and storage. Unfortunately, this is beyond the scope of this study. The effectiveness of vaccine depends on the source of procurement and proper maintenance of cold chain, which is largely affected by the place where the vaccine was given. In a tropical country like ours, adherence to the recommended vaccine storage of refrigerator temperature between 2-8C remains a challenge among local health centers. In a study in 2011, 100% seroprotection was observed in children who received vaccine from a private source[20], in contrast with our findings of equal seroprotection and nonseroprotection rate among patients from both private and local health center. Although majority of the seroprotected group were patients who received vaccine from local health centers, this did not show any significant difference.

The WHO recommends that the first dose of vaccine be administered within 24 hours of birth to prevent mother to child transmission of infection[21]. Our data shows that only 48% (53/110) of the study population received their initial vaccine dose within 24 hours as recommended despite being covered by the Mandatory Infants and Children Health Immunization Act of 2011. Though some authors reported lower proportions of individuals with anti-HBs ≥ 10 mIU/mL if the first vaccine dose had been given directly after birth[22, 23], findings in this study are in accordance with other observations[24, 25] that this is not the case, hence, vaccination schedules starting at birth is supported in order to attain timely vaccination and higher vaccination rates.

Dosing schedule is another important factor in the development of antibody response and titer level. There should be a minimum gap of 8 weeks between the 2nd and 3rd doses, and at least 16 weeks between the 1st and 3rd doses of Hepatitis B vaccination[26]. However, to minimize frequency of healthcare visits and not to miss patient's compliance, the dosing schedule in the EPI is at 6-10-14 weeks. The WHO has recommended 0-1-2 schedule for highly endemic countries like the Philippines however, in this study, 68% of those who followed this schedule were seronegative, similar to the findings of Mapandi of 43% seroprotection rate[27]. Yao et al in 2015 demonstrated a lower seropositive anti-HBs level with other schedules compared to 0-1-6 schedule[28], likewise our findings in this study showed 100% seroprotection among the 0-1-6 group. Although some studies have shown the 6-10-14 schedule to be effective, other studies demonstrated a low seroprotection rate of 68% compared to the 0-1-6 schedule[20]. Among those who followed this 0-6-10-14 schedule (subjects from the local health centers), this study found a 68% seroprotection rate, although overall, the vaccination schedule had no significant association with seroprotection.

Studies have compared combination and monovalent vaccines and have shown little difference between the two types in terms of immunogenicity after a dose of HBV at birth[29]. Combination vaccine is reported to have shown good immunogenicity and good long term anti-HBs persistence which could advantageously replace separate monovalent vaccines in areas of high Hepatitis B endemicity in terms of clinical, economic and strategic benefits[30]. In our study, majority from the seroprotected group were given combination vaccine although this did not show significant difference on multi-logistic regression. A higher seroprotection of 65% was also seen among those who were given combination vaccine with or without monovalent dose. Interestingly, 59% of those who only received monovalent vaccine were nonseroprotected. The variation in vaccine brands used may contribute to the difference in immunogenicity of the monovalent vaccine and could be a possible explanation for this finding. Vaccine brands that were used were not explored in this study as records from the patients mostly did not include this information.

In this study, 1% of the subject participant was positive to HBsAg that was a case of vertical transmission. This is closely similar to two separate local and unpublished studies in 2014 and 2015 reporting an HBsAg positivity rate of 0.3% and 0.6% among preschool and school aged children in Cebu City and Cagayan de Oro, respectively[11,12]. According to Wong et al in 2013, the Philippines is still highly endemic for Hepatitis B with a prevalence rate of 16.7%[31]. Since this is a single-center study in pediatrics with limited sample size, the finding of 1% Hepatitis B infection rate is not reflective of the true national HBsAg prevalence and cannot be used to conclude the effectiveness of our country's universal immunization program. However, with our findings of decreasing seroprotection rate at <50% 5 years and beyond after vaccination, there is a need to review our Hepatitis B immunization program, consider serologic anti-HBs level testing as early as 5 years post vaccination to monitor response, and probably

revisit the current recommendation regarding Hepatitis B booster administration.

The HBsAg reactive patient and her mother were advised further hepatitis serologic and liver function tests with follow up at subspecialty clinic. It is unfortunate that even if this patient was given the recommended Hepatitis B vaccine and hepatitis B immunoglobulin at birth as recommended to babies born to HBsAg reactive mothers, the patient still turned out HBsAg positive.

The two other study participants who tested reactive to total anti-HBc and were also reactive to anti-HBs but nonreactive to HBsAg, could be an indication of protective antibodies from natural infection. However, maternal transfer of antibody cannot be excluded in one patient who was 4 months of age. Both were advised specific testing of anti-HBc IgM and IgG and complete serologic viral hepatitis tests including maternal HBsAg work up.

CONCLUSION

In this study, 52% of patients among different age groups were found to have seroprotective anti-HBs levels after complete primary Hepatitis B vaccination. A decrease in seroprotection rate was demonstrated at < 50% 5 years and more post-vaccination with statistical significance. Gender, geographic area, age at first dose, place of vaccination, schedule and type of vaccine were among the other factors analyzed which, in this study, did not conclusively affect seroprotection rate.

RECOMMENDATION

There is a need to review the implementation of Hepatitis B immunization program in our country as well as the recommended hepatitis B vaccine schedule and booster recommendation. Further studies in the community, or among different hospital institutions should be carried out for larger study population to determine seroprotection rate and factors affecting it 1-2 months after vaccination. Subjects with hepatitis B booster can also be a good population to determine seroprotecton levels amongst them. Determination of anti-HBs levels 5 years or more post vaccination may be considered to detect the need for booster dose and prevent breakthrough Hepatitis B infection. Follow-up studies on the subjects who were not seroprotected should be done, with repeat anti-HBs testing post completion of booster doses to identify the true nonresponders.

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ORIGINAL ARTICLE

RELATIONSHIP BETWEEN IMMATURE PLATELET FRACTION AND PLATELET COUNT AMONG PEDIATRIC PATIENTS WITH DENGUE FEVER: A PROSPECTIVE CROSS-SECTIONAL STUDY

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Background and Objectives: Immature platelet fraction (IPF) is a new hematologic parameter that reflects the rate of thrombopoiesis. It has been suggested to be a predictor of platelet recovery in patients with thrombocytopenia. This study aimed to determine the relationship between IPF and platelet count among pediatric patients with thrombocytopenia due to dengue fever.

Methods: This was a prospective cross-sectional study of 77 thrombocytopenic pediatric dengue fever patients. IPF was included in the daily complete blood count extraction. Baseline and daily IPF, platelet count, hematocrit, white blood cell count and presence of fever were recorded according to day of illness. The pattern of IPF in relation to the pattern of platelet count was analyzed. The proportion of patients showing platelet recovery at different time points was also determined. A receiver operating characteristic analysis was done to determine an IPF cut-off value predictive of platelet recovery within 24 hours.

Results: The IPF increased as the platelet count decreased. The highest increase in IPF coincided with the trough of platelet count. Eighty-seven percent of the patients showed platelet recovery after the increasing trend of IPF, 87% after the peak value and 95% after the decreasing trend. An IPF value of more than 6.6% was found to be predictive of platelet recovery within 24 hours, with a sensitivity of 45% and specificity of 70%.

Conclusion: There was an observed inverse relationship between IPF and platelet count but with a statistically weak correlation. The decreasing trend of IPF can be a possible good predictor of an increasing trend in platelet count. These findings suggest a possible role of IPF as an additional parameter to predict platelet recovery in pediatric dengue fever patients.

KEYWORDS: *Immature platelet fraction, thrombocytopenia, dengue fever*

INTRODUCTION

Dengue fever is a mosquito-borne illness that is a major public health concern internationally and locally due to its high morbidity and mortality.[1] In the Philippines, there were 200,415 suspected cases of dengue and 598 deaths in 2015, majority of whom were children.[2]

The clinical course of dengue virus infection ranges from an inapparent infection, mild febrile illness, to severe dengue hemorrhagic fever. Hematologically, the most common abnormalities are hemoconcentration, thrombocytopenia, prolonged bleeding time, and a moderately decreased prothrombin level.[3] Thrombocytopenia in dengue fever is multifactorial with bone marrow hypocellularity followed by immune-mediated peripheral destruction of platelets as the most common proposed mechanisms.[4,5,6,7]

Immature platelet fraction (IPF) is a new hematologic parameter that determines the percentage of new platelets released by the bone marrow by measuring reticulated platelets in peripheral blood. It reflects the rate of thrombopoiesis, as IPF level increases as the production of platelets increases.[8,9,10,11] IPF range is 4.1 $\pm$ 7 in neonates, 2.7 $\pm$ 1.3 in children and 1.1 to 6.1 in adults.[10] It is proven to differentiate between consumptive (peripheral destruction) versus productive (bone marrow failure) etiology of thrombocytopenia and has been suggested to be an early predictor of platelet recovery in various clinical conditions including dengue fever.[8,11,12,13,14,15]

There are no clear guidelines regarding blood transfusion for thrombocytopenia due to dengue fever. The World Health Organization (WHO) recommends that blood transfusion should be given as soon as severe bleeding is suspected or recognized in a hemodynamically unstable dengue patient.[16] However, there is no evidence that supports the practice of transfusing platelet concentrate and/or fresh frozen plasma for thrombocytopenia or severe bleeding in hemodynamically stable dengue patients.[16,17] It has been a common practice to consider blood transfusion when the platelet count is less than 50,000/µL in the presence of significant bleeding or when less than 10,000/µL with no bleeding.[7]

IPF as of today has no role in the management nor in the monitoring of dengue patients due to lack of available information and research. If IPF is proven to have significant association with platelet count recovery, it may have a role in the monitoring and management of dengue fever which can possibly lead to less unnecessary blood transfusion, blood test monitoring, financial cost and a shorter hospital stay.

This study aimed to determine the relationship between IPF and platelet count and whether IPF can be utilized as an indicator of platelet recovery among pediatric patients with dengue fever. Specifically, to describe the pattern of IPF in relation to the pattern of platelet count, hematocrit, white blood cell count (WBC) and fever throughout the course of dengue fever illness; to determine the proportion of patients showing platelet recovery within 24 hours after the increasing IPF trend, peak IPF and decreasing IPF trend; and to identify the IPF value that predicts platelet recovery within 24 hours.

METHODOLOGY

Subject Selection and Data Collection

This prospective cross-sectional study was conducted at Makati Medical Center, a tertiary private healthcare facility in the Philippines, from August 1 to October 31, 2016.

All private and service pediatric patients aged zero to 18 years old of any gender admitted with a diagnosis of dengue fever, positive dengue NS1 antigen and thrombocytopenia of less than 150,000/µL, were included in this study. Pediatric patients with a concomitant disease that may

cause thrombocytopenia such as primary immune thrombocytopenia, connective tissue disease, myelodysplastic syndrome, and those with intake of medications that may increase the risk of bleeding such as anticoagulants, antiplatelet agents, acetylsalicylic acid and ibuprofen were excluded. Those who had any blood product transfusion (fresh whole blood, packed red blood cell, platelet concentrate and fresh frozen plasma) prior to admission or during the course of illness were also excluded.

Upon the approval of the Institutional Review Board, all patients who satisfied the inclusion criteria were enrolled in the study. A written informed consent from the parents or guardian of legal age, and assent from patients seven years old and above were secured prior to enrollment. The cost of the IPF determination was shouldered by the study proponents. The study participants were allowed to drop out at any point during the study period.

General demographic and clinical data (age, sex, day of illness, presence of fever, platelet count, hematocrit, WBC count and presence of warning signs) upon enrollment to the study were taken. On subsequent days, the day of illness, presence of fever, IPF, platelet count, hematocrit and WBC count were recorded. IPF was requested to be included in the daily routine complete blood count (CBC) ordered by the attending physician. The blood samples were extracted daily at the patient's room by a certified medical technologist from the time of study enrollment until a platelet count of 150,000/µL or more was achieved, and/or until the attending physician ordered to discontinue CBC monitoring. Only one blood sample per day was required to determine both the CBC and IPF. When blood samples were drawn more frequently, IPF was included only on the first blood sample for the day. The study proponents decided to only include IPF on the first blood sample for the day for consistency and to minimize costs. Standard WHO dengue fever management was instituted, including the use of isotonic intravenous fluids.

IPF in this study was measured using the automated hematology analyzer, Sysmex XE-2100. It uses a carefully designed gating system in the optical (fluorescence) reticulocyte/platelet channel to quantify the IPF. This flow cytometric IPF determination uses a patented fluorescent dye containing polymethine and oxazine or oxadine. These dyes penetrate the cell membrane and stain the RNA in the red cell and platelet reticulocytes.[11,13,14]

Sample Size and Statistical Analysis

A minimum of 90 patients were needed for this study using the Cochran's equation ($n = z^2 * pq / error^2$) for determining sample size for proportions with precision of $\pm$ 5% at 95% level of confidence, where: n = sample size, z-value at 0.05 alpha level of significance is 1.96, p = 0.9375 (previous knowledge based on the study by Dadu et al.)[14], q = 1-p and error = 0.05.

Descriptive statistics were used to summarize the clinical characteristics of the patients. Frequency and proportion were used for nominal variables, median and range for ordinal variables, and mean and standard deviation for interval/ratio variables. Spearman's rank correlation was used to determine the correlation of the platelet count and IPF. The missing variables were neither replaced nor estimated. No imputation was performed so as not to underestimate nor overestimate percentage changes or trends. A receiver operating characteristic analysis was done to determine whether IPF was predictive of platelet recovery within 24 hours. The null hypothesis was rejected at 0.05 α-level of significance. STATA 12.0 was used for data analysis.

Operational Definition

1. Increasing IPF trend – an increase in IPF by more than 10% from its previous value.[14]
2. Decreasing IPF trend – a decrease in IPF by more than 10% from its previous value.[14]
3. Peak IPF value – the maximum IPF value reached while monitoring the IPF.[14]
4. Platelet recovery – the first day of increase in platelet by more than 14.1% without any blood transfusion.[11]

RESULTS

A total of 77 pediatric patients admitted for dengue fever were included in this study, which was 86% of the minimum sample size required. The mean age was 10.5 years and 52% were male. The subjects were enrolled on different days from the onset of illness, with 34 (44%) entering at the 4th day of illness. Sixty-seven percent of the patients were febrile upon enrollment. Their baseline platelet count ranged from 17,000 to 149,000. Table 1 provides the demographic and clinical characteristics of the enrolled patients.

TABLE 1. Demographic and clinical characteristics.

	Frequency (%); Mean ± SD; Median (Range)
Age	10.51 ± 4.86
1 to 5 years old	15 (19)
6 to 10 years old	19 (25)
11 to 18 years old	43 (56)
Sex	
Male	40 (52)
Female	37 (48)
Day of illness upon entry into the study	
Day 3 of illness	5 (7)
Day 4 of illness	34 (44)
Day 5 of illness	26 (34)
Day 6 of illness	12 (15)
Presence of fever upon entry into the study	67 (87)
Day 3 of illness	5 (7)
Day 4 of illness	34 (44)
Day 5 of illness	20 (26)
Day 6 of illness	8 (10)
Baseline platelet count (/µL)	106,000 (17,000 to 149,000)
Baseline hematocrit (%)	41.1 (33.2 to 52.8)
Baseline WBC count (x10^3/µL)	2.77 (0.93 to 9.4)
Presence of warning signs*	28 (36.4)
Abdominal pain/ tenderness	14 (50)
Persistent vomiting	10 (35.7)
Mucosal bleeding	5 (17.9)
Fluid accumulation (edema, ascites, pleural effusion)	1 (3.6)
Lethargy/restlessness	0
Liver enlargement > 2 cm	0
No urine output > 6 hours	0

** Multiple Response Variable*

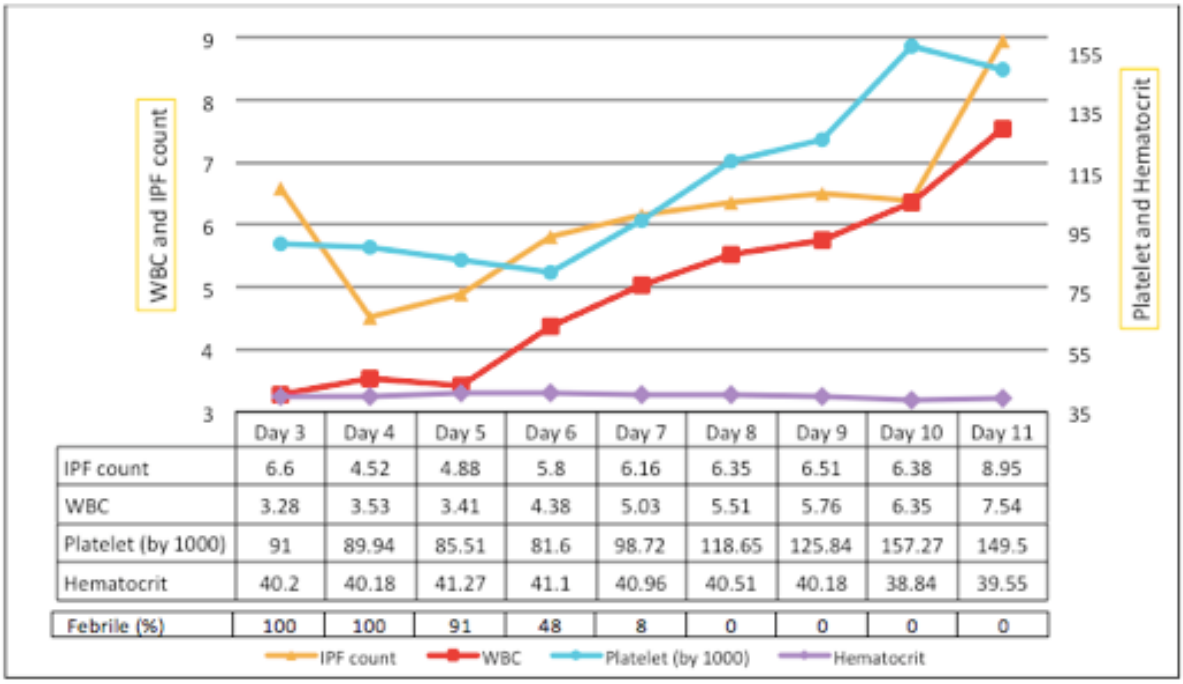
IPF, Platelet, Hematocrit, WBC and Fever Patterns

The average IPF increased steadily from day four to 11 of illness. The platelet count had reached its trough at day six of illness. The hematocrit count steadily decreased and the WBC count steadily increased. Resolution of fever started by day five of illness and by day eight, all patients were afebrile (Table 2).

TABLE 2. Average IPF, platelet count, hematocrit, WBC count and fever status according to day of illness.

Day of Illness	IPF (%)	Platelet (by 1000/µL)	Hematocrit (%)	WBC (x10^3/µL)	Febrile (%)
	Mean ± SD; Frequency (%)				
Day 3 (n=2)	6.6 ± 2.83	91 ± 33.94	40.2 ± 7.78	3.28 ± 1.22	2 (100)
Day 4 (n=17)	4.52 ± 2.32	89.94 ± 35.15	40.18 ± 3.84	3.53 ± 1.95	17 (100)
Day 5 (n=45)	4.88 ± 2.09	85.51 ± 33.08	41.27 ± 3.39	3.41 ± 1.74	41 (91)
Day 6 (n=67)	5.8 ± 2.11	81.6 ± 32.33	41.1 ± 3.55	4.38 ± 2.08	32 (48)
Day 7 (n=72)	6.16 ± 2.36	98.72 ± 44.9	40.96 ± 4.15	5.03 ± 1.99	6 (8)
Day 8 (n=55)	6.35 ± 2.23	118.65 ± 48.75	40.51 ± 4.2	5.51 ± 2.37	0
Day 9 (n=25)	6.51 ± 2.05	125.84 ± 57.96	40.18 ± 4.47	5.76 ± 1.89	0
Day 10 (n=11)	6.38 ± 2.76	157.27 ± 77.55	38.84 ± 5.45	6.35 ± 1.91	0
Day 11 (n=2)	8.95 ± 3.04	149.5 ± 7.78	39.55 ± 5.59	7.54 ± 2.48	0

Figure I illustrates the trend of IPF in relation to the trend of fever, platelet count, hematocrit and WBC count over days three to 11 of illness. Overall, there was a steady increase in IPF from day four to 11 of illness. The largest increase was observed on day six, where the median percentage change was at 32% from the previous day (Table 3). This coincides with the trough of platelet count (Table 2, Figure I).



	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
IPF count	6.6	4.52	4.88	5.8	6.16	6.35	6.51	6.38	8.95
WBC	3.28	3.53	3.41	4.38	5.03	5.51	5.76	6.35	7.54
Platelet (by 1000)	91	89.94	85.51	81.6	98.72	118.65	125.84	157.27	149.5
Hematocrit	40.2	40.18	41.27	41.1	40.96	40.51	40.18	38.84	39.55
Febrile (%)	100	100	91	48	8	0	0	0	0

FIGURE I. Pattern of IPF in relation to the platelet count, hematocrit, WBC count and fever according to day of illness.

TABLE 3. Percentage change in IPF from previous day according to day of illness.

Day of Illness	IPF (%)	% Change from previous day
	Mean ± SD; Median (Range)	
Day 3	6.6 ± 2.83	-
Day 4 (n=2)	4.52 ± 2.32	3 (2 to 5)
Day 5 (n=17)	4.88 ± 2.09	12 (-25 to 67)
Day 6 (n=44)	5.8 ± 2.11	32 (-73 to 274)
Day 7 (n=63)	6.16 ± 2.36	8 (-36 to 57.5)
Day 8 (n=55)	6.35 ± 2.23	2 (-57 to 96)
Day 9 (n=25)	6.51 ± 2.05	-10 (-34 to 55)
Day 10 (n=11)	6.38 ± 2.76	-16 (-63 to 27)
Day 11 (n=2)	8.95 ± 3.04	-19 (-29 to -9)

**Percentage change in IPF = [(current day IPF – previous day IPF)/previous day IPF]*100*

Since it is a common practice to consider blood transfusion when the platelet count is less than 50,000/µL in the presence of significant bleeding, or when less than 10,000/µL with no bleeding, a subgroup analysis for patients who had a platelet count of 50,000/µL and below was done to determine if there is a difference in the patterns of IPF, platelet count, hemocrit, WBC count and fever status among those patients. It was observed that the patterns of IPF, platelet count, hematocrit, WBC count and fever status of those with platelet count of 50,000/µL and below were the same when compared to those with platelet counts of more than 50,000/µL.

Of the 21 patients in the subgroup, nine presented with warning signs: four had abdominal pain, two had epistaxis, two had persistent vomiting and one had minimal pleural effusion. There were no cases of severe dengue, shock or death. And none of them were given fluid resuscitation or blood transfusion.

IPF and Platelet Recovery

The proportion of patients who showed platelet recovery at different time points is shown in Table 4. An increasing trend of IPF started to be evident by day seven of illness and was observed in 51% of the patients by day nine of illness. Twenty-four hours after the start of the increasing trend of IPF, 87% of the patients demonstrated platelet recovery.

For the decreasing trend of IPF, it started to be evident by day eight of illness and was observed in 39% of the patients. Twenty-four hours after the decreasing trend began, 95% of the patients demonstrated platelet recovery.

By day seven of illness, 62% of the patients had reached peak IPF value and 58% had recovering platelet counts. Twenty-four hours after the patients had reached peak IPF value, 87% of them demonstrated platelet recovery.

For all the time points mentioned, it was observed that the platelet and WBC counts showed an increasing trend while the hematocrit count showed a decreasing trend (Table 2, Figure I). Using the Spearman's correlation, there is a weak and indirect correlation between IPF and platelet count with a correlation coefficient of -0.23 (p-value of 0.000).

To determine the IPF value predictive of platelet recovery within 24 hours, a receiving operating characteristic analysis was done. From the 77 patients, a total of 296 readings of IPF were obtained. A receiver operating characteristic analysis was also done to determine the sensitivity and specificity of the different IPF cut-off points. The optimal criterion was an IPF of more than 6.6%, with a sensitivity of 45%, specificity of 70%, positive likelihood ratio of 1.49 and negative likelihood ratio of 0.79. This prescribed cut-off point has a Youden index J of 0.1548. The area under the curve is 0.592 (95% CI = 0.53 to 0.65, p value = 0.006).

TABLE 4. Cumulative frequency of patients that achieved increased or decreased IPF trend, peak IPF count, and proportion of patients that showed platelet recovery by day of illness.

Day of Illness	Increasing IPF trend	Reached peak IPF value	Decreasing IPF trend	With platelet recovery
	Cumulative frequency (%)			
Day 3	-	-	-	-
Day 4	-	1 (1)	-	-
Day 5	2 (3)	6 (8)	-	1 (1)
Day 6	11 (14)	25 (32)	-	14 (18)
Day 7	25 (32)	48 (62)	5 (6)	45 (58)
Day 8	35 (45)	69 (90)	10 (39)	67 (87)
Day 9	39 (51)	76 (99)	14 (18)	73 (95)
Day 10	-	77 (100)	19 (25)	74 (96)
Day 11	-	-	-	-

DISCUSSION

This prospective cross-sectional study of 77 hospitalized children with dengue fever who were dengue NS1 antigen test positive and thrombocytopenic (platelet count less than 150,000/µL) demonstrated an inverse relationship between IPF and platelet count, although this relationship showed a statistically weak correlation. Throughout the course of illness, measured by day, the IPF increased while the platelet count decreased. The trend of IPF at different time points (when it shows an increasing trend, peak value and decreasing trend) also suggested platelet recovery within 24 hours. An IPF value of more than 6.6% was found to be predictive of platelet recovery within 24 hours, although the sensitivity of this finding was at 45%.

There was an observed inverse relationship between IPF and platelet count. However, the correlation between IPF and platelet count was found to be statistically weak probably because the enrolled number of subjects in this study was below the minimum sample size required. Briggs et al. in 2004 assessed IPF in peripheral thrombocytopenia. Their study included 22 patients with immune thrombocytopenic purpura, 11 patients with thrombotic thrombocytopenic purpura, 12 pregnant women and 13 patients undergoing chemotherapy with decreasing platelet counts. There was a statistically significant inverse correlation of platelet count with IPF, as they saw that a decrease in platelet count was accompanied by an increase in IPF.[13] In 2014, a study by Dadu et al. showed that IPF has a strong correlation with recovery of platelet counts in patients with dengue fever. They evaluated the relationship of IPF with platelet recovery in 32 patients with dengue fever confirmed by dengue IgM and/or dengue NS1 antigen and with a complete blood count showing thrombocytopenia (defined as less than 150,000/µL) and concluded that IPF can be used to evaluate platelet recovery in patients with dengue fever.[14] In dengue fever, a rapid decrease in the platelet count is evident during the critical phase which can be between day three to seven of illness.[16,17] The thrombocytopenia in dengue fever is most commonly due to impaired thrombopoiesis and peripheral platelet destruction.[4,5,6,7] During the early phase of dengue fever (two to four days of dengue virus infection), the bone marrow shows hypocellularity and decrease in megakaryocyte maturation caused by direct infection of the

progenitor cells by the dengue virus and changes in bone marrow regulation.[4,6,7] During the defervescence phase, the progressive thrombocytopenia is due to the increase in peripheral platelet destruction caused by several factors which include autoimmune-induced platelet activation and destruction by anti-platelet autoantibodies, platelet consumption during an ongoing coagulopathy process and increased peripheral sequestration or hemophagocytosis.[5,6,7] As IPF measures young, reticulated platelets in the blood, it can be used as a marker for platelet production.[8,9,10,11] A high IPF indicates increased platelet production, while a low IPF indicates decreased platelet production as evident in this study wherein the largest increase in the IPF coincided with the lowest value of the platelet count, indicating an increase in thrombopoiesis.

In this study, it was also observed that as the IPF increased, the pattern of WBC count showed an increasing trend, while the hematocrit showed a decreasing trend throughout the days of illness. During the critical phase (day three to seven of illness) of dengue fever, aside from rapid decrease in platelet count, a decrease in WBC count and an increase in hematocrit count are also observed.[16,17] Damage on the structure of the endothelial cells by direct virus invasion or immune-mediated pathology results in increase in capillary permeability, plasma leakage, hemoconcentration, fluid effusion and consequently, the increase in hematocrit.[16,17,18] The decrease in WBC count is caused by bone marrow depression and hypocellularity during the period of viremia in dengue fever.[18]

The trend of IPF at different time points suggested platelet recovery within 24 hours. When the IPF reached an increasing trend, peak value and decreasing trend, 87%, 87%, and 95% of the patients showed platelet recovery after 24 hours respectively. The decreasing trend of IPF can be a possible good predictor of an increasing trend in platelet count since 95% of the patients showed platelet recovery 24 hours after this phase. This is similar to a study done by Dadu et al., wherein 94% of the 32 patients demonstrated platelet recovery within 24 to 48 hours during the increasing trend, 84% during the peak of IPF, 100% during the decreasing trend and 94% at a value of 10% or more.[14] These observed values can be explained by the presumed pathogenetic mechanisms of thrombocytopenia in dengue fever. The impaired thrombopoiesis and peripheral platelet destruction reduces the platelet count which leads to the production of more platelets by the bone marrow and subsequently causing an increase in the IPF.[4,5,6,7,14]

An IPF value of more than 6.6% was found in this study to be predictive of platelet recovery within 24 hours with a specificity of 70% and sensitivity of 45%. A study in 2014 involving 16 autologous stem cell transplant patients by Van der Linden et al. determined an IPF cut-off value of 5.3% (specificity of 98%, sensitivity of 47%, positive predictive value of 93%) to predict platelet recovery within two days.[11] On the other hand, an IPF cut-off value of 6.25% (specificity of 63%, sensitivity of 77%, positive predictive value 67%) was determined in a study by Suman et al. that same year to be statistically significant in predicting platelet recovery within 48 hours.[15] A known IPF cut-off value to predict platelet recovery would be useful in monitoring dengue fever patients and in helping physicians to decide if blood transfusion may be necessary since there are no clear guidelines for blood transfusion in pediatric dengue patients. According to the WHO, the prophylactic blood transfusion for severe thrombocytopenia in hemodynamically stable dengue patients are not effective and needed.[16] Observational studies have shown that preventive transfusions of platelet concentrate and fresh frozen plasma in dengue fever patients were not able to maintain the platelet counts or improve the coagulation

profile.[7,16,17] Moreover, the incidence of pulmonary edema and increased length of hospital stay was higher among patients who received transfusion.[7] Blood transfusion should be given in cases with suspected or severe bleeding in a hemodynamically unstable patient.[16] However, there is no defined recommendation for severely thrombocytopenic patients who are hemodynamically unstable but without severe bleeding. In these patients, IPF may be a potentially helpful parameter, though still needs further study, to aid in the decision-making process regarding its utility in deciding on blood transfusion.

CONCLUSION

There was an inverse relationship between IPF and platelet count, but this relationship showed a statistically weak correlation. The decreasing trend of IPF can be a possible good predictor of an increasing trend in platelet count based on the observed patterns. An IPF value of more than 6.6% was found to be predictive of platelet recovery within 24 hours but the sensitivity was only 45%. These findings provide support on the possible role of IPF as an additional parameter to predict platelet recovery in pediatric dengue fever patients.

LIMITATIONS AND RECOMMENDATIONS

Due to the relatively decreased number of dengue fever patients compared to previous years at Makati Medical Center, this study did not meet the required minimum sample size of 90. For future studies, a larger sample size and longer study period are recommended to increase the findings' reliability and accuracy. A clinical trial in a multicenter setting can be done to validate the IPF cut-off value identified in this study and possibly establish a role for IPF in pediatric dengue fever monitoring and management.

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Pardo J, Mendoza KI, Wong J. Compliance to the National Immunization Program: A Review of Immunization Records of Grade 1 Students in a Public Elementary School in Manila for the Academic Year 2017-2018

ORIGINAL ARTICLE

COMPLIANCE TO THE NATIONAL IMMUNIZATION PROGRAM: A REVIEW OF IMMUNIZATION RECORDS OF GRADE 1 STUDENTS IN A PUBLIC ELEMENTARY SCHOOL IN MANILA FOR THE ACADEMIC YEAR 2017-2018

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

BACKGROUND: Vaccination is a cost-effective primary preventive measure against infectious diseases. However, protection for specific diseases may wane over time. The National Immunization Program was launched to improve vaccine coverage but despite this, some countries including the Philippines have erratic vaccine coverage.

OBJECTIVE: To determine the compliance to the National Immunization Program of Grade 1 students in a public elementary school

METHODOLOGY: The study utilized a descriptive cross-sectional design. Simple random sampling of students enrolled in first grade for A.Y. 2017-2018 was done to determine the study respondents. Primary and secondary data were obtained through a pretested structured questionnaire with interview of the students' caregiver and verification via the students' immunization records. Compliance to immunization was correlated with the subjects' age, birth rank, primary caregiver and socio-demographic profile of the caregiver, place of birth and place of vaccination. Data were analyzed using descriptive statistics and logistic regression was used to assess factors for increased vaccination compliance.

RESULTS: Most respondents had their mothers as primary caregivers. Majority were institutional deliveries and immunized at a health center. Mean compliance to vaccination was 69%. Among the factors, only place of birth, specifically, hospital delivery, was associated with increased compliance to vaccination (OR = 0.3312, 90% CI 0.1496 to 0.7333, p value 0.0064). Subjects whose primary caregivers were the mothers and whose parents had higher educational attainment or were both employed were shown to have higher vaccination compliance, although this was not statistically significant. Vaccination coverage was observed to decrease over time as the subjects grew older. Most common reasons cited for missing vaccinations were vaccine unavailability (68%), financial constraints 46%), and lack of information (40%).

CONCLUSION: Compliance to vaccination in this study was 69% and is affected by multiple factors. Policymakers and stakeholders should address these barriers to improve vaccination coverage and overall health status.

KEYWORDS: *immunization, vaccine, children, national immunization program, school-based immunization, EPI*

INTRODUCTION

Vaccination is a cost-effective and proven primary preventive measure against a number of childhood infections. It prevents more than 2.5 million child deaths yearly with an additional 2 million deaths that could be prevented with the utilization of currently available vaccines. In spite of this, universal vaccination coverage has not been fully achieved [1,2,3]. In the Philippines, the Expanded Program on Immunization (EPI) was established in 1976 to ensure access of infants and children, as well as mothers to routinely recommended vaccines. Initial vaccine-preventable diseases included in the program were tuberculosis, poliomyelitis, diphtheria, tetanus, pertussis and measles. As new studies and epidemiological data emerged, additional vaccines were added to the program including the pentavalent DPT-HepB-HiB vaccine in 2010 and rotavirus and pneumococcal vaccines in 2012 – 2014. The expanded program on immunization was updated to the National Immunization Program (NIP) in 2016. This included immunization of school-aged children, adolescents, and senior citizens. Under the NIP, the Department of Health together with the Department of Education issued guidelines on the implementation of school-based immunization. This program provided free catch-up doses for Grade 1 and 7 students in public schools against measles, rubella, tetanus and diphtheria. The future of the NIP is dynamic, with additional recommended vaccines being introduced into the program. However, these vaccines are not always readily available due to inadequate supply.[4,5,6,7]

National Immunization Programs are great public health achievements in our history. In the Philippines, the NIP includes the following vaccines: 1) Bacille-Calmette Guérin (BCG) vaccine as a single dose at birth or prior to the first month of life, 2)Hepatitis B (HepB) vaccine at birth, 3) Three doses of DPT-HiB-HepB vaccines, 4) Three doses of oral polio vaccine (OPV) and a single dose of inactivated polio vaccine (IPV) with the third dose of OPV, 5)Three doses of pneumococcal conjugate vaccine (PCV), 7) A dose of measles containing vaccine at 9 months old (MCV1), 8) Measles-mumps-rubella vaccine at 12 months old (MMR), and 9) Two or three doses of Rotavirus vaccine. It also recommends booster doses as part of the school-based immunization program which include 1) Measles-Rubella (MR) and Tetanus-diphtheria (TD). This immunization program follows the recommended routine immunizations for children by the World Health Organization. Although closely patterned to the WHO routine recommended vaccines for age, the vaccine coverage in the country has been erratic.[3,7,8,9]

A report released by WHO and UNICEF shows that although some of the immunization trends have remained stable throughout the years – such as in the case of BCG – there is still a long way to go to achieve the ideal global coverage in certain vaccines.[9,10] Around 19.4 million infants globally are not receiving the full complement of vaccines recommended in the Expanded Program on Immunization and 60% of these infants live in 1 of 10 countries which include the Philippines. According to the Department of Health, 69% of the target population of the EPI have been fully immunized through the National School-Based Immunization Campaign vaccinating 60% of Grade 1 students with Measles Rubella (MR) vaccine and 73% with Tetanus-diphtheria (TD).[6]

This study aims to assess the immunization coverage in grade 1 students in one public elementary school in Manila and to determine factors that affect vaccination compliance.

METHODS

Study Design:

This study utilized a descriptive cross-sectional design.

Study Population and Sampling:

Grade 1 students from Aurora A. Quezon Elementary School, a public elementary school in Manila comprised the study population. Based on a 90% level of confidence corresponding to a normal z-deviate of 1.645 and assuming an immunization rate

of 69% from the Department of Health's Performance Report for 2016, a sample size of 58 study participants is needed to assure validity and reliability of study results.[6]

Simple randomized sampling using an online randomizer was done to determine the study respondents. Parental consent was obtained prior to data collection. Students with no immunization records were excluded from the study.

Definition of Study Terms

a. ***National Immunization Program (NIP)*** - An update on the Expanded Program on Immunization, NIP was instituted in 2016 and includes immunization of school-aged children, adolescents, and senior citizens.

b. ***Fully immunized child (National Immunization Program)*** - Children who received one dose of BCG, three doses each of OPV, DPT, and Hepatitis B vaccine, Hib-containing vaccine, Pneumonia conjugate vaccine, two or three doses of rotavirus vaccine, rubella containing vaccine and booster doses of measles-rubella and tetanus-diphtheria vaccine.

c. Compliance

Compliance to the NIP is defined by the percentage of vaccines acquired by the participant. However, since pneumonia conjugate vaccine and rotavirus vaccine were only included in the EPI in 2012 and 2014, respectively, provision for not acquiring these vaccines were given as these two vaccines were introduced after the average age of the study population were eligible for these vaccines.[7]

Compliance is defined in this study as follows:

Table 1. Study Definition of Compliance Level

Level	Remarks
100% (23 out of 23 vaccines)	Excellent compliance
83%-99% (19-22 out of 23 vaccines)	Very good compliance
69% - 82.9% (16-18 out of 23 vaccines)	Good compliance
56%-68.9% (13-15 out of 23 vaccines)	Satisfactory compliance
43%-55.9% (10-12 out of 23 vaccines)	Fair compliance
30%-42.9% (7-9 out of 23 vaccines)	Poor compliance
< 30% (6 or less out of 23 vaccines)	Very poor compliance

DATA COLLECTION AND ANALYSIS

Data Collection

This study utilized primary and secondary data obtained through a pretested structured questionnaire with interview of the students' primary caregiver and verification via the students' immunization records. The interview tool that was used has been pretested and is available in both English and Filipino. The interview was conducted by the researcher followed by review of the subjects' vaccine records.

Data Analysis

Upon collection, data was encoded and tabulated. Descriptive statistics including frequency and percentage, were used to ascertain the population profile distribution.

Compliance to immunization was correlated with the subjects' age, birth rank, primary caregiver and socio-demographic profile of the caregiver, place of birth and place of vaccination. Data was then statistically analyzed using logistic regression analysis with Stata software.

Ethical Considerations

Prior to data collection, the research protocol was reviewed and approved by the Hospital Ethics Review Board. Appropriate permissions from respective agencies were also acquired.

RESULTS AND DISCUSSION

Out of the 719 grade 1 students enrolled at the study area, 58 were randomly chosen to be study participants. As shown in Table 2, most of the respondents were between 6-7 years old (86%, n = 54) with majority being female (54%, n = 34) and Roman Catholic (83%, n = 52). Birth rank was distributed more heterogenously with majority being first-born (37%, n = 23), followed by second- born children (24%, n = 15) and third-born (21% n = 13).

Table 2. Socio-Demographic Profile of Grade 1 Students in a Public Elementary School in Manila

Profile	Number of Subjects (n = 58)	Percentage (%)
Gender		
Male	24	38
Female	34	54
Age (years)		
6 – 7	54	86
8 – 10	2	3
11 and above	2	3
Birth rank		
1	23	37
2	15	24
3	13	21
4	4	6
5 and above	3	5
Religion		
Roman Catholic	52	83
Christian	2	3
Islam	3	5
Iglesia ni Cristo	1	2

Table 3 shows that most subjects have their mothers as the primary caregiver (70%, n = 44) and most caregivers were young adults aged 20-39 (62%, n = 39). Most primary caregivers were married (44%, n = 28), graduated or reached high school level (46%, n = 29) with at least one parent employed (48%, n = 30).

Table 3. Socio-Demographic Profile of the Primary Caregivers of Grade 1 Students in a Public Elementary School in Manila

Profile	Number of Subjects (n = 58)	Percentage (%)
Primary Caregiver		
Mother	44	70
Father	0	0
Grandparent	8	13
Other	6	10
Age of Caregiver		
13-19	0	0%
20-39	39	62%
40-64	18	29%
65 and above	1	2%
Civil Status of Caregiver		
Married	28	44
Single	10	16
Widow	2	3
Common-in-law	18	29
Educational attainment		
College graduate	7	11
College level/ Vocational	20	32
High school level	29	46
Elementary level	2	3
No formal education	0	0
Employment status		
Both employed	18	29
One caregiver is employed	30	48
No fixed employment	4	6
Unemployed	6	10

As shown in Table 4, majority of the respondents were born institutionally, with 65% (n=41) born in a hospital and 13% (n = 8) born in a lying-in clinic. Majority also had their vaccinations at their local health center (78%, n = 49).

Table 4. Place of Birth and Place of Vaccination Distribution of Grade 1 Students in a Public Elementary School in Manila

Profile	Number of Subjects (N = 58)	Percentage (%)
Place of Birth		
Hospital	41	65
Lying-in clinic/Health center	8	13
Non-institutional (Home)	9	14
Place of vaccination		
Health center or hospital	49	78
Private practitioner	3	5
Both of the above	6	10

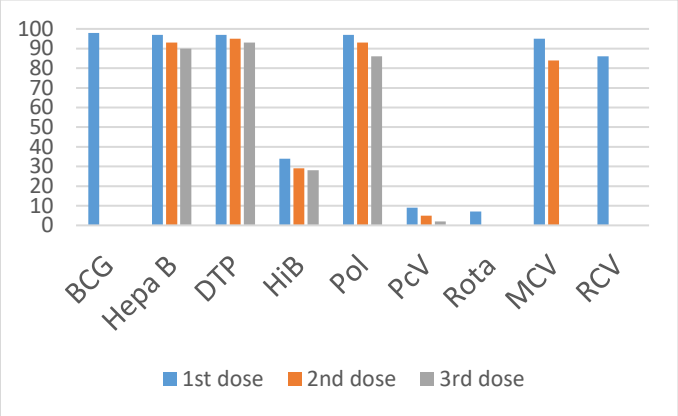
In terms of vaccination compliance, only 1 of the respondents completed the immunizations recommended by the National Immunization Program (1.7%, n = 1). However, it should be emphasized that the rotavirus vaccine and pneumococcal vaccine were included in the national program in the years 2012 and 2014 only, past the age of eligibility of the respondents. As seen in Table 5, 22% (n = 14) of the respondents had excellent compliance, having at least 19 of the 23 vaccines, followed by 35% who received 16 to 18 of the recommended vaccines and 24% (n = 15) who received 13 to 15 of the recommended vaccines. The mean compliance to vaccination was 69%.

Table 5. Vaccine Compliance Distribution of Grade 1 Students in a Public Elementary School in Manila

Remarks	Compliance	Number of Respondents (n = 58)	Percentage (%)
Excellent compliance	**83% and above** (19 and above out of 23 vaccines)	14	22
Very good compliance	**69% - 82.9%** (16-18 out of 23 vaccines)	22	35
Satisfactory compliance	**56%-68.9%** (13-15 out of 23 vaccines)	15	24
Fair compliance	**43%-55.9%** (10-12 out of 23 vaccines)	4	6
Poor compliance	**30%-42.9%** (7-9 out of 23 vaccines)	3	5

Among the 23 recommended vaccinations, most respondents were able to receive BCG (98%, n = 57) as well as the first dose of Hepatitis B vaccine, DTP1, and Pol1 (97%, n = 56). The trend of immunization coverage with age is shown below (*see Figure 1*).

Figure 1. Trend of Immunization Coverage with Age in Grade 1 Students in a Public Elementary School in Manila



The respondents' average compliance to the expanded program on immunization, school-based immunization program and overall compliance to the national immunization program are shown in Table 7 below.

Table 7. Average Compliance to the Expanded Program on Immunization, School-based Immunization Program and National Immunization Program of Grade 1 Students in a Public Elementary School in Manila

Program	Average Compliance (%)
Expanded Program on Immunization	67%
School-based Immunization Program	95%
National Immunization Program	69%

The primary caregivers had varying reasons for missed vaccinations as shown in Table 8. Among these, the most common cited were vaccine unavailability, lack of funds for vaccines not given for free, and lack of information regarding other recommended vaccines.

Table 8. Percentage Distribution of Reason for Missed Vaccinations of Grade 1 Students in a Public Elementary School in Manila

Reason for missed vaccination	Number of respondents (n = 58)	Percentage %
Vaccine unavailability	43	68%
Lack of time to visit vaccine provider	4	6%
Lack of funds for vaccines not given for free	29	46%
Forgot to bring child to follow-up	8	13%
Change of location	6	10%
Lack of information	25	40%
Sickness	6	10%
Caregiver opted not to have the child vaccinated	0	0%
Other reason	0	0%

Using logistic regression analysis with a confidence interval of 90% and $p < 0.05$, only the place of birth was statistically significant in the outcome of compliance to vaccination. Children born in hospitals were more compliant to vaccination compared to children born in health centers, lying-in clinics and non-institutional births. Mothers as primary caregivers who were married and those belonging to the young adult age group (20-39 years old) were found to be more compliant to vaccination, but this was not statistically significant. Data also show that as the birth rank increases, the compliance to vaccination decreases. Vaccinations of the subjects were primarily given at the local health center. The odds of compliance to vaccination is higher in parents with higher educational attainment with both parents employed but this was also not statistically significant.

Table 9. Correlation of Various Sociodemographic and Health Related Factors with Compliance to Immunization of Grade 1 Students in a Public Elementary School in Manila

Factor	Odds Ratio	90% CI	P
Birth Rank	1.0364	0.6549 to 1.6403	0.8786
Gender	0.5216	0.1718 to 1.5833	0.2506
Religion	0.6562	0.3103 to 1.3880	0.2704
Place of Birth	0.3312	0.1496 to 0.7333	**0.0064**
Place of Vaccination	3.1627	0.6377 to 1.568	0.1587
Primary Caregiver	0.8757	0.5369 to 1.4282	0.5948
Age of Caregiver	1.0067	0.9556 to 1.0606	0.8019
Civil status of Caregiver	0.8032	0.5369 to 1.2017	0.2854
Educational attainment of Caregiver	1.2761	0.6270 to 2.5970	0.5013
Employment of Caregiver	1.2384	0.6676 to 2.2971	0.4976

DISCUSSION

A mean compliance rate to immunization of 69% was shown in the study. This is congruent with the national data from the Department of Health 2016 Progress Report.[4] Although there were several factors that could affect vaccination compliance, this study showed that only place of birth was significant. Children born in hospitals had higher vaccination compliance compared to children born in health centers, lying-in clinic and non-institutional settings. Although children whose primary caregivers were the mothers who were married and belonged to the age group 20-39 years and whose parents had higher educational attainment or were both employed had higher vaccination compliance, this was not shown to be statistically significant.

In a local study conducted in a private hospital in a city outside of Manila, it was shown that children with higher birth order (first or second-born) and those whose parents are the primary care takers had a higher compliance rate to vaccination.[11] However, in that study, the subjects' place of birth was not shown to have an effect on vaccination compliance because immunization coverage was similar regardless of place of delivery, whether in an institutional or non-institutional setting [11]. This is in contrast to the results of a large-scale study conducted in India which determined place of delivery to be a predictor for vaccination. The researchers noted that children born in private institutions were at higher risk of non-vaccination than those born in government institutions. This was attributed to stricter policies for government institutions to ensure that children receive appropriate and timely immunizations [12].

In this current study, most subjects were able to obtain booster doses for measles, rubella, tetanus, and diphtheria as part of the school-based program. Those who were not able to obtain these vaccines were usually ill or absent during the scheduled vaccination. School-based vaccination was instituted by the Department of Health in collaboration with the Department of Education and Department of Interior and Local Government in 2015 due to the need for booster doses for certain vaccines whose protections wane over time. The higher compliance rates in school-based immunization program compared to the expanded program on immunization may be due to the convenience of the location for vaccination as well as availability of vaccines [5, 6].

Although statistically not significant, the odds of having better compliance to vaccination were found to be higher in children whose primary caregivers had higher educational attainment or those with both parents employed. This may be due to increased understanding of the importance of vaccination, as well as extra finances to cover vaccine costs. This is in congruence with a survey conducted in local health departments in Georgia which found that noncompliance was higher in children whose parents had low educational attainment [13].

A study conducted in 2014 showed that respondents agreed that they do not have enough money allocated for health concerns, including vaccination. Other factors contributing to non-compliance in the above study were lack of time and forgetting to return on scheduled follow-up.[14] Other reasons given for missed immunizations were lack of information regarding immunization. Most

caregivers were unaware that not all of the recommended childhood vaccinations were available at their local health center. Furthermore, some were not aware that they could avail of these vaccines through a private practitioner.

The study results also showed a progressive decline in the trend of vaccination coverage with age. This was also seen in both studies conducted by Lim and Shrivasta. This was mainly attributed to difficulty in accessing health services, lack of information regarding the need for subsequent vaccination, lack of time or loss of motivation to bring the child to the vaccine provider [11, 12]

Considering the study results, compliance to vaccination can still improve to achieve the national goal of 95% coverage. Barriers to immunization compliance should be addressed, especially at the local level. Reliable supply and timely distribution of vaccines, enhanced access to vaccination services, reduced financial barriers to families and increased funding for vaccines as well as continued health education regarding vaccination should be emphasized to the stakeholders.

CONCLUSION

Several factors have been studied as to compliance and non-compliance to vaccination, with the most commonly noted associations including place of delivery, caregiver education and employment status, as well as birth rank.

Drop-out rates have also been noted, and is an area of concern as the protection of certain vaccines wane over time. Often, there is a progressive decline in vaccine coverage as the subjects grow older. The low immunization coverage of 69% in the respondents is disconcerting. However, the good coverage for school-based immunization of 95% suggests that the convenience and availability of vaccines have a positive impact on compliance. Therefore, this practice should be continued and strengthened.

The common reasons for missed vaccinations – namely unavailability of vaccines, lack of funds for vaccines not given for free, and lack of information regarding other vaccines – should be addressed. Adequate health education and provision of affordable vaccines should be a priority of the government and healthcare force to achieve the national goal of vaccine coverage.

LIMITATIONS

The comparison of immunization coverage of urban and rural communities, and among different socioeconomic groups are not within the scope of this study.

RECOMMENDATIONS

The author recommends a larger scale study which may include other variables including private or public schooling, rural vs urban living, as well as monthly income. Multivariate analysis of data with a larger sample will be useful to determine additional factors affecting compliance at the national level.

A prospective study utilizing different methods to improve compliance, either via reminders using SMS or social media, incentives to caregivers, etc. may also be done as these could help the government in increasing compliance to immunization.

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Perez MA & Anacleto F. Diagnostic Accuracy of Renal Angina Index in Predicting Acute Kidney Injury in Pediatric Patients with Sepsis: A Philippine Tertiary Hospital Experience

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ORIGINAL ARTICLE

DIAGNOSTIC ACCURACY OF RENAL ANGINA INDEX IN PREDICTING ACUTE KIDNEY INJURY IN PEDIATRIC PATIENTS WITH SEPSIS: A PHILIPPINE TERTIARY HOSPITAL EXPERIENCE

ABSTRACT

Background: The coexistence of acute kidney injury (AKI) in sepsis contributes significantly to morbidity and mortality rates. Traditional diagnostic markers still pose variable limitations in early AKI prediction. The use of renal angina index (RAI) as a clinical predictive tool for AKI is an emerging concept.

Objectives: To determine the diagnostic accuracy of RAI in predicting AKI in patients with sepsis

Methodology: This is a five-year retrospective cohort study conducted at the Philippine General Hospital (PGH). Records of eligible patients with sepsis were reviewed. RAI was calculated based on the composite of risk factors and clinical evidence of injury on day 0 of admission stratifying subjects into two groups: RAI (-) and RAI (+) for those with scores ≥ 8. Prediction of AKI with the RAI was analyzed.

Results: A total of 222 patients were enrolled. The RAI (+) group (score ≥ 8) consisted 95 patients (43%). AKI incidence rate was 40.5 % (90/222) and 87/90 patients (91.6%) were classified in the RAI (+) group. The use of RAI in predicting AKI has a sensitivity of 96.7%, specificity of 94.0%, positive predictive value (PPV) of 91.6%, negative predictive value (NPV) of 97.7%, positive likelihood ratio (LR) of 15.95, negative LR of 0.04 and area under the curve-receiver operating characteristic (AUC-ROC) of 0.953 (95% CI 0.92-0.98).

Conclusions: RAI is a good screening tool in predicting sepsis-associated AKI among pediatric patients. It provides early recognition of AKI and is a practical method which can be used at bedside.

KEYWORDS: *Renal Angina Index, Acute Kidney Injury, Sepsis*

INTRODUCTION

The global pooled incidence rate of pediatric AKI across all clinical settings was 33.7% with a corresponding mortality rate of 13.8% in a meta-analysis by Susantitaphong et. al. in 2013.[1] On the other hand, a study conducted in Korea by Suh et. al. showed that sepsis-associated AKI developed in 57.7% of enrolled pediatric subjects.[2] Despite the availability of accepted definitions, parameters being relied upon for AKI seem to be highly confounded by variability in body mass and sex and by inherent time lag to response to injury.[3,4] Novel urinary biomarkers as promising candidates in the early prediction of injury have emerged. However, studies failed to show their robust efficacy in children especially when used in isolation and outside of the cardiopulmonary bypass population.[5]

The RAI is a composite of an individual's AKI risk and early signs of injury and this is designed to guide the risk stratification of patients for whom the use of AKI biomarker would be most optimal.[6] Risk for AKI as part of the index includes the need for pediatric intensive care unit (PICU) admission, history of transplantation (solid organ or bone marrow), and need for ventilation and inotropic support which correspond to scores of 1, 3 and 5 respectively. On the other hand, clinical signs of injury make use of the degree of change in estimated creatinine clearance (eCCl) or percent of fluid overload (FO). A score of 1 is given for < 5% FO or no change in eCCl, 2 is given for 5-10% FO or less than 25% decrease in eCCl, 4 is given for 10-15% FO or 25-50% decrease in eCCl, and 8 is given for 15% FO or at least 50% decrease in eCCl.[4,6] In a derivation and validation study conducted by Basu and colleagues in 2014, a RAI of at least 8 showed higher AKI rate, longer PICU length of stay, higher renal replacement therapy (RRT) provision and higher hospital mortality rates. Corollary to this, RAI of less than 8 had a high negative predictive value of 92%.[7] RAI utility as a pretest probability assessment tool in AKI appears to have good performance metrics which can improve the efficiency in predicting AKI by biomarkers leading to expedited early therapy.[8,9]

At present, there is still no single diagnostic marker that can accurately predict the occurrence of AKI in critically ill patients, and this poses variable limitations that greatly affect monitoring and the expedited institution of therapy in the affected population. To address this diagnostic challenge in pediatric AKI, the use of RAI as a clinical predictive tool is an emerging concept. This study aims to determine the diagnostic accuracy of RAI in predicting AKI in patients with sepsis and evaluate its utility to clinical practice.

METHODOLOGY

This is a 5-year retrospective cohort study approved by the University of the Philippines Manila Research Ethics Board (UPMREB) Panel at PGH. A list of eligible subjects was obtained from the census files of the different units of the Department of Pediatrics (emergency room, in-patient wards, hematology-oncology unit, neonatal intensive care unit and pediatric intensive care unit) and compiled in a database. Supplemental lists were also obtained from records of the Sections of Infectious and Tropical Diseases and Nephrology as referral services. Charts of these subjects were retrieved from the records section of the institution. The following were the inclusion and exclusion criteria:

Inclusion Criteria

Pediatric patients aged between one month and less than 19 years old admitted at PGH from January 2012 to December 2016 with sepsis or septic shock were included in the study. In the case of patients with multiple admissions in the institution, only the initial admission was considered to eliminate confounders and bias.

Exclusion Criteria

Excluded from the study are the following:

Patients on maintenance RRT (hemodialysis or peritoneal dialysis); patients with preexisting chronic kidney disease with estimated glomerular filtration rate (GFR) of < 15 ml/min/1.73 m^2; patients who underwent kidney transplantation within 90 days of admission; cardiac patients who immediately underwent cardiac catheterization; patients who

underwent surgical corrections requiring cardiopulmonary bypass; and patients who died within the 1st 48 hours of admission

Baseline demographic information, comorbidities, use of any medication prior to admission, clinical signs and symptoms, anthropometric measurements, available values of requested diagnostics, vital signs and admitting diagnosis were recorded. Subsequent results of diagnostic tests during the course of admission were also recorded. Variables including need for inotropic support, mechanical ventilation and daily fluid balance in the first three days of admission were documented. Pediatric Risk for Mortality (PRISM) III was scored based on physiological and clinical variables from the studies of Pollack et al and Tan et al. [10-11] Outcomes which included duration of hospital stay, development of AKI on Day 3 of admission, need for RRT during the course of admission and mortality were recorded. Collected patient information were kept anonymous and confidential by removing identifiers. Only the data necessary for the study were obtained from retrieved charts.

Subjects were classified into two groups based on the calculated RAI defined as the composite of risk factors and clinical signs of injury using FO percentage or change in eCCl. Data used in the composite score were collected on the first calendar day of admission (Day 0) with a minimum of 8 hours stay in the facility. FO percentage was computed as a function of the difference of total fluid input and total fluid output (in liters) divided by the subject's weight on admission, multiplied by 100. Documentation of fluid balance was on a daily basis using the subject's working weight for the first three days. On the other hand, the eCCl was calculated using the modified Schwartz formula defined as the product of 36.5 (constant value) and length or height (in centimeters) divided by the serum creatinine (in µmol/L). The lowest creatinine level of each subject up to three months before the present admission was searched during the review. If no available baseline serum creatinine value was noted, subjects were assigned baseline values of 27 µmol/L for infants < one year old, 44 µmol/L for children one to nine years old and 66 µmol/L for adolescents 10-18 years old based on the mean value of the normal range of serum creatinine by age determined enzymatically by means of either creatininase or creatininase-/creatinase-based assays.[12-13] Corresponding computation of RAI were documented with an RAI score of at least 8 interpreted as fulfillment of the index.[7] Absence or fulfillment of the index were denoted as RAI (-) and RAI (+) respectively.

Sample Size

A priori, four variables which were known risk factors for AKI were included in a comparative prediction model in this study: age, the RAI index, PRISM III scores[10] and the presence of sepsis.[9] Designation of 10 events per variable would yield 40 events (patients developing AKI).[4] Based on the latest meta-analysis of the incidence of AKI across all clinical settings, a 33.7% incidence rate yields an estimate of at least 120 eligible patients for study enrollment.[1]

Statistical Analysis

Data retrieved from each patient were tabulated, and normally-distributed quantitative variables were presented as means and standard deviation with the application of independent t-test for comparison. Non-normally distributed variables were expressed in medians with interquartile ranges and the Mann-Whitney U test was used for comparison. Categorical variables were reported as frequencies and proportions and the Fisher's exact test used for comparison. Accuracy of RAI ≥ 8 in detecting AKI among sepsis patients was computed in terms of its sensitivity, specificity, PPV, NPV, positive and negative LR and AUC-ROC. The association between RAI and AKI among sepsis patients was analyzed using logistic regression. The level of significance was set at 5%.

Primary and Secondary Outcome

The primary outcome of the study is to determine the diagnostic accuracy of RAI in terms of sensitivity, specificity, PPV, NPV, positive and negative LR and AUC-ROC in the prediction of AKI in pediatric patients with sepsis. The secondary outcome is to determine the association between RAI and AKI.

RESULTS

Demographic data and clinical characteristics are shown in Table 1. A total of 222 patients were enrolled and 95 subjects representing approximately 43% of the total population fulfilled the RAI with a score of at least 8. The population was predominantly male (59%) with a median age of two years. The state of nutrition of all subjects in terms of weight for age, length/height for age and weight for length/height was documented with no significant difference between the two groups. Majority of patients (60%) had no known comorbidities. More than 80% of the subjects denied any history of medication use prior to admission. Respiratory, gastrointestinal and neurologic complaints comprised the top three most common reasons for admission with a median presentation time to the hospital of six days.

Table 2 shows a summary of diagnostic results requested and available for both groups. RAI (+) patients have significantly lower values for platelet count (p = < 0.001) and prothrombin time activity (p = 0.025). As expected, the RAI (+) patients had higher BUN (p = <0.001) and creatinine (p = < 0.001) values with a corresponding lower estimated GFR (p = < 0.001) computed using the Modified Schwartz formula. Other variables which showed significant differences between the two groups include calcium values (p = < 0.001), blood pH (p = 0.002), pCO2 (p = 0.002) and bicarbonate levels (p = < 0.001). Results of other variables were comparable between the two groups.

Table 1. Demographic and Clinical Characteristics Stratified by Day 0 Renal Angina Fulfillment

Category	Overall N= 222	RAI (-) n= 127	RAI (+) n= 95	*p* value
Age on Admission (in years)	2 (0.5, 7)	2 (0.5, 5)	3 (0.5, 14)	0.124
Sex				
Male	130 (58.6)	67 (30.2)	63 (28.4)	
Female	92 (41.4)	60 (27.0)	32 (14.4)	0.054
Weight for Age (z-score)				
Normal	99 (44.6)	53 (23.9)	46 (20.7)	
Underweight	123 (55.4)	74 (33.3)	49 (22.1)	0.122
Length/Height for Age (z-score)				
Normal	132 (59.5)	77 (34.7)	55 (24.8)	
Stunted	90 (40.5)	50 (22.5)	40 (18.0)	0.816
Weight for Length/Height (z-score)				
Normal	110 (49.6)	59 (26.6)	51 (23.0)	
Wasted	94 (42.3)	55 (24.8)	39 (17.5)	0.321
Overweight/Obese	18 (8.1)	13 (5.9)	5 (2.2)	
Comorbidities				
None	135 (60.8)	79 (35.6)	56 (25.2)	
Cardiovascular	27 (12.2)	12 (5.4)	15 (6.8)	
Respiratory	4 (1.8)	3 (1.3)	1 (0.5)	
Gastrointestinal	4 (1.8)	2 (0.9)	2 (0.9)	0.827
Hematologic	5 (2.3)	3 (1.3)	2 (0.9)	
Oncologic	6 (2.7)	3 (1.3)	3 (1.3)	
Neurologic	24 (10.8)	15 (6.7)	9 (4.1)	
Others	17 (7.6)	10 (4.5)	7 (3.1)	
Medication Use				
Present	41 (18.5)	19 (8.5)	22 (9.9)	
Absent	181 (81.5)	108 (48.6)	73 (32.9)	0.161
Site of Infection				
Unknown	18 (8.1)	6 (2.7)	12 (5.4)	
Respiratory	122 (55.0)	75 (33.8)	47(21.2)	
Genitourinary Tract	2 (0.9)	0	2 (0.9)	
Gastrointestinal Tract	32 (14.4)	11 (5.0)	21 (9.4)	*0.002**
Skin/Soft Tissue	17 (7.6)	13 (5.9)	4 (1.8)	
Bone	1 (0.5)	1 (0.5)	0	
Central Nervous System	30 (13.5)	21 (9.4)	9 (4.1)	
Time to Present to Hospital (days)	6 (3, 8)	7 (3, 11)	5 (3, 7)	*0.045**
Blood Pressure (percentile)				
< P5	35 (15.8)	7 (3.2)	28 (12.6)	
P5-P95	172 (77.5)	113 (51.0)	59 (26.6)	*< 0.001**
P95	15 (6.7)	7 (3.2)	8 (3.6)	
Baseline Heart Rate				
Bradycardia	1 (0.5)	1 (0.5)	0	
Normal for age	73 (32.9)	37 (16.7)	36 (16.2)	0.245
Tachycardia	148 (66.6)	89 (40.1)	59 (26.6)	
Baseline Respiratory Rate (N=221)				
Bradypnea	2 (0.9)	0	2 (0.9)	
Normal for age	75 (33.9)	43 (19.5)	32 (14.5)	0.317
Tachypnea	144 (65.2)	84 (38.0)	60 (27.0)	
Baseline Temperature (N=214)				
Hypothermia	9 (4.2)	9 (4.2)	0	
Normal	115 (53.7)	66 (30.8)	49 (22.9)	*0.025**
Hyperthermia	90 (42.1)	50 (23.4)	40 (18.7)	
Baseline mental status				
Normal	187 (84.2)	115 (51.8)	72 (32.4)	
Decreased sensorium	35 (15.8)	12 (5.4)	23 (10.4)	*0.005**
Pupillary reflexes				
Reactive	218 (98.2)	127 (57.2)	91 (41.0)	
Non-reactive	4 (1.8)	0	4 (1.8)	*0.032**
Daily Fluid Overload (%)	2.0 (0, 4.8)	1.2 (0, 3.5)	4.0 (0.4, 7.5)	*< 0.001**
Need for Mechanical Ventilation				
Absent	104 (46.8)	80 (36.0)	24 (10.8)	
Present	118 (53.2)	47 (21.2)	71 (32.0)	*<0.001**
Need for Inotropes				
Absent	135 (60.8)	103 (46.4)	32 (14.4)	
Present	87 (39.2)	24 (10.8)	63 (28.4)	*< 0.001**

Data were expressed as n (%) or median (interquartile range). RAI (+) was assigned for index fulfillment (RAI score of ≥ 8). P value compared RAI (-) versus RAI (+) cohorts. Level of significance set at p < 0.05.

Table 2. Laboratory Data on Admission Stratified by Day 0 Renal Angina Fulfillment

Category	Overall N= 222	RAI (-) n= 127	RAI (+) n= 95	*p* value
Hemoglobin (g/L)	109 (93, 124)	107 (93, 125)	110 (92, 124)	0.572
Hematocrit (n= 220)	0.34 (0.29, 0.39)	0.34 (0.29, 0.39)	0.34 (0.3, 0.39)	0.62
White Blood Cell Count (x 10^9/L)	15.2 (10.5, 23.6)	15.1 (10.5, 22.4)	15.3 (10.5, 24.6)	0.457
Platelet Count (x 10^9/L) (N=221)	340 (183, 459)	393.5 (249, 478)	286 (159, 415)	*<0.001**
Prothrombin Time (activity) (N=127)	78 (60, 94)	82 (66, 98)	74 (44, 89)	*0.025**
International normalized ratio (N=127)	1.2 (1.0, 1.4)	1.1 (1.0, 1.3)	1.2 (1.1, 1.7)	0.068
BUN (mmol/L) (N=210)	4.3 (2.5, 8.7)	3.1 (1.9, 4.7)	9.5 (4.4, 22.3)	*< 0.001**
Creatinine (umol/L)	37 (26, 78)	28(22, 36.5	89 (47, 238	*<0.001**
Estimated GFR on admission (ml/min per 1.73 m^2)	80 (39, 112)	105 (82, 126)	35 (12, 60)	*< 0.001**
Sodium (mmol/L) (N=220)	139 (132, 143)	140 (134, 143)	138 (128, 143)	0.087
Potassium (mmol/L) (N= 220)	4.4 ±1.3	4.5 ± 1.0	4.4 ± 1.5	0.885
Chloride (mmol/L) (N=207)	103 (96, 107)	103 (98, 106)	102 (92, 110)	0.819
Total Bilirubin (umol/L) (N=36)	19.4 (7.0, 90.1)	15.5 (7.6, 29.2)	30.9 (6.4,180.1)	0.399
Glucose (mmol/L) (N=140)	4.9 (3.8, 6.5)	4.8 (4.2,6.2	5.4 (3.8,7.1	0.645
Albumin (g/L) (N= 139)	30.6 ± 1.05	31.4 ± 8.4	29.9 ± 8.9	0.319
Calcium (mmol/L) (N=163)	2.2 (2.02, 24	2.3(2.2, 2.5	2.1(1.9, 2.2	*< 0.001**
Blood Culture (N=198)				
No Growth	134 (67.7)	71(35.9)	63 (31.8)	0.975
Gram Positive	29 (14.6)	15 (7.6)	14 (7.1)	
Gram Negative	34(17.2)	19 (9.6)	15 (7.6)	
Fungi	1 (0.5)	1 (0.5)	0	
Blood pH (N=183)	7.42 (7.37, 7.47)	7.44 (7.4, 7.48)	7.4 (7.33, 7.47)	*0.002**
pCO2 (mm Hg) (N=182)	29.2 (23.5, 34.9)	31.6 (25.7, 36.9)	25.9 (21.4,32.2)	*0.002**
pO2 (mm Hg) (N=182)	110 (75, 203)	104 (70, 195)	120 (79, 217)	0.278
Bicarbonate (mmol/L) (N= 183)	19.5 ± 6.2	21.9 ± 4.8	17.1 ± 6.5	*<0.001**

Normally-distributed data were expressed as mean ± standard deviation. Other data were expressed as n (%) or median (interquartile range). RAI (+) was assigned for index fulfillment (RAI score of ≥ 8). P value compared RAI (-) versus RAI (+) cohorts. Level of significance set at p < 0.05.

A summary of clinical outcomes is presented in Table 3. All outcomes showed significant differences between the two groups. The overall incidence of AKI was 40.5% with the RAI (+) group having significantly higher incidence. PRISM III scores were also much higher in the RAI (+) group with a median score of seven. The number of patients needing RRT was also higher in the RAI (+) group. Overall mortality rate was high at 29% and majority belonged to the RAI (+) group. In terms of hospital stay, the RAI (+) group had a shorter duration of stay with a median admitted days of 12 compared to the RAI (-) group of 20 days.

Table 3. Clinical Outcomes Stratified by Day 0 Renal Angina Fulfillment

Category	Overall N= 222	RAI (-) n= 127	RAI (+) n= 95	p value
Length of Hospital Stay (in days)	14 (8, 30)	20 (10, 36)	12 (5,19)	< 0.001*
PRISM III Score	5 (0, 7)	0 (0, 4.5)	7 (5, 10)	< 0.001*
Presence of AKI	90 (40.5)	3 (1.4)	87 (39.2)	< 0.001*
Need for RRT	54 (24.3)	2 (0.9)	52 (23.4)	< 0.001*
Mortality	64 (28.9)	10 (4.5)	54 (24.3)	< 0.001*

Data were expressed as n (%) or median (interquartile range). RAI (+) was assigned for index fulfillment (RAI score of ≥ 8). P value compared RAI (-) versus RAI (+) cohorts. Level of significance set at p < 0.05.

The diagnostic accuracy of RAI is presented in Table 4. AUC-ROC was at 0.953 (95% CI 0.92-0.98). Figure I shows the prediction model and its corresponding AUC- ROC plot.

Table 4. Diagnostic performance of renal angina index in prediction of acute kidney injury

Sensitivity (%)	96.7 (90.6-99.3)
Specificity (%)	94.0 (88.4-97.4)
Positive Predictive Value (%)	91.6 (84.1-96.3)
Negative Predictive Value (%)	97.7 (93.3-99.5)
Positive Likelihood Ratio	15.95 (8.1–31.3)
Negative Likelihood Ratio	0.04 (0.01-0.11)
AUC ROC	0.953 (0.92-0.98)

Data were presented as percentage (95% confidence interval). RAI (+) was assigned for index fulfillment (RAI score of ≥ 8). P value compared RAI (-) versus RAI (+) cohorts. The absolute RAI value (range, 1-40) was used to derive the AUC-ROC which was expressed with 95% confidence interval.

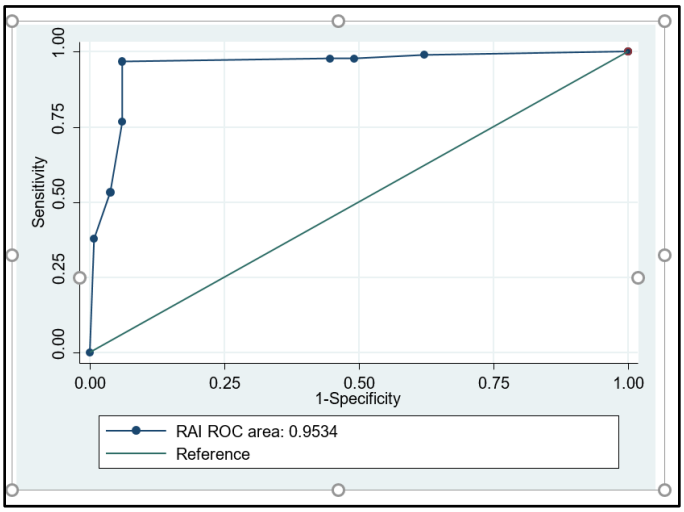

Figure I. AUC-ROC Plot for AKI Prediction Using RAI. The RAI AUC-ROC area was at 0.9534 and showed clear separation of the two distributions

The fulfillment of RAI with a score of at least 8 on day of admission was independently associated with the occurrence of AKI (odds ratio 449.5, p value < 0.001) as presented in Table 5.

Table 5. Logistic Regression of Renal Angina Index for Acute Kidney Injury

	Odds Ratio	95% Confidence Interval	p-value
RAI	449.5	116 - 1742.5	<0.001*

Odds ratio was expressed with 95% confidence interval. Level of significance set at p < 0.05.

DISCUSSION

The burden brought about by the occurrence of AKI remains to be high across all regions of the globe with geographic variations noted between countries and their economies. A published meta-analysis by Susantitaphong et al. in 2013 reported a high AKI incidence rate of 33.7% in the pediatric population encompassing all clinical settings such as critical care, trauma and cardiac surgery.[1] Sepsis and septic shock have been shown as the most significant predictors of AKI in critically ill patients as supported by a study in 2008 by Bagshaw et al.[14] In this present retrospective study, 90 of 222 subjects developed AKI, roughly a rate of 40.5% which was double than the reported incidence of 20% among a pediatric cohort with severe sepsis in a study conducted by Fitzgerald et al. in 2016.[15] This alarmingly high incidence rate was coupled by an overall mortality rate of 28.9%. Early recognition and management of AKI is the most logical way to address its dreaded complications. However, the use of creatinine or clinical symptom such as oliguria as a marker of AKI remains to be a hindrance in achieving this goal since these parameters are often late markers of injury. Unlike other systemic diseases with successful breakthroughs in early diagnosis with the use of biomarkers, available breakthrough diagnostics for AKI provide inconsistent to fair performance. The introduction of the RAI concept paved the way in predicting AKI in vulnerable patients. The use of RAI as a risk-stratification model optimizes the pre-test probability of the disease which will be complemented by novel biomarkers once necessary to have an improved post-test probability of detecting AKI.[4] A composite score of at least 8 signify fulfillment of the index which reflected marked discriminatory utility.

Given that risk tranches and clinical evidence of injury are basic components of the RAI definition, it is not surprising to have a greater number of patients in the RAI (+) group needing inotropes and mechanical ventilation with a concomitant higher percentage of fluid overload and higher values of BUN and creatinine. The negative effect of sepsis is manifested by substantial derangements in other organ systems such as thrombocytopenia (p = < 0.001), lower prothrombin activity (p = 0.025), lower calcium level (p = < 0.001) and lower blood pH, pCO2 and bicarbonate levels (p = < 0.001). These changes which were significantly noted in the RAI (+) group suggest possible risk factors in the development of sepsis-associated AKI. The blood gas parameters reflected the presence of metabolic acidosis combined with respiratory alkalosis supporting the increased need for hemodynamic and ventilatory support in the RAI (+) group. Moreover, the higher AKI incidence reflected poorer clinical outcomes such as increased need for RRT and mortality

thereby suggesting the proposed additive detrimental effect of having both sepsis and the subsequent AKI rather than having sepsis alone. PRISM III scores turned out to be higher in the RAI (+) group supporting its association with higher mortality rate. Several studies have reported that RAI (+) patients had a longer hospital stay. [4,6-7] This is in contrast with what was seen in this study where RAI (-) patients had significantly longer duration of stay compared to RAI (+) subjects with a median of 20 and 12 days respectively. This significant result can be hypothesized to be due to the sicker state of RAI (+) patients, a more complicated and stormier course and earlier demise.

The utility of RAI as a clinical guide is brought about by integration of baseline, contextual and clinical evidence of injury which identifies patients at risk for AKI.[9] Several studies have concluded that RAI is a good screening tool due to its high NPV and acceptable AUC-ROC.[5-7]

In this study, the diagnostic accuracy of RAI tested in pediatric patients with sepsis yielded good results in all parameters for the validation of an assessment tool. The high sensitivity and NPV of RAI further confirm its beneficial role as a screening tool in ruling out AKI and preventing further indiscriminate testing such as use of biomarkers. The high specificity and PPV suggest that RAI can successfully detect those who need further investigation and for whom an AKI biomarker will be most beneficial and cost-effective. With the study's sample size, the utility of RAI as a predictive tool is supported by positive and negative LR. A high positive LR of 15.95 supports AKI consideration while its low negative LR aids in ruling out the possibility of AKI in each subject. The discriminatory nature of RAI is further supported by the high AUC ROC value of 0.953 and a plot showing separation of the two distributions. Inherent validity of the diagnostic tool is therefore appreciated. Logistic regression computation showed a very high odds ratio thus RAI can be considered as an independent risk factor associated with the occurrence of AKI.

There are several potential limitations of this study. First, the study design has inherent restrictions especially in the analysis and data extraction of variables not available upon review of the medical records. Second, being a single-center study, the management of patients can be subject to institutional bias especially in the provision of work-ups and in the use of management algorithms. Lastly, no transplant patients were enrolled since this patient subset is usually referred and followed-up in another tertiary institution. The inclusion of these groups in future studies will refine the RAI stratification and will make comparison between high risk and very high-risk tranches possible.

CONCLUSION

RAI is a good screening tool in the prediction of sepsis-associated AKI in the pediatric population. Its application provides early AKI recognition upon admission. It is a clinically practical and feasible method which can be used at bedside with relatively simple calculations. Its discriminatory utility may reduce arbitrary use of expensive biomarkers providing context to their use. Its pragmatic nature and good performance holds promise to its future integration into clinical practice.

RECOMMENDATION

The call for a larger pediatric population and for prospective studies involving serum and urinary biomarkers in conjunction with RAI stratification of children with sepsis is recommended to aid in targeted and early management of AKI which involves hemodynamic intervention, vigilance in renal monitoring, avoidance of nephrotoxins, aggressive sepsis treatment, and provision of adequate nutrition and target goals for fluid balance.

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Verzosa G, Yu MMC, Senen KA, & Quilendrino MI. Granulocyte Colony Stimulating Factor in Improving Outcomes of Neonatal Sepsis: A Meta-Analysis

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

3RD PRIZE 2018 PIDSP RESEARCH CONTEST

ORIGINAL ARTICLE

GRANULOCYTE COLONY STIMULATING FACTOR IN IMPROVING OUTCOMES OF NEONATAL SEPSIS: A META-ANALYSIS

ABSTRACT

Background: Neonatal sepsis complicated with neutropenia increases risk of mortality by 50%. The immature neutrophil production of neonates is often overwhelmed by severe infection. Granulocyte colony stimulating factor (G-CSF), a naturally occurring cytokine used to support neutrophil recovery during chemotherapy, is a possible treatment that can improve outcomes of neonatal sepsis.

Objectives: To determine the efficacy of G-CSF in decreasing mortality and morbidity in septic neonates.

Methodology: Electronic searches were conducted on online journal databases. Unpublished or ongoing studies were sought in training institutions accredited by the Philippine Pediatric Society. The investigators included randomized control trials using G-CSF on septic neonates.

Results: Twenty-two trials were identified and thirteen were assessed to be eligible for review. The studies had a total of 530 participants, with the largest having 78 subjects. Relative risks (RR), mean differences (MD) and standard mean differences (SMD) with 95% confidence intervals (CI) using the fixed effect model and random effects model were reported in the results. There was a significant decrease in mortality (RR 0.69, 95% CI 0.48 to 0.99) with a greater reduction for preterm neonates, low birth weight neonates and neutropenic neonates. There was no significant reduction in morbidities caused by neonatal sepsis.

Conclusions: There is moderate quality evidence that suggests that G-CSF as an adjunct treatment for neonatal sepsis significantly decreases mortality with greater benefit to preterm neonates, low birth weight neonates and those with baseline neutropenia. The studies did not show any benefit in reducing sepsis-related morbidity.

KEYWORDS: *granulocyte colony stimulating factor, neonatal sepsis, neutropenia*

INTRODUCTION

Neonatal sepsis is one of the principal causes of morbidity and mortality worldwide. More than one-third of the 2.7 million deaths in the neonatal period is attributed to severe infection[1]. It also leads to permanent disability such as cerebral palsy and chronic lung disease[2,3]. Incidence is much higher in developing countries; but even in more sophisticated settings, neonatal sepsis still proves to be difficult to manage[4]. This is attributed to the immature immune system of neonates that is more profound in the susceptible groups of preterm neonates and those with low birth weights. When sepsis is accompanied with severe neutropenia, risk of mortality increases to more than 50%[5]. Neutropenia occurs when the immature neutrophil production of neonates is overwhelmed by severe infection. Another factor that contributes to increased mortality and morbidity is the functionally immature neonatal neutrophils[6]. Even with advancements in antibiotic and adjunctive therapies, the presence of neutropenia in the context of neonatal sepsis poses a difficult challenge to the clinician. These coupled with the alarming rise of antibiotic resistance stresses the need to explore alternative or adjunct therapies for neonatal sepsis[7].

Granulocyte colony stimulating factor (G-CSF) is a naturally occurring cytokine often used in cancer patients after chemotherapy to hasten neutrophil recovery[8]. Use of G-CSF is relatively safe with common side effects including headache, loss of appetite, bone pain, diarrhea, constipation and mild liver changes[9]. In previous studies, there was no associated increase in mortality or morbidity among neonates administered with G-CSF[10,11].

It is hypothesized that use of G-CSF in neutropenic neonates will increase numbers of circulating neutrophils as well as improve their phagocytic function. In a previous meta-analysis done by Carr et al. in 2003, it was concluded that there was insufficient evidence supporting the use of G-CSF in decreasing mortality in septic neonates. The studies that were reviewed included 257 neonates with suspected bacterial infection. However, each study had small sample sizes with 60 subjects at the most[10]. Since 2003, more studies have been conducted with larger populations recruited from multiple centers. This additional data could help determine if the administration of G-CSF will improve outcomes of neonatal sepsis. Hence, this study aimed to determine the efficacy of G-CSF in decreasing mortality and morbidity from neonatal sepsis as well as in increasing absolute neutrophil count (ANC) in septic neonates.

METHODS

Criteria for Considering Studies for Review

The following criteria were used to identify studies for inclusion:

Types of Studies

- randomized control trials
- with or without blinding
- with or without placebo control

Types of Participants

Newborn infants (0-28 days old) with culture proven or suspected sepsis fulfilling one or more of the following criteria:

- admitted to a neonatal intensive care unit or hospital ward
- with neutropenia (ANC < 1,500)
- at high risk for developing sepsis (i.e. preterm, low birth weight, small for gestational age)

Types of Interventions

Administration of G-CSF in any dose alongside conventional medical treatment compared with standard care with or without placebo.

Types of Outcome Measures

Primary Outcomes

1. mortality
2. morbidities caused by neonatal sepsis (i.e. chronic lung disease, necrotizing enterocolitis, cerebral palsy, etc.)

Secondary Outcomes

1. absolute neutrophil count
2. leukocyte count
3. immature: total neutrophil ratio (I:T Ratio)
4. duration of hospital stay
5. duration of ventilatory support
6. adverse effects that can be attributed to the administration of G-CSF

Search Methods for Identification of Studies

Electronic searches were conducted on online medical journal databases (Cochrane Library, PubMed, MEDLINE, Embase, WHO International Clinical Trials Registry, Herdin) as well as on other online journal databases (Google Scholar, Jstor, Directory of Open Access Journals, Science Direct) to identify relevant studies. The following search strategy was utilized: ("G-CSF" OR "rhG-CSF" OR "granulocyte colony stimulating factor") AND "neonatal sepsis". There was no limitation in terms of language or publication period. In the articles retrieved, the reference lists were searched for other relevant trials.

Local pediatric training institutions accredited by the Philippine Pediatric Society were also contacted to inquire about any unpublished or ongoing studies that fulfill the inclusion criteria.

Data Collection and Analysis

Selection of Studies

Two review authors separately searched for all available articles that meet the inclusion criteria. Meta-analyses and systematic reviews were also scanned for eligible studies. Full-text articles of all potentially eligible studies were obtained and reviewed. Studies that were published multiple times only had one final report included in the review. For articles with data that are either insufficient or unclear, authors were contacted for clarification. Such articles whose authors could not be contacted were excluded from the review. For articles that are written in languages other than English, a translated paper was searched for or requested from the author/s. If there was none available, then the study was excluded. In case of disagreements between the authors, issues were resolved through discussion.

Data Extraction and Management

From each of the eligible articles, data was extracted independently by the reviewers and organized into a standard database. A modified data collection form based on the one published by The Cochrane Collaboration was used to organize the extracted data that included[12]:

1. General Information: title, primary investigator, year of publication
2. Population: age (in weeks), birth weight, sex
3. Sample Size
4. Characteristics of Intervention: dose, route
5. Characteristics of Control: standard care, placebo
6. Blinding: treatment allocation, intervention, outcome measure assessment

The data collected was summarized and entered into the Review Manager ver. 5.3 program (Cochrane Collaboration software)[13].

Assessment of Risk of Bias in Included Studies

The reviewers independently evaluated the overall risk of bias and assessed the quality of evidence based on indicators of internal validity. The criteria for evaluating the articles were based on the Cochrane Handbook for Systematic Reviews of Interventions[14]. The articles were assessed as low risk, high risk or unclear based on the following indicators:

1. Sequence Generation (screening for selection bias)

   For each eligible study, the method used to generate treatment allocation was described. The methods were assessed as:

   - Low Risk - any form of randomization (ex. random number table, computer generated)

- High Risk - non-randomized processes (ex. alternating case numbers, odd or even date of birth)
- Unclear - insufficient information

2. Allocation Concealment (screening for selection bias)

For each eligible study, the method used to conceal treatment allocation was described. The methods were assessed as:

- Low Risk - opaque and sealed envelopes, sequentially numbered drug containers, central randomization
- High Risk - open label trials, predictable allocation (ex. alternation, rotation), clear or unsealed envelopes
- Unclear - insufficient information

3. Blinding (screening for performance bias and detection bias)

For each eligible study, the blinding of patients, personnel and/or outcome assessors were described. Whether or not placebo was used in the study was also indicated. The methods were assessed as:

- Low Risk - no/incomplete blinding but the outcome is not likely to be influenced by the lack of blinding, blinding was ensured and was unlikely to be broken
- High Risk - no/incomplete blinding and the outcome is likely to be influenced by the lack of blinding, blinding was done but was likely to be broken
- Unclear - insufficient information

4. Incomplete Outcome Data (screening for attrition bias)

For each eligible study, the completeness of data including reasons for exclusions, withdrawals, dropouts and protocol deviations was evaluated. The final number of included participants in the analysis was also compared to the initial randomized participants. The studies were assessed as:

- Low Risk - complete outcome data, reasons for missing outcome data are unlikely to be related to the true outcome, missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups
- High Risk - reasons for missing outcome data are likely to be related to the true outcome, missing outcome data are not balanced in numbers across intervention groups
- Unclear - insufficient information

5. Selective Outcome Reporting (screening for reporting bias)

For each eligible study, the presence of possible selective reporting bias was evaluated. The studies were assessed as:

- Low Risk - published reports include all expected outcomes that have been pre-specified
- High Risk - one or more primary outcomes were not pre-specified, outcomes of interest are not reported completely, does not include results of a key outcome which was expected of the study
- Unclear - insufficient information

6. Other sources of bias

For each eligible study, the presence of other sources of bias that were not clearly stated above was assessed. The studies were assessed as:

- Low Risk - appears to be free of other sources of bias
- High Risk - has at least one important risk of bias
- Unclear - insufficient information

<u>Data Synthesis and Measures of Treatment Effect</u>

The collected data from all articles that are included in the final data analysis were run through the Review Manager ver. 5.3 software. Dichotomous data included incidence of mortality, morbidity and adverse events while continuous data included absolute neutrophil count, leukocyte

count, immature: total neutrophil ratio, duration of hospital stay and duration of ventilatory support. For continuous data, the mean difference was used if outcomes were measured using the same method and unit of measure. Otherwise, the standardized mean difference was used to combine trials using different methods and units to measure the same outcome.

Dealing with Missing Data

It was anticipated that some trials did not report all relevant figures to the study. Where there is significant loss of data, trial authors were contacted. In cases where missing data could not be retrieved, imputation methods were utilized and such studies underwent sensitivity analysis.

Assessment of Heterogeneity

The heterogeneity of data included in the analysis was measured through the chi-square test, the I2 statistic and visual inspection of forest plots. Significant heterogeneity is defined as P-value of < 0.10 in the chi-squared test and an I2 value > 50%.

Assessment of Reporting Biases

For eligible studies included in the study, funnel plots were drawn to investigate if there is an association between the sample size and the effect estimates. When an association was detected, the studies were further examined and possible reasons for such an association were reported.

Subgroup Analysis and Investigation of Heterogeneity

The study aimed to do the following subgroup analyses should there be enough data for these to be conducted:

1. preterm infants (gestational age less than 37 weeks) versus term (gestational age less than 37 weeks)
2. birth weight (low - < 2500g, very low - < 1500g, extremely low - <1000g)
3. initial Absolute Neutrophil Count (ANC) = (%Neutrophils + %Bands) x WBC/100
4. culture-positive sepsis versus suspected sepsis dose and duration of G-CSF treatment

Sensitivity Analysis

To assess the impact of the quality of the studies included in the study as well as the imputation of missing data on the results of the meta-analysis, sensitivity analysis was conducted. The presence or absence of significant association was included in the report.

RESULTS

Results of the Search

The search identified 22 trials, 13 of which were included in the review while 9 were excluded.

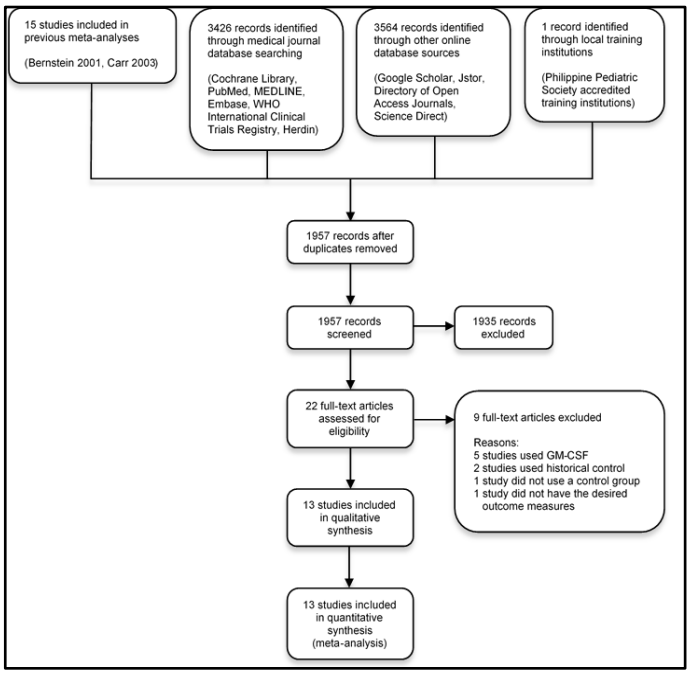

Figure 1. The PRISMA flow diagram showing the process of study selection

Included Studies

Fifteen studies from previous meta-analyses (Bernstein 2001, Carr 2003) were originally planned to be included; however, 6 were excluded due to reasons enumerated in the next segment. There was a total of 530 participants in all 13 studies included for review, the largest having 78 participants (Chaudhuri 2012). Three were conducted in multiple centers (Aktas 2013, Gillan 1994, Schibler 1998) while the rest were conducted in single institutions. Eight trials specified that they were conducted in

neonatal intensive care units (Ahmad 2002, Bedford Russell 2001, Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002). All studies recruited neonates with either proven or suspected sepsis. Certain studies had the following common characteristics in their inclusion criteria: neutropenia in 10 studies (Ahmad 2002, Aktas 2013, Bedford Russell 2001, Borjianyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, Drossou-Agakidou 2002, Gathwala 2011, Miura 2001, Schibler 1998), prematurity in 9 studies (Ahmad 2002, Aktas 2013, Bedford Russell 2001, Borjianyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, Gathwala 2011, Miura 2001, Sezer 2002), low birthweight in 5 studies (Bedford Russell 2001, Chaudhuri 2012, Gathwala 2011, Miura 2001, Sezer 2002). Nine studies used a placebo of equal volumes of either normal saline or the same diluent used for administering G-CSF (Ahmad 2002, Bedford Russell 2001, Borjianyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 2002, Gillan 1994, Miura 2001, Schibler 1998, Sezer 2002). The rest of the studies compared the treatment group with standard medical care. (Aktas 2013, Drossou-Agakidou 1998, El-Ganzoury 2012, Gathwala 2011).

Excluded Studies

There were 9 excluded studies. Reasons for exclusion were:

1. Five studies used granulocyte-macrophage colony stimulating factor instead of granulocyte colony stimulating factor.
2. Two studies used historic control subjects instead of randomizing patients into treatment and control groups.
3. One study compared the effects of two different doses of G-CSF. There was no control group to which the treatment groups were compared to
4. One article discusses the neuropsychological development and anthropometrics of children included in the PROGRAMS trial conducted in the UK. The article did not include the necessary outcome measurements.

Risk of Bias in Included Studies

Randomization

Eight studies (62%) specified the method of randomization used for treatment allocation (Bedford Russell 2001, Borjianyazdi 2013, Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002). The studies used either a computer-generated randomization or a table of random numbers. The rest of the studies (38%) stated that the patients included in their trials were randomized; however, the method used was not specified.

Allocation Concealment

Among the included studies, only 3 studies (23%) adequately described how treatment allocation was concealed (Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011). The studies used either opaque sequentially numbered sealed envelopes or allocation numbers concealed in the cover of each medication/placebo. The other 10 studies (77%) did not mention allocation concealment methods in their articles.

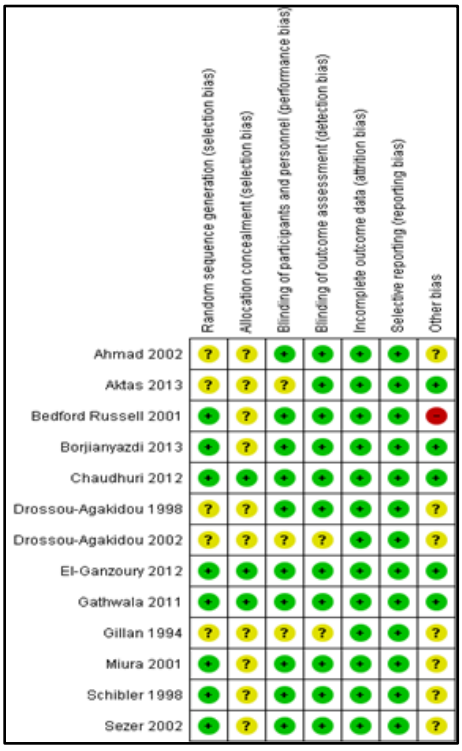

Figure 2. Risk of Bias Summary: review authors' judgements about each risk of bias item for each included study

Blinding

Ten studies (77%) followed a double-blinded study design (Ahmad 2002, Bedford Russell 2001, Borjianyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002) while 3 studies (23%) had insufficient information regarding blinding (Aktas 2013, Drossou-Agakidou 2002, Gillan 1994).

Incomplete Outcome Data

Most of the studies included all enrolled participants in the final analyses. In cases where there are withdrawals or exclusions in analyses, there was sufficient information explaining why certain participants were not included in the final analyses. The most common reason for exclusion was mortality. Patients who died during the trial were excluded from the analysis of the duration of hospital stay as well as the duration of ventilatory support.

Selective Reporting

It is difficult to assess true selective reporting bias since the protocols for the included studies could not be retrieved. Instead, judgment was based on the outcomes mentioned in the methods section compared with the final results reported in the article

Other Potential Sources of Bias

The authors of one article (8%) stated that their study was funded by the pharmaceutical company that manufactured the G-CSF used in the trial (Bedford Russell 2001). Seven studies (54%) mentioned the pharmaceutical companies who manufactured the G-CSF used in the trial; however, they did not declare any connections to those companies mentioned (Ahmad 2002, Drossou-Agakidou 1998, Drossou-Agakidou 2002, Gillan 1994, Miura 2001, Schibler 1998, Sezer 2002). One study (8%) did not mention any pharmaceutical company at all. Four studies (31%) explicitly mentioned that they do not have any conflicts of interest to declare.

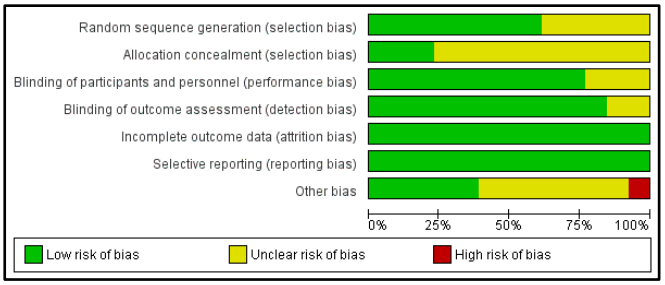

Figure 3. Risk of Bias Graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

Effects of Interventions

Primary Outcomes

Mortality

All thirteen included studies reported data on all-cause mortality. The studies exhibited homogenous results, as shown in Figure 4.

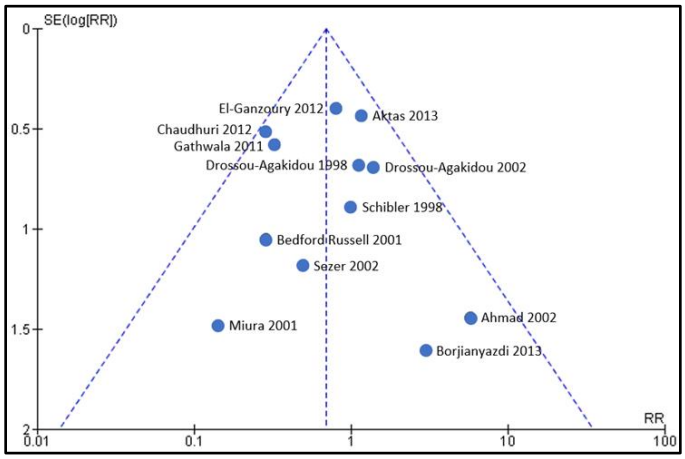

Figure 4. Funnel Plot of Comparison: Mortality Rate

Five hundred thirty participants were included in the analysis. There were 41 deaths from the 282 participants in the treatment group while there were 54 deaths from the 248 participants from the control group. There was a significant decrease in mortality in the treatment group [RR 0.69 (0.48, 0.99)] (Figure 5).

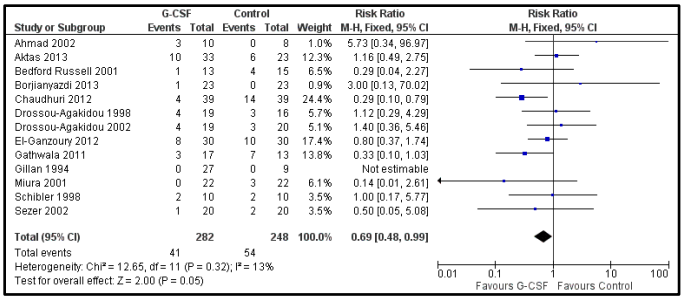

Figure 5. Forest Plot: Mortality Rate

There was a greater reduction of mortality rate for preterm neonates [RR 0.60 (0.39, 0.94)] and neonates with low birthweight [RR 0.29 (0.15, 0.57)]. However, there was no significant reduction of mortality in neonates who had baseline neutropenia [RR 0.68 (0.45, 1.02)].

Morbidity

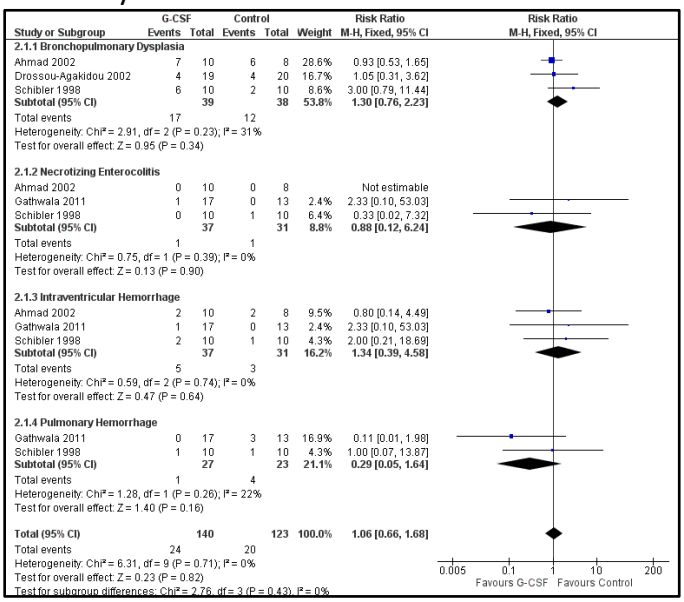

Figure 6. Forest Plot: Morbidities (Bronchopulmonary Dysplasia, Necrotizing Enterocolitis, Intraventricular Hemorrhage, Pulmonary Hemorrhage)

Four studies reported morbidities related to neonatal sepsis (Ahmad 2002, Drossou-Agakidou 2002, Gathwala 2011, Schibler 1998). There was no significant reduction of morbidities reported: Bronchopulmonary Dysplasia [RR 1.30 (0.76, 2.23)], Necrotizing Enterocolitis [RR 0.88 (0.12, 6.24)], Intraventricular Hemorrhage [RR 1.34 (0.39, 4.58)], Pulmonary Hemorrhage [RR 0.29 (0.05, 1.64)], Overall Morbidity [1.06 (0.66, 1.68)] (Figure 6).

Secondary Outcomes

Duration of Hospital Stay

There were six studies that reported the duration of hospital stay (Ahmad 2002, Bedford Russell 2001, Borjianyazdi 2013, Drossou-Agakidou 2002, El-Ganzoury 2012, Gathwala 2011). There was a reduction in the duration of hospital stay for the treatment group [MD -4.91 (-6.92, -2.90)]. However, the studies analyzed showed heterogenous results with a P-value of 0.0004 and an I2 value of 78% (Figure 7).

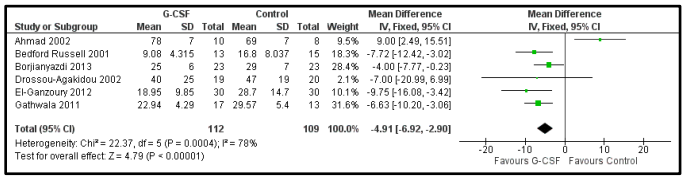

Figure 7. Forest Plot: Duration of Hospital Stay in Days

Duration of Ventilatory Support

Three studies reported the duration of ventilatory support (Bedford Russell 2001, Drossou-Agakidou 2002, El-Ganzoury 2012). There was a reduction in the duration of ventilatory support for the treatment group [MD -3.72 (-6.94, -0.50)]. The studies analyzed also showed heterogenous results with a P-value of 0.003 and an I2 value of 83% (Figure 8).

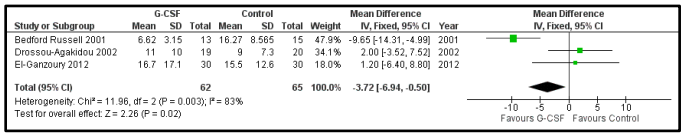

Figure 8. Forest Plot: Duration of Ventilatory Support in Days

Increase in Absolute Neutrophil Count

There were nine studies that reported ANC; however, they did not report the means and standard deviations for change from the baseline counts (Ahmad 2002, Aktas 2013, Borjianyazdi 2013,

Chaudhuri 2012, El-Ganzoury 2012, Gathwala 2011, Miura 2001, Schibler 1998, Sezer 2002). To derive this data from the results, the following formula from the Cochrane Handbook for Systematic Reviews of Interventions was used: SDE-change = √ [SDE-baseline2 + SDE-final2 - (2 x 0.5 x SDE-baseline x SDE-final)][14]. One study reported the medians and range of values in their results (Miura 2001). The authors were contacted to retrieve the values of the means and standard deviations; however, the investigators did not receive a reply. Instead, the formulas developed by Hozo et al. were used to derive the necessary data[15]. Sensitivity analysis was done which showed that the imputation of missing data did not have a significant effect on the final analysis.

In the analyzed studies, ANC was usually measured during the 1st, 2nd and 3rd day of treatment. There was an increase in ANC for the G-CSF treated group on the first three days of treatment: 1st day [SMD 0.99 (0.70, 1.28)], 2nd day [SMD 1.11 (0.74, 1.49)], 3rd day [SMD 2.05 (1.71, 2.38)]. There were different treatment periods between the studies ranging from 3-14 days with the most common treatment period lasting 3 days. There was an increase in ANC in the G-CSF group after treatment regardless of duration [SMD 0.73 (0.52, 0.95)]. There was a smaller effect for those who already had baseline neutropenia [SMD 0.71 (0.46, 0.95)]. However, the studies showed significant heterogeneity with P-values < 0.10 (0.003, 0.003, < 0.00001, < 0.00001) and I2 values > 50% (75%, 78%, 91%, 89%) (Figure 9).

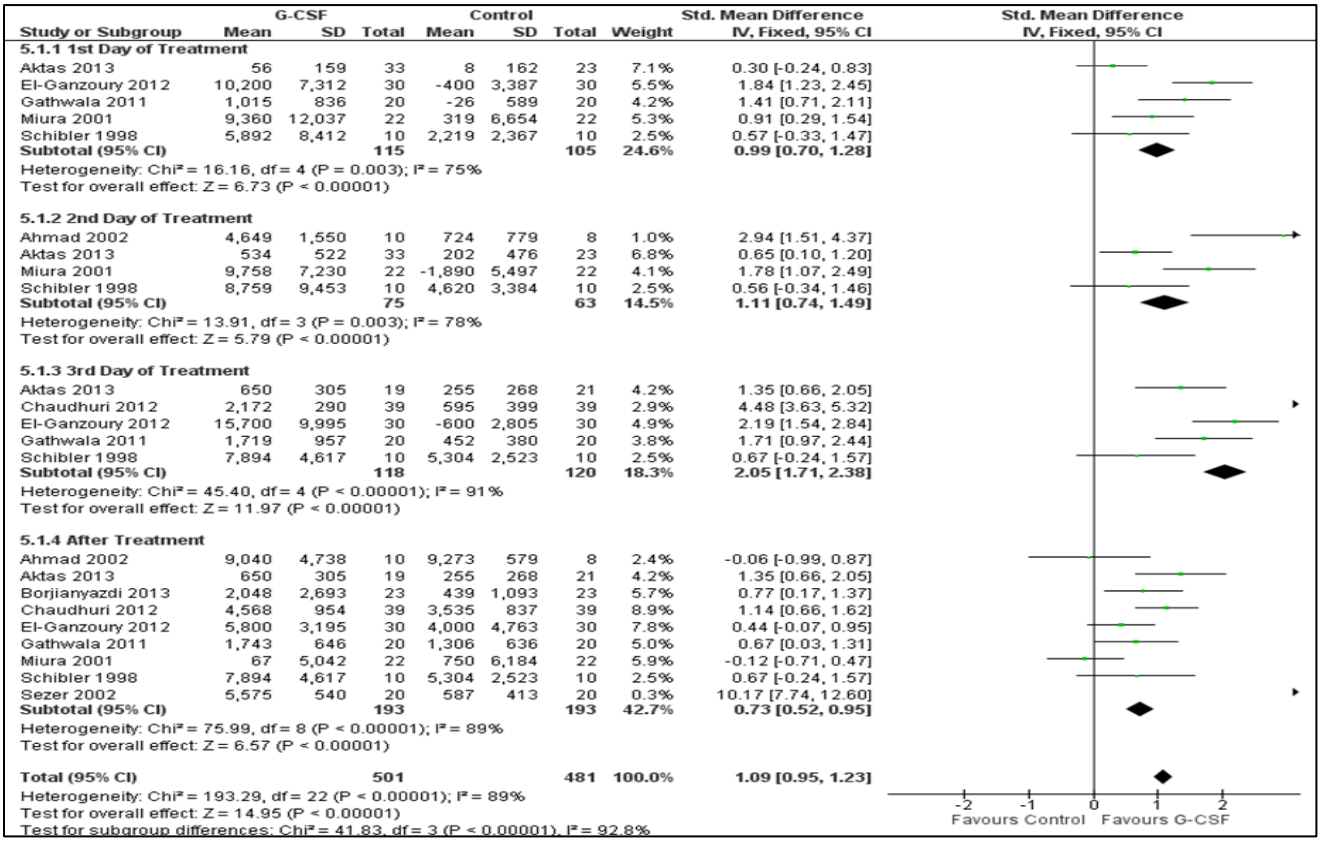

Figure 9. Forest Plot: Increase in Absolute Neutrophil Count

Increase in Leukocyte Count

Three studies reported leukocyte counts of the participants but did not report the means and standard deviations for change from the baseline counts (El-Ganzoury 2012, Gathwala 2011, Miura 2001). The same imputation methods mentioned above were used to derive the necessary data. The studies show that there was a significant increase in leukocyte counts in the treatment group [SMD 0.41 (0.08, 0.74)] (Figure 10).

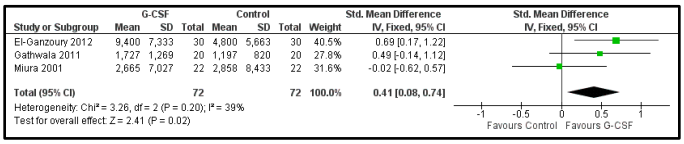

Figure 10. Forest Plot: Increase in Leukocyte Count

Decrease in Immature:Total Neutrophil Ratio

There were three studies that reported I:T ratios of the participants (Aktas 2013, El-Ganzoury 2012, Sezer 2002). However, they did not include the means and standard deviations for change from the baseline ratios. The same formula mentioned above was used to derive the necessary data. The studies show that there is no significant difference in I:T ratios between the treatment group and the control group [SMD 0.23 (-0.10, 0.57)] (Figure 11).

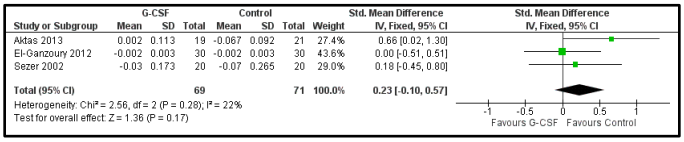

Figure 11. Forest Plot: Decrease in Immature: Total Neutrophil Ratio

Adverse Effects Caused by G-CSF

Twelve studies mentioned in their methods that they monitored for toxicity or adverse effects caused by G-CSF administration (Ahmad 2002, Aktas 2013, Bedford Russell 2001, Borjianyazdi 2013, Chaudhuri 2012, Drossou-Agakidou 1998, Drossou-Agakidou 2002, El-Ganzoury 2012, Gillan 1994, Miura 2001, Schibler 1998, Sezer 2002). The drug was found to be well tolerated by the participants in the treatment group as no toxicity or adverse effects were noted. However, there was no data regarding long-term adverse effects in any of the included studies.

DISCUSSION

The purpose of this meta-analysis was to integrate and examine the data collected from published reports on the effect of G-CSF on the outcome of neonatal sepsis. There were 13 randomized control trials included with a total of 530 neonates. The studies reviewed had relatively small population sizes with the largest having only 78 participants (Chaudhuri 2012). This greatly affected the quality of data as 9 out of the 17 outcomes analyzed only had low to very low quality of evidence partly due to small population size.

The studies had participants with different ranges of age of gestation, birthweight, severity of neutropenia, as well as severity of sepsis. The age of gestation of the neonates ranged from 24-40 weeks while their birth weights ranged from 530-3667g. The definition of neutropenia also varied greatly between studies. The inclusion criteria used in the studies usually had ANCs < 1000 cells/µL, < 1500 cells/µL or < 5000 cells/µL while one study had an inclusion criterion with ANC < 20000 cells/µL (Drossou-Agakidou 2002). Only three studies used the Score for Neonatal Acute Physiology (SNAP) in order to score the severity of sepsis (El-Ganzoury 2012, Miura 2001, Schibler 1998). The dose, frequency and duration of G-CSF administration were also different between studies and some compared these in their results. The most common dose across the studies was 10 µg/kg/day given either q12h or OD for 3-5 days. These may be significant confounding factors to the final analysis.

There was a significant reduction in all-cause mortality. There was a greater benefit for participants who were preterm and had low birth weight as compared to those who had baseline neutropenia. This is contrary to the conclusion of the previous meta-analyses[10,14]. This may be because some of the excluded studies from the previous meta-analyses showed a greater reduction

in mortality in neutropenic infants. Another likely source of bias would be the different severity of neutropenia between the studies as mentioned above.

There was no significant reduction in sepsis-related morbidities. For bronchopulmonary dysplasia and necrotizing enterocolitis, this may be due to the inflammatory role of neutrophils in the pathogenesis of these diseases themselves[16,17]. It is notable that the data collected for morbidity had low quality of evidence due to unclear risk for selection bias, sparse data and lack of agreement between studies.

There was a reduction in the duration of hospital stay and the duration of ventilatory support; however, the studies analyzed showed heterogenous results. A possible confounding factor may be different nosocomial infection rates in different hospitals that may prolong hospitalization as well as ventilatory support.

As was expected, there was an increase in the ANC for the treatment group from the first three days of G-CSF administration up to the end of the studies. A majority of the studies analyzed showed results favoring the administration of G-CSF; yet, there was a smaller increase in those who had baseline neutropenia. This may have a significant correlation with the minimal effect of G-CSF on the mortality rate of neutropenic patients. However, the studies had significant statistical heterogeneity, hence there is only low to very low-quality evidence supporting this. One possible cause for this heterogeneity may be the different baseline ANC of the participants in the different studies. Another would be the different treatment durations in the studies analyzed.

There was a significant increase in total leukocyte count that could be attributed to the increase in neutrophil production. As for the I:T ratio, there was no significant difference between the groups.

No toxicity or adverse effects attributed to G-CSF administration were reported in any of the studies included in the review. However, there were no studies which followed through long enough to report long term effects of G-CSF.

CONCLUSIONS

Current evidence shows that administering G-CSF to septic neonates could possibly reduce mortality rates. Preterms and low birthweight newborns are shown to be the populations that could benefit the most from this treatment. However, there was minimal improvement of outcomes for septic neonates with concomitant neutropenia. Nevertheless, treatment for this population warrants further study.

Further investigation on the effects of G-CSF on septic neonates should be done with standardized protocols and larger populations. Future trials should have a consistent set of inclusion criteria with the same definition of terms (i.e. neutropenia) across all studies. Stratification of patients according to gestational age, birth weight, baseline ANC and severity of sepsis may help pinpoint the populations that will most likely benefit from the treatment. The effects of G-CSF on culture-positive sepsis may also be compared to its effects on suspected sepsis. Different doses, frequencies and treatment durations may also be compared in future trials. Sepsis-related morbidities should be correlated with the ANC of the patient at the time of onset. This could determine the effect of increase in ANC on morbidities linked to neutrophilia (i.e. bronchopulmonary dysplasia, necrotizing enterocolitis). A more extensive follow-up should be done to elicit possible long-term adverse effects of the drug.

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ORIGINAL ARTICLE

A RETROSPECTIVE STUDY ON THE OUTCOME OF CHILDREN WITH EXTENSIVELY DRUG-RESISTANT GRAM-NEGATIVE INFECTION TREATED WITH COLISTIN VS OTHER ANTIMICROBIALS

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The authors declare that the data presented are original material and has not been previously published, accepted or considered for publication elsewhere; that the manuscript has been approved by all authors, and all authors have met the requirements for authorship.

ABSTRACT

Introduction: The increasing trend of extensively drug-resistant gram-negative infections led to the reconsideration of colistin as a valuable therapeutic option.

Objectives: To describe the clinical profile and treatment response of children with extensively drug-resistant (XDR) Gram-negative infections given colistin versus other antimicrobials.

Methods: This retrospective descriptive study involved patients treated for XDR Gram-negative infections from January 2014 to June 2017 in a tertiary hospital in Metro Manila. Descriptive statistics were used to summarize clinical characteristics of subjects. Treatment response to colistin versus other antimicrobial agents were compared in terms of success, failure, and toxicity. The Fisher-exact and Mann Whitney U tests were used to assess statistical differences between the colistin and non-colistin groups.

Results: Majority of patients with XDR Gram-negative infections had previous antibiotic exposure. More patients in the colistin group received TPN 43.2% vs 23.7% (p=0.035), had a longer hospital stay prior to the onset of XDR Gram-negative infection, 27 days vs. 15.5 days (p=0.001), and had a longer total hospital stay with a median of 52 days vs 30 days ($p < 0.001$). Treatment success was significantly higher in the colistin group at 70.3%, as against 46.5% in the non-colistin group (p=0.014). There was no difference in the treatment duration of both groups. The colistin group had longer time to clinical response, with a mean of 6.27 ($\pm$ 3.57) days compared with those from the non-colistin group, with a mean of 4.36 ($\pm$ 1.77) (p=0.008). The colistin group had more fungal infections during the course of treatment (p=0.001).

Conclusion: Based on our institutional experience, colistin is considered relatively effective and safe in treating XDR Gram-negative infections in children.

KEYWORDS: *extensively drug-resistant gram-negative infection, healthcare associated infection, colistin*

INTRODUCTION

Antibiotic resistance among Gram-negative bacteria has reached critical levels.[1] The World Health Organization (WHO) continues to warn on the rise of new resistance mechanisms threatening our ability to treat common infectious diseases. This results in prolonged illness, increased cost of therapy, disability, and even death. For these serious infections, carbapenems are usually the recommended treatment. Recently, the emergence of carbapenemases has led to high level antibiotic resistance leaving colistin as the only treatment option. Gram-negative bacteria use two main mechanisms to develop phenotypic resistance to carbapenems: the production of carbapenemases, or a combination of structural mutations and production of other β-lactamase enzymes.[1] The following risk factors have been identified to contribute to carbapenem resistance in children: hospitalization for more than 48 hours, receipt of antibiotics, underlying medical conditions such as pulmonary disease, prematurity, oncologic and cardiac disease, solid-organ or stem-cell transplantation, history of surgery particularly gastrointestinal procedures, intake of immunosuppressants and presence of an indwelling device.

A similar trend is seen locally. Compared to 2015, the 2016 Antimicrobial Resistance Surveillance Program of the Department of Health reported a statistically significant increase in Extended spectrum beta-lactamase producing *Klebsiella pneumoniae* from 27% to 40% while the Carbapanem-resistant *Klebsiella* decreased from 11.9 – 15.3% to 9.1 – 11.4%. There was a slight decrease in *Pseudomonas* MDR and XDR rates from 22% and 18% to 21% and 16% respectively. However, there was a significant increase in resistance to amikacin from 7.3 to 8.6% and ciprofloxacin from 13.14 to 14.9%. *Acinetobacter* MDR rate for 2016 decreased from 66% to 61% but the XDR rate increased from 48% to 50%. There was a significant increase in *Acinetobacter* resistance to amikacin from 31% to 38%.[2]

The emergence of Gram-negative bacteria resistant to most classes of antibiotics and the lack of effective new antibiotics led to the reconsideration of colistin as a valuable therapeutic option in the early 1990s. In recent studies on the use of colistin in the pediatric population, a favorable outcome was observed in 65% – 89% of patients who received the drug.[3][4][5][6] Nephrotoxicity rates from studies involving pediatric patients including one study on neonates ranged from 1.6% to 22%.[3][4][5][6][7][8] Neurotoxicity is less common and ranged from 0% to 4%.[3][4][5][8][9]. Colistin however is not exempt from antibiotic resistance. The exact mechanism is still unclear but it has been suggested that resistance is related to lipopolysaccharide (LPS) modification.[10] At present, studies and experience on the use of intravenous colistin in extensively drug-resistant Gram-negative infections in the pediatric population are limited and there are no available local data or published research in the Philippine setting. This paper aims to describe the clinical profile and treatment response of children with extensively drug-resistant (XDR) Gram-negative infections given colistin versus other antimicrobials.

METHODOLOGY

Ethical Considerations

The study commenced upon the approval of the Institutional Review Board and Ethics Committee of the institution. The board and committee were likewise

informed of revisions during the research process. All information collected from the laboratory and patients' charts were used for this research only. Confidentiality was maintained during and after the study.

This retrospective descriptive study was conducted in a tertiary hospital in the Philippines from January 2014 to June 2017.

Subject and Sample Size Computation

Inclusion Criteria

Included in the study were children 0-18 years old admitted at the service and pay wards of a tertiary hospital who received colistin or other antimicrobials for the treatment of culture-proven extensively drug-resistant Gram-negative infection.

Exclusion Criteria

Patients 0-18 years old with culture proven extensively drug-resistant Gram-negative infection who received less than 6 doses of antimicrobial therapy, was not admitted, or expired prior to treatment were not included in the study. Patients with XDR Gram-negative culture isolates from the urine or tracheal aspirate but with Gram-positive sepsis or candidemia as the predominant infection in the blood, CNS and other sterile sites were likewise excluded.

Sample Size

A minimum of 346 subjects was required for this study based on a level of significance of 5%, a prevalence of 65.8%, and with a desired width of confidence interval of 10%, as noted from the reference article by Ozsurekci et. al. in 2016. [5] Since, extensively drug-resistant infections are rare, the desired sample size was not attained. The sample size requirement may be precluded by the fact that our primary aim is to describe rather than to estimate prevalence.

Description of the Study Procedure

Data Collection Method

Culture and sensitivity results from the Microbiology Section logbooks were reviewed and charts of patients with extensively drug-resistant cultures were retrieved from the Medical Records for review. Patients with culture proven extensively drug-resistant Gram-negative infection who were treated were included in the study and the following data were obtained:

Microbiologic data which included the culture source, organism and antimicrobial susceptibility of the Gram-negative XDR isolates; patient's demographic data included the age to further qualify if term or preterm if neonate, and sex. Clinical data noted in this study included the type of infection whether community or hospital acquired, the patient's nutritional status, co-morbid illness, surgical intervention and other medical procedures (invasive vascular access, receipt of TPN, use of mechanical ventilator, blood transfusion and foley catheter insertion). Intake of Immunosuppressants were also noted. Receipt of prior antibiotics within the last 90 days prior to XDR Gram-negative infection, and antibiotic regimen and duration were noted. Duration of hospital stay, days of hospitalization prior to onset of XDR gram negative infection, as well as the wards where patients were admitted were also recorded.

Patients were classified into the colistin and non-colistin group based on antibiotics received. The primary outcome was treatment success or failure. Treatment success was based on clinical and/or microbiological response after treatment while treatment failure was demonstrated by poor clinical response, persistence of the organism in the specimens (blood, urine, CSF, tracheal

aspirate, wound or surgical site), relapse or mortality. The secondary outcomes measured were time to clinical response (in days), total treatment duration (in days), occurrence of adverse events (nephrotoxicity, neurotoxicity, others) and occurrence of treatment related infection (example: fungal infection, other Gram-negative or Gram-positive infection)

Data Analysis

Frequency and proportion were used to summarize the number of Gram-negative isolates and the ratio of extremely drug-resistant isolates per year. Descriptive statistics was used to summarize the overall general and clinical characteristics of the subjects in the non-colistin and colistin groups. The number in count and percentages were presented for categorical data. The mean, median, standard deviation, and interquartile range (IQR) were presented for continuous data.

The Fisher-exact test was used to assess statistical differences between the non-colistin and colistin groups. Given the non-parametric distribution of continuous variables in the study population (negatively skewed), the comparative statistical analysis of Mann Whitney U test was performed to determine the differences between the non-colistin and colistin groups.

All complete records were considered valid and were included in the final analysis. Incomplete data and missing charts were excluded. Null hypothesis was rejected at 0.05 α-level of significance. SPSS 22.0 was used for data analysis. [13]

RESULTS

The total number of Gram-negative isolates from January 2014 to June 2017 was 4,571. Among these Gram-negative isolates, 228 XDR isolates were initially identified as potential eligible cases. There were 204 charts available for review. Fifty-three were excluded due to findings of Gram-positive sepsis (5), fungal infection (2), no treatment (6), not admitted (3), expired in less than 48 hours of treatment (4) and MDR infection (33). A total of 151 eligible patients were included in the study. Of the 151 patients with XDR infection, 114 were included in the non-colistin group and 37 in the colistin group.

For the period of January 2014 to June 2017, there was an increasing trend in the proportion of XDR isolates over the total number of Gram-negative isolates. In this study, there were 14 XDR isolates out of 788 Gram-negative isolates for 2014 (1.7%), 36 XDR out of 1,490 isolates for 2015 (2.4%), 66 out of 1,816 isolates for 2016 (3.6%) and 35 out of 477 isolates for the first half of 2017 (7.3%). Table 1 summarizes the microbiologic data of the XDR Gram-negative isolates. The top 3 sources of the isolates were blood (38.4%), urine (19.2%) and tracheal aspirate (18.5%). Sixty (39.7%) out of 151 XDR isolates were sensitive to colistin and amikacin, followed by 47 (31.1%) isolates sensitive only to colistin. (Appendix B) The most common XDR isolate was *Klebsiella* with 53 isolates (35.1%), followed by *Acinetobacter*, 40 isolates (26.5%) and *Stenotrophomonas* and *Pseudomonas aeruginosa* with 16 isolates each (10.6%).

Table 1. Microbiologic Data of Treated XDR Gram-negative Infection

		Overall n = 151 (%)	Non-colistin n = 114 (%)	Colistin n = 37 (%)	p-value
Culture source	Blood	58 (38.4)	42 (36.8)	16 (43.2)	0.347
	Urine	29 (19.2)	18 (15.8)	11 (29.7)	
	Tracheal Aspirate	28 (18.5)	24 (21.1)	4 (10.8)	
	CSF	11 (7.3)	8 (7)	3 (8.1)	
	Wound	11 (7.3)	10 (8.8)	1 (2.7)	
	Peritoneal Fluid	7 (4.6)	5 (4.4)	2 (5.4)	
	Surgical Site	5 (3.3)	5 (4.4)	0 (0)	
	Pleural Fluid	2 (1.3)	2 (1.8)	0 (0)	
Culture and sensitivity	Colistin Only	47 (31.1)	21 (18.4)	26 (70.3)	<0.001
	Colistin + Fluoroquinolone	13 (8.6)	12 (10.5)	1 (2.7)	0.188
	Colistin +Carbapenems	6 (4)	5 (4.4)	1 (2.7)	1.00
	Colistin + Aminoglycosides	60 (39.7)	51 (44.7)	9 (24.3)	0.034
	Others	25 (15.6)	25 (100)	0 (0)	0.001
Culture Isolate	*Klebsiella*	53 (35.1)	36 (31.6)	17 (45.9)	0.066
	Acinetobacter	40 (26.5)	31 (27.2)	9 (24.3)	
	Stenotrophomonas	16 (10.6)	16 (14)	0 (0)	
	Pseudomonas aeruginosa	16 (10.6)	9 (7.9)	7 (18.9)	
	E. cloacae	6 (4)	3 (2.6)	3 (8.1)	
	Elizabeth kingae	4 (2.6)	4 (3.5)	0 (0)	
	E. Coli	4 (2.6)	4 (3.5)	0 (0)	
	Burkholderia cepacia	3 (2)	3 (2.6)	0 (0)	
	E. aerogenes	3 (2)	2 (1.8)	1 (2.7)	
	Serratia	2 (1.3)	2 (1.8)	0 (0)	
	Citrobacter	2 (1.3)	2 (1.8)	0 (0)	
	Chryseobacterium	2 (1.3)	2 (1.8)	0 (0)	

There was no significant difference noted between the colistin and non-colistin groups in terms of age, proportion of preterms among neonates and sex. (Table 2)

Table 2. Demographic Data of Patients Treated for XDR Gram-negative Infection

		Overall	Non-colistin Group	Colistin Group	p-value
Age in years	Mean (SD)	3.9 (± 5.78)	3.4 (± 5.16)	5.4 (±7.26)	0.210
	Median (IQR)	0.69 (0.85-5.55)	0.57 (0.08-4.78)	1.08 (0.10 - 10.95)	
Neonates	neonates	32 (21.2%)	28 (24.6%)	4 (10.8%)	0.104
	Preterm among neonates	8 (25%)	8 (28.6%)	0 (0%)	0.550
Sex	Male	86 (57%)	61 (53.5%)	25 (67.6%)	0.181
	Female	65 (43%)	53 (46.5%)	12 (32.4%)	

Table 3 summarizes the clinical data of patients included in the study. The clinical profile between the non-colistin and colistin groups were not statistically significant except for receipt of TPN which was noted to be higher in the colistin group, 43.2% vs 23.7% (p=0.035).

Table 3. Clinical Data of Patients Treated for XDR Gram-negative Infection

		Overall n= 151 (%)	Non-colistin n =114 (%)	Colistin n=37 (%)	p-value
Nutritional status	Severely wasted	31 (20.5)	24 (21.1)	7 (18.9)	0.371
	wasted	18 (11.9)	13 (11.4)	5 (13.5)	
	normal	91 (60.3)	71 (62.3)	20 (54.1)	
	Overweight	11 (7.3)	6 (5.3)	5 (13.5)	
Co-morbidities	None	23 (15.2)	17 (14.9)	6 (16.2)	0.165
	Malignancy	33 (21.9)	29 (25.4)	4 (10.8)	
	Congenital Anomalies	95 (62.9)	68 (59.6)	27 (73)	
Type of infection	Hospital acquired	122 (80.8)	89 (78.1)	33 (89.2)	0.157
	Community acquired	29 (19.2)	25 (21.9)	4 (10.8)	
Surgical intervention		49 (32.5)	36 (31.6)	13 (35.1)	0.691
Accessory medical	Invasive Vascular access	58 (38.4)	43 (37.7)	15 (40.5)	0.846
	TPN	43 (28.5)	27 (23.7)	16 (43.2)	0.035
	Mechanical ventilator	70 (46.4)	51 (44.7)	19 (51.4)	0.570

procedure	**Blood transfusion**	**84 (55.6)**	**63 (55.3)**	**21 (56.8)**	**1.00**
	Foley catheter placement	**43 (28.5)**	**28(24.6)**	**15 (40.5)**	**0.092**
Prior Antibiotics	**None**	**12 (7.9)**	**10 (8.8)**	**2 (5.4)**	**0.045**
	Monotherapy	**92 (60.9)**	**71 (62.3)**	**21 (56.8)**	
	Combination therapy (non colistin)	**44 (29.1)**	**33 (28.9)**	**11 (29.7)**	
	Combination therapy with colistin	**3 (2)**	**0 (0)**	**3 (8.1)**	
Immunosuppression	**None**	**111 (73.5)**	**80 (70.2)**	**31 (83.8)**	**0.297**
	Steroids	**27 (17.9)**	**23 (20.3)**	**4 (10.8)**	
	Chemotherapy	**13 (8.6)**	**11 (9.6)**	**2 (5.4)**	
Ward Admitted	**NICU**	**41 (27.2)**	**28 (24.6)**	**13 (35.1)**	**0.669**
	PICU	**25 (16.6)**	**21 (18.4)**	**4 (10.8)**	
	SICU	**7 (4.6)**	**5 (4.4)**	**2 (5.4)**	
	Service ward	**73 (48.3)**	**56 (49.1)**	**17 (45.9)**	
	Pay	**5 (3.3)**	**4 (3.5)**	**1 (2.7)**	

Table 4 shows the temporal data of patients treated for XDR infections. Overall, patients with XDR Gram-negative infections have received antibiotics for a median duration of 15 days with interquartile percentile (IQR) of 6 to 26 days. The median hospital stay prior to developing XDR Gram-negative infection was 18 days with an IQR of 8 – 30 days. The hospital stay of patients with XDR Gram-negative infection was significantly longer in the colistin group with a median of 27 days, IQR of 14.5 – 56.5 days, compared to the non-colistin group with a median of 15.5 days, IQR of 5.8 – 27 days (p=0.001). Among the 49 patients who underwent surgery, a median of 14 days, IQR of 6 – 23 days post op was noted prior to developing XDR Gram-negative infections. Overall, the total hospital stay of patients was at a median of 36 days with IQR of 18 – 71 days. Patients in the colistin group had a statistically significant longer hospital stay with a median of 52 days, IQR of 36 – 123 days compared to the non-colistin group with a median of 30 days, IQR of 15.5 – 53.3 days (p=0.001).

Table 4. Temporal Data of Patients Treated for XDR Gram-negative Infections

		Overall	Non-colistin	colistin	p-value
Prior antibiotic treatment duration (days)	**Mean (SD)** **Median (IQR)**	**20.1 (24.9)** **15 (6-26)**	**16.5 (15.8)** **13 (6-24)**	**31.2 (40.3)** **17 (5.5-48)**	**0.085**
Hospital stay to XDR infection (days)	**Mean (SD)** **Median (IQR)**	**27.42 (44.13)** **18 (8-30)**	**20.63 (33.5)** **15.5 (5.8-27)**	**48.32 (63.23)** **27 (14.5-56.5)**	**0.001**
post Surgery days to XDR infection (49 cases)	**Mean (SD)** **Median (IQR)**	**15.0 (9.44)** **14 (6-23)**	**14.3 (9.36)** **13.5 (6-22)**	**17.2 (9.7)** **19 (9-25)**	**0.395**
Total Hospital Stay (days)	**Mean (SD)** **Median (IQR)**	**57.9 (66.78)** **36 (18-71)**	**46.9 (57.4)** **30 (15.5-53.3)**	**91.9 (81.56)** **52 (36-123)**	**0.001**

Treatment success of XDR Gram-negative infections was significantly higher in the colistin group at 70.3% vs 46.5% in the non-colistin group (p=0.014), but mortality is almost the same at 73.8% in the non-colistin and 72.7% in the colistin group (p=0.010). Persistence of XDR infection was higher in the non-colistin group at 26.2% vs. 9.1% in the colistin group (p=0.010). There was no significant difference in the duration of treatment among those with treatment success with a median of 10 days (IQR 10-14) for the non-colistin and 10.5 days (IQR 10-14) in the colistin group (p=0.279). However, clinical response is noted to be longer by 2 days in the colistin group, with mean of 6.27 (± 3.57) days vs 4.36 (± 1.77) days in the non-colistin group (p=0.008).

Table 5. Outcome of Patients Treated for XDR Gram-negative Infection

		Overall n =151	Non-colistin n = 114 (%)	Colistin n = 37 (%)	p-value
Treatment outcome	**Success**	**79 (52.3%)**	**53 (46.5%)**	**26 (70.3%)**	**0.014**
	Failure	**72 (47.7%)**	**61 (53.5%)**	**11 (29.7%)**	
Clinical response (days)	**Mean (SD)** **Median (IQR)**	**4.99 (± 2.64)** **4 (3-6)**	**4.36 (± 1.77)** **4 (3-5)**	**6.27 (± 3.57)** **5 (4-7)**	**0.008**

	Median (IQR)				
Success treatment duration (days)	**Mean (SD)** **Median (IQR)**	**12.27 (± 4.96)** **10 (10-14)**	**11.5 (± 3.03)** **10 (10-14)**	**13.8 (± 7.34)** **10.5 (10-14)**	**0.279**

Table 5 shows the treatment outcome of patients included in the study. Treatment in the non-colistin group comprised of meropenem + aminoglycoside (22.8%), meropenem as monotherapy (13.2%), ciprofloxacin as monotherapy (14%) or combined with aminoglycoside (14%), and cefepime (3.3%). A total of 26 out of 37 patients (70.3%) in the colistin group were given colistin + meropenem, colistin + ciprofloxacin (16.2%), colistin + amikacin (2.7%), and colistin + piperacillin tazobactam (2.7%), (Appendix D). The dose of colistin had a median of 4mg/Kg/day, IQR of 1.58 – 4.44 mg/Kg/day. The treatment duration was a median of 10 days with IQR of 10 – 14 days.

No adverse event was noted with the administration of colistin but fungal infection during the course of treatment was significantly higher at 18.9% in patients treated in the colistin group compared to 1.8% in the non-colistin group (p=0.001). Nephrotoxicity was noted in one patient in the non-colistin group. The patient was a 16-year-old female with Non-hodgkins Lymphoma who received meropenem, vancomycin, amikacin and amphotericin B simultaneously for 12 days prior to the development of acute kidney injury**.** (see Table 6)

Table 6. Treatment Related Adverse Events

		Overall n = 151 (%)	**Non-colistin n = 114 (%)**	**Colistin n = 37 (%)**	**p value**
Post Treatment Infection	**None**	**135 (89.4)**	**107 (93.9)**	**28 (75.7)**	
	Gram-positive (blood)	**1 (0.7)**	**1 (0.9)**	**0 (0)**	
	Fungal (blood)	**9 (6)**	**2 (1.8)**	**7 (18.9)**	**0.001**
	HCAP	**1 (0.7)**	**0 (0)**	**1 (2.7)**	
Toxicity	**none**	**113 (.99)**	**112 (0.98)**	**0**	
	Nephrotoxicity	**1 (0.88)**	**1 (0.88)**	**0**	
	Neurotoxicity	**0**	**0**	**0**	

DISCUSSION

The problem of increasing antibiotic resistance continues to be a threat with catastrophic consequences. There is a rising trend of extensive drug-resistance among Gram-negative isolates in our institution. The top XDR Gram-negative isolates *Klebsiella* (35.1%), *Acinetobacter* (26.5%), *Pseudomonas aeruginosa* (10.6%) with the addition of *Stenotrophomonas* (10.6%), were

consistent with XDR species identified by the WHO and the 2016 Antimicrobial Resistance Surveillance Program of the Philippines. [2]

In this study, 92.1% of the patients received antibiotics for a median duration of 15 days with interquartile percentile (IQR) of 6 to 26 days. The patients were receiving Meropenem ± aminoglycoside and Ciprofloxacin prior to developing XDR Gram-negative infection. Current studies have identified that the selective pressure of broad-spectrum antibiotics was a major risk factor for developing XDR Gram-negative infections. [4] [5] [6]

This study noted malignancy, gastrointestinal congenital anomalies, Chiari II malformation, and gastrointestinal surgery as the most common underlying conditions of patients with XDR gram negative infections, compared to Weintstein and Logan's study where pulmonary disorders, prematurity and malignancy were the top co-morbidities. [1] There was no significant difference noted between the colistin and non-colistin groups in terms of demographic and clinical data, consistent with previous studies. [1] [7]

Treatment success of XDR infections was significantly higher in the colistin group at 70.3% vs 46.5% in the non-colistin group (p=0.014). This is comparable with previous studies with colistin success rate ranging from 65 – 89%. [3][4][5][6] There was no significant difference in the duration of treatment among those with successful treatments, with a median of 10 days (IQR 10-14) for the non-colistin and 10.5 days (IQR 10-14) for the colistin group (p=0.279). The patients in our study received colistin for a shorter duration compared to Ozsurekci's study where patients received the drug with a median duration of 17 days IQR 9-14 days. However, clinical response is noted to be longer in the colistin group, with mean of 6.27 (± 3.57) days vs 4.36 (± 1.77) days in the non-colistin group (p=0.008). This finding can guide us in monitoring our treatment of XDR infections.

Previous studies, including one study involving neonates reported nephrotoxicity rates ranging from 1.6% to 22%. There were no reports of nephrotoxicity among patients who received colistin in this study. However, it is worth mentioning that one neonate had an episode of decreased urine output for 6 hours, occurring within 3 days after the last dose of colistin. The patient responded to hydration. There was no recurrence of oliguria noted and no laboratories were requested. One neonate had increased creatinine levels which occurred on the 6th day of colistin. The patient was evaluated by the nephrology service and acute kidney injury was attributed to prolonged shock. This patient received only 20,000 IU/kg/day of colistin. As mentioned earlier, one patient from the non-colistin group had nephrotoxicity and the patient received vancomycin, amphotericin B deoxycholate and amikacin, all known nephrotoxic drugs, and received at the same time. The lower nephrotoxicity rates in recent colistin studies can be attributed to closer monitoring of renal function and advances in intensive care unit monitoring as mentioned by Cagan et al. [3] Fungal infection during the course of treatment was significantly higher in the colistin group at 18.9% vs.1.8% in the non-colistin group (p=0.001). This finding can also be explained by selective pressure but it needs to be further validated since this is not a usual complication of colistin noted in other studies.

As to colistin dosing, the recommended dose of colistimethate for

patients with normal renal function is 2.5 to 5 mg/kg of ideal body weight not to exceed 300 mg daily. [11] A loading dose is recommended in critically ill adult patients. [11] Patients in this study received an average of 3.5 (±2.1) mg or 43,698 (±26,157) units per Kg/day in 3 divided doses, with no loading dose given. The IV infusion rate cannot be determined. The Adult and Pediatric Guideline for South Africa 2016 recommends 50 – 75, 000 IU/kg/day in three divided doses to be infused for 30 minutes for neonates and 50 – 75, 000 IU/kg/day in three divided doses for infants and children. [9] While colistin showed a favorable outcome, determining the optimal dose for the best treatment outcome is of clinical relevance for future studies.

Given the retrospective nature of this study, the authors acknowledge that the major limitation is the small number of subjects in the colistin group. However, to the authors' knowledge, this is the first descriptive study on the use of colistin among children in the Philippines. Similar to other retrospective studies, some clinical variables are uncontrolled and may affect treatment outcome analysis. The authors recommend a prospective study to validate the findings in our study.

CONCLUSION AND RECOMMENDATION

This retrospective comparative study showed that in the treatment of XDR Gram-negative infection in children, colistin, compared to other antimicrobials, is considered relatively effective and well tolerated. Though colistin is now available in our setting, preventive measures against the occurrence of XDR gram negative infections are of prime importance. In this study, majority of patients have previous or current exposure to broad-spectrum antibiotics prior to developing extensively drug-resistant Gram-negative infection. Hence, it is urgent and critical to continue efforts to prevent healthcare associated infections and to implement antimicrobial stewardship while new therapeutic options are evaluated.

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