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An Official Publication of the Philippine Children's Medical Center

EDITORIAL STAFF

Welcome to the first issue of The PCMC Journal for 2017!

In line with the recent accreditation and re-accreditation of PCMC by national bodies, and with its original thrust to be a research institution, the Office of Research Development, which manages the journal, has taken steps to show its seriousness in achieving these goals.

Over the past years, there has been a serious lack of manpower and infrastructure (including information technology and access to scientific researches), as well as a focus on the equally pressing needs of service and training, that have hobbled the hospital's plans to progress as a research institution. And yet in spite of that, the trainees and staff have continued to produce groundbreaking research and managed to achieve recognition of its research program, as well as accreditation of its Institutional Review Board (IRB) by the Philippine Health Research Ethics Board (PHREB). The hospital has also continued to attract pharmaceutical industry sponsored researches, which ensures funding and training for its participants.

The same problems of manpower and infrastructure have plagued the journal, which have impaired our ability to be included and maintained in various electronic databases, such as the Western Pacific Region Index Medicus (WPRIM). Hopefully the same impetus from the hospital administration shall result in more regular production of the journal, and its eventual indexing in other more established databases such as PubMed and Scopus. The long term goal is that the journal shall be seen as a worthy avenue for our staff and trainees to publish in, equal to any international publication.

The Editor

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Instruction to Authors:

The Philippine Children's Medical Center Journal (PCMC Journal) is a peer-viewed journal that is published bi-annually and publishes original scientific papers in the field of basic and clinical pediatric research. The articles it accepts for publication may be in the form of collective and current reviews, original papers, case reports, lectures, essays, editorials, abstracts or letters to the editor.

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Cover Letter

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General Guidelines

1. One original and two duplicate manuscripts should be submitted. An electronic copy must also be submitted on a floppy or compact disc. The manuscript should be typed double-spaced throughout with 1 ¼ cm (½ inch) paragraph indentation, using only one side of each 22x28 cm (8 ½ x 11 inch) opaque bond paper with 3-cm margins (1 ¼ inch) all around. Preferred font styles and sizes are: Times New Roman 12, Arial 11, Tahoma 11, & Verdana 11.

2. The manuscript should be arranged in sequence as follows: (1) Title Page (2) Abstract (3) Text (4) References (5) Tables (6) Figures & Illustrations. A manuscript for an original article should not exceed 25 typewritten pages (including tables, figures, Illustrations and references). The text for case reports should not exceed 10 pages, including the visual aids and references.

3. References should be selective and pertain directly to the work being reported.

4. All the sheets of the manuscript should be labelled with the family name of the main/first author (all in capital letters) and page number (in Arabic Numeral) printed on the upper right corner.

Title Page

1. The title should be as concise as possible. Include only the full names of the authors directly affiliated with the work starting with the first name, middle initial if any, and last name. The highest educational attainment or title of the authors should be included as an attachment whenever appropriates; name and location of no more than three institutional affiliations may be included.

2. If the paper has been presented in a scientific program or convention, provide a footnote giving the name, location and date of the meeting.

Abstract

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

Text

1. Generally, the text should be organized consecutively as follows: Introduction, Materials and Methods, Results and Discussion (and Conclusion).
2. All References, tables, figures and illustrations should be cited in the text, in numerical order.
3. Even if commonly employed, all abbreviations should be spelled out once, the first time they are mentioned in the text, followed by the abbreviations enclosed in parentheses. Subsequently, the same abbreviations may be used instead of the long names.
4. All measurements and weights should be System International (SI) units.
5. Acknowledgements to individuals/ groups of persons, or institution/s should be included at the end of the text just before the references. Grants and subsidies from government or private institutions should also be acknowledge

References

1. References in the text should be identified by Arabic Numerals in enclosed parentheses on the same line as the preceding sentence.
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3. All references should provide inclusive page numbers.
4. Journal abbreviations should conform with those used in the Index Medicus.
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6. The style/punctuation conforms to that recommended by the ICMJE, which is the ANSI standard style used by the NLM, and should follow the format of the examples shown below:

Journal Article

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Book

Huth EJ. How to write and publish papers in the medical sciences. Philadelphia: ISI Press, 1982.

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1. Cite all tables consecutively in the text and number them accordingly. Create tables preferably using a spreadsheet program such as MS Excel with one table per worksheet. Tables should not be saved as image files. The content of tables should include a table number (Arabic) and title in capital letters above the table, and explanatory notes and legends as well as definitions of abbreviations used below. Recommended font is Arial Narrow size 8.

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1. All illustrations/photographic prints should be submitted in duplicate and placed in a separate envelope.
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THE EFFECT OF SHORT MESSAGING SERVICE (SMS) REMINDER ON THE IMMUNIZATION RATES OF PATIENTS AT BARANGAY PINYAHAN HEALTH CENTER: A RANDOMIZED CONTROLLED DOUBLE BLIND FIELD TRIAL

JASON E. ABELLO, MD, MICHAEL RESURRECCION, MD,
SORAYA ALVARADO, MD

ABSTRACT

INTRODUCTION: There is an increasing interest and funding for the use of Information and Communication Technologies in the field of health. However, studies have shown conflicting results of Short Messaging Service (SMS) or Text reminder in the improvement of health care services such as immunization. This paper aims to determine the effect of SMS reminder on the immunization rate, which includes the dropout, delayed and prompt immunization rate.

METHODS: Newly registered children for immunization at Barangay Pinyahan Health Center were enrolled in randomized controlled double blind field trial. 144 subjects were exposed to treatment 1 (regular follow-up with no SMS reminder; treatment 2 (with SMS reminder); and treatment 3 (with blank SMS reminder). Immunization rate which included dropout, delayed and prompt immunization rates were determined. The effects of the confounding factors such as family income, barangay location, and number of children, parental education and immunization status of siblings were also determined.

RESULTS: The prompt immunization rate is inversely proportional to the dropout and delayed immunization rates. As the subjects progress to the third dose of the immunization, the dropout rate increases. The dropout rate is highest on the third dose of Hepatitis B and Measles vaccination which are given at a later age of a child. Only 6% of the total subjects were classified as Fully Immunized Child. This data is in contrast to the national data of Fully Immunized Child coverage at 80%. SMS reminder has no significant effect on the immunization rate for DPT, OPV, HPV and Measles in Barangay Pinyahan Health Center in comparison to the regular follow-up. However, in Treatment Group 3 (SMS Reminder), the dropout rates per type of vaccine are significantly different from each other. The dropout of DPT and OPV which is expected to be equal or higher than Hepatitis B and Measles vaccine is lower.

Variation in immunization rates among different income groups and geographic barangay location is particularly evident. Low income families and living distant from the health center have a significant propensity to dropout from immunization.

CONCLUSION: SMS reminder and regular follow-up have no significant difference on the immunization rate. Information communications technologies such as SMS reminder are more likely to affect immunization rate when health systems offer a supportive environment of consistently available vaccine such as DPT and OPV, and confounding barriers such as low family income, which affects HPV immunization rate, and distance to the health center, which affects measles immunization rate, are at the minimum.

INTRODUCTION

One of the most challenging Millennium Development Goals is the reduction of mortality by two thirds among children under five by 2015. The indicators to measure reduction of mortality rates includes: under-five mortality rates, infant mortality rates and proportion of one-year-olds immunized against measles. Efforts to increase immunization coverage are delayed by weak health systems, conflict, and unaffordable cost of some vaccines.[1]

Health related campaigns are commonly executed through word of mouth, flyers and posters, which are however ineffective in remote areas and mobile families. These families could be reached through the increasing use of information and communication technologies which include internet and mobile phones. At present, ICT projects in the Philippines are developed and funded by nongovernment agencies such as USAID and ADB to help achieve the MDGs.[2,3]

The use of mobile phones for healthcare has proven to be far reaching as the rapid distribution of mobile telephones has made it possible for poor people to have easy access to useful information. SMS then may be used to advocate programs of the Department of the Health such as immunization. However, this is not a one-shot implementation. In the Philippines, the lead institution for health informatics is the National Telehealth Center in the University of the Philippines – Manila. Their applications include internet and mobile teleconferencing and teleconsultation. The use of mobile phones to remind parents on a child's immunization schedule is a prospective project. However, this needs to be studied prior to implementation as beneficiary community have a tendency to fall short of the main objective, which is improved immunization rate, with the lack of resources or education.[1]

Immunization is an effective intervention that has achieved dramatic reductions in illness including disability and death from diphtheria, hepatitis B, measles, pertussis, poliomyelitis and tetanus. Available vaccines could prevent some 5% of the more than 500 000 deaths of children under 5 that occur every year in the Western Pacific Region such as the Philippines.[3]

Researches have been exploring health reminders through SMS. Immunization reminders delivered by text message interventions have shown promising results in some populations, but other studies have mixed results from medical practitioners and parents. In addition, organizational barriers and logistical issues were identified in some studies that need to be addressed for SMS reminder to be effective. The impact of text-message reminders on adherence to the EPI program is explored in this study. Children should be immunized as early as possible to the scheduled date to avoid preventable diseases. Understanding the confounding factors such as geographic location, parental education, and number of children, family income and immunization status of other children are important factors to consider in addressing immunization gaps and missed opportunities.[2]

In spite of the wide acceptance and routine use of immunization, there are reasons on the lack of adherence among patients and parents. A community setting, significant pockets of hard to reach rural villages and mobile urban poor population have not been provided immunization through routine services due lack to the geographical limitation, lack of health workers and information campaigns.[4] In a hospital setting, the major reason for failure to attend appears to be patients forgetting their

appointments. Many methods of reminding patients of appointments have been studied in a variety of clinical settings. The most common methods of immunization reminders are paper-based vaccination records, letters and personalized phone calls from the physician's secretary.[5]

The patient's failure to attend hospital or health center appointments has significant impact on the ability of the health system to provide efficient and effective outpatient services. Poor adherence to consultation schedules result in suboptimal utilization of clinical equipment and staff, leading to prolonged storage or expiration of vaccines. It reduces revenue opportunities for the hospitals.[5]

In a focus group discussion and individual interviews in any diverse population of parents in New York, text message immunization reminders revealed greater acceptance by many of the respondents and is found to be more effective than standard phone or mail reminders. Parents preferred text message reminders which are brief and personalized. Most parents were able to retrieve sample text messages but many had difficulty with interactive texting. [10]

In a study conducted at the Royal Children's Hospital in Melbourne, FTA rate was reduced from 23.4% to 14.2% with the use of SMS reminder three days before consultation. In a study conducted at the Philippine General Hospital, failure to show (FTS) to Genetics consultation was significantly reduced from 60% to 46% by using SMS reminder 2 days prior to consultation.[11] The ease with which large numbers of messages can be customized and sent by SMS text messaging, along with its availability and comparatively low cost, suggest it may be a suitable means of improving patient attendance.[14]

The Pinyahan Health Center, the community of PCMC, is also no stranger to the delayed immunization and dropout rates among their patients. Only 50-60% complete their vaccination regimen. Adherence is likely dependent to the aggressiveness of the health workers in implementing the immunization program. An interview with them revealed residents in their area who forget immunization schedules and of mobile patients with no permanent homes. These concerns require them to do door to door announcements.[15]

General Objective:

To determine the effect of Short Message Service (SMS) Reminder on Immunization Rates among Patients of Barangay Pinyahan Health Center.

Specific Objectives:

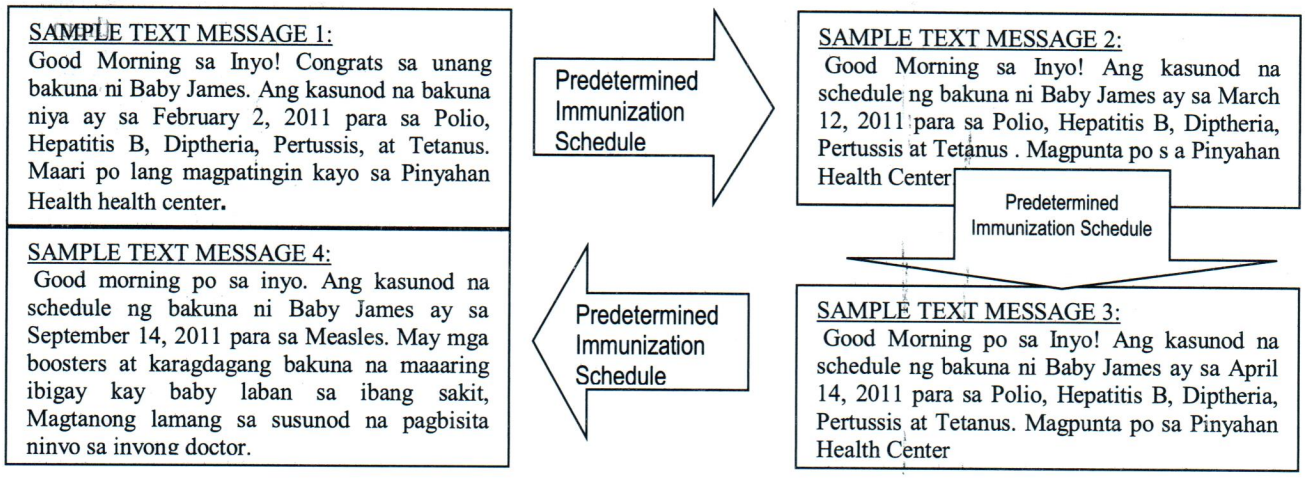
1. To determine the sociodemographic profile of the patients and parents attending the Immunization at Pinyahan Health Center.
2. To evaluate the effect of targeted SMS reminders against regular check-up (control negative) and empty text message (control positive) on the Immunization rates
    a. Prompt Immunization Rate
    b. Delayed Immunization Rate
    c. Dropout Rate
3. To determine the effects of confounding factors namely barangay location, family income, maternal education, paternal education, number of children and immunization status of other siblings on the immunization rates.

METHODOLOGY

Validation of Consent Forms and Text Messages

A focus group discussion and interview was conducted at PCMC Outpatient Clinic and Pinyahan Health Center. The mothers validated the consent forms and text messages. All the respondents understood the consent and text messages, and were willing to be participants of the research.

They suggested that the reminders be sent 3 days and 1 day before the immunization date. Seventy percent of them preferred non-interactive reminders which will not require them to text back. Text abbreviations were also minimized for better comprehension.



This was a randomized Controlled Double-Blind Field Trial conducted in a community setting, Pinyahan Health Center, 35 Malakas Street, Barangay Pinyahan, Diliman Quezon City. The health center caters to a population of 59,303 people. Approximately 200 children are immunized every month.

Inclusion Criteria:

1.) Children on their first dose of vaccination, specifically BCG
2.) Children at birth to 12 weeks old (1 day old to 3 months old)
3.) Children with parent/s or guardians who have mobile phones

Exclusion Criteria:

1.) Children on their second, third or booster doses of vaccination
2.) Children with or who develop allergic reaction to vaccines
3.) Children with illnesses which the health workers, midwife or nurse deem unfit for vaccination

The patients and parents name were concealed by replacing them with numerical codes. The numerical codes were then designated randomly between the SMS group, control negative group (on the regular follow-up) and the control positive group (which receive blank text message) through a table of random numbers. The groups received both verbal
and written reminders during the time of visit. They received a vaccination record which the nurse or BHW filled out during their visit. The nurse or BHW is blinded to the intervention of the patient. Another staff, blinded to the coding, sent SMS reminder as determined by the grouping and scheduling. The statiscal analysis was also done by a statistician who was blinded of the group designation.

The patients who were included were the new registrants from November 2011 to January 2012. An immunization registry was developed for all the patients, the succeeding vaccination schedules were determined. The

schedules for sending SMS reminder were also determined. The SMS group received an SMS reminder on the scheduled date and place of immunization 3 days and 1 day prior to the immunization schedule. The control negative group did not receive an SMS reminder. The control positive group received an empty text message. The mobile numbers were contacted and updated for validity and continued functionality prior to sending of messages. On October 2012, the vaccination records from all subjects were gathered and counterchecked to the health center's logbook. All the subjects were also contacted to confirm their immunization status.

The primary outcomes were prompt immunization rate, delayed immunization rate and dropout rate. Blinding was done to the staff who was sending text messages. The nurses who were giving the vaccines were blinded or uninformed to the intervention done to the patients. She or he filled out the vaccination record given to all patients regardless of the intervention. The vaccination records were then collected.

Multivariate analysis was used to determine significant difference between the intervention and the control groups. Analysis of variance was also used to determine differences of dropout pattern within treatment groups. Pearson correlation was used to establish relationship of confounding factors to the immunization rates. [18]

RESULTS AND DISCUSSION

For the 11 month period of the study, 160 subjects were recruited. However, upon checking the phones, only 96 continued to function for the SMS Group and Control Positive group. Those no longer receiving text messages for these two groups were excluded from the study.

The highest parental education is commonly secondary education or high school. There is equal distribution of socioeconomic strata among subjects as evident by the near equal number of subjects per income group. Most of the subjects are the first child in the family. Seven is the largest number of children in the family.

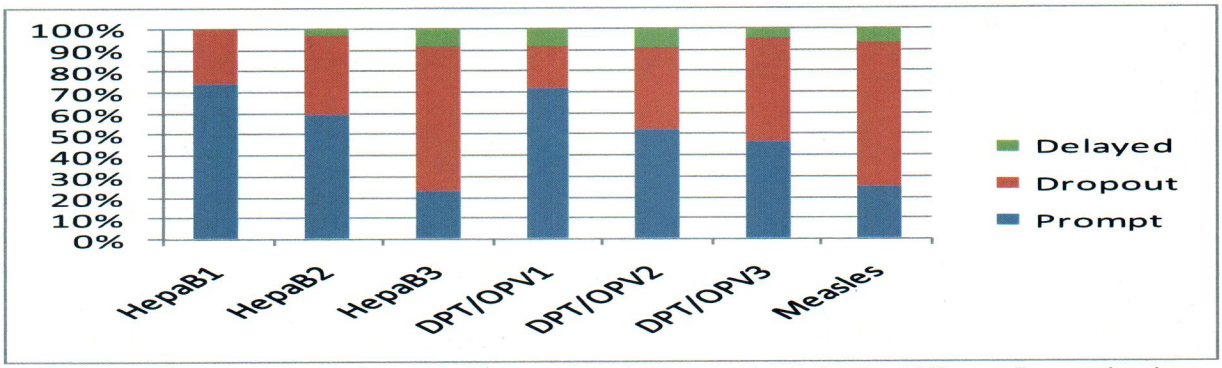


Figure 1: Distribution of Delayed, Dropout, and Prompt Rates in the different Immunizations

Figure 1 above shows the distribution of delayed, dropout, and prompt rates in the different immunizations The DPT and OPV are grouped as one in this study due to the constant provision of the vaccines at the same time. The Prompt immunization rate is inversely proportional to the dropout and delayed immunization rates. BCG is often given at birth. If not given, it is the first vaccine given which does not require reminding. There is almost no dropout rate in the first dose of Hepatitis B due to the birth dose given upon delivery of the child. As the subjects progress to the third dose of the immunization, the dropout rate increases. The dropout rate is highest on the third dose of Hepatitis B and Measles vaccination which are given at a later age of a child. The third dose reflects the cumulative nondelivery of immunization to the subjects. Once noncompliance is started, it eventually progresses to incomplete immunization. [19]Although some caregivers may opt to

immunize the subjects at a later time, this is reflected by the delayed immunization rate. This however corresponds only to a minimal percentage.[20]

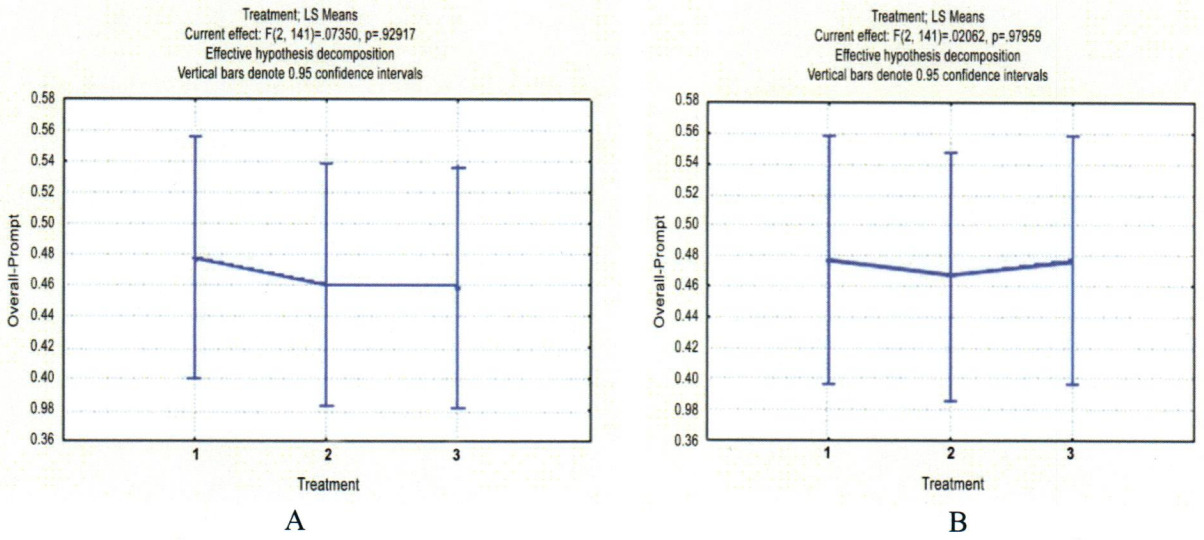
Table 1: Prompt, Dropout, Delayed Rates on the Immunizations given at Pinyahan Health Center

	HepaB1	HepaB2	HepaB3	DPT/OPV1	DPT/OPV2	DPT/OPV3	Measles
Prompt	44	78	33	79	75	67	35
Dropout	15	49	99	22	56	71	95
Delayed	0	4	12	9	13	6	9
Given on First Visit / NA	85	13	0	34	0	0	5
Total	144	144	144	144	144	144	144

*NA-status not yet determined due to the time constraint of the study

There are 5 subjects whose disposition is not yet determined since their scheduled Measles vaccination, to be considered dropout, falls beyond the time of study (Table 1). For Hepatitis B vaccine, there is 69% dropout. For Measles, there is 66% dropout. For DPT and OPV, dropout is only at 15%. These dropouts eventually lead to an incomplete immunization.

A complete immunization of the 4 types of immunizations (BCG, DPT, HPV and Measles) translates to a fully immunized child which is only 6% of the total subjects (9 out of 144 subjects). This data is in contrast to the national data of FIC coverage at 80%.



A B

Figure 2 A & B: Treatment 1 (Regular Follow-up) Treatment 2 (SMS Reminder) and Treatment 3 (Blank SMS) in relation to Prompt Immunization Rate (A) and Dropout Rate (B)

An ANOVA of the treatment groups showed no significant difference between the groups. SMS Reminder has no significant effect to the immunization rate in comparison to regular follow-up and blank text message. This is true for the dropout and prompt immunization rates between treatment groups (Figure 2). The Pearson Coefficient also showed no significant correlation between a specific treatment and immunization rate.

The initial vaccine given in the Philippines is BCG and Hepatitis B, the remaining doses are given thereafter together with DPT and OPV. The last of the EPI component to be an FIC is the Measles Vaccine. There is a general trend of increasing dropout

rate as the immunization requirement progresses (See Figure 10).

An analysis of the dropout pattern is done. A dropout in Hepatitis B progresses to an equal or increased dropout rates in DPT, OPV and Measles. For Treatment, regular follow-up with no SMS reminder, the dropout rate for Hepatitis B vaccine is 0.51, which is almost similar to the dropout for DPT/OPV at 0.42 and 0.63 for Measles (p-value 0.08). Comparison of these dropout rates shows no significant difference. There is also no significant difference for Treatment 3 which is the group sent with blank text message.

However, the dropout trend in treatment group 2, sent with SMS reminder, is different. The dropout in DPT and OPV is significantly different compared to the dropout rate for Hepatitis B and Measles (p-value 0.001) (Figure 3). This suggests that the SMS reminder may affect the dropout trend within a cohort of children being immunized. SMS reminder have a significant effect to an immunization that is readily available. DPT and OPV are the vaccines with most financial allocation as DPT three doses of vaccine against (diptheria, pertussis, and tetanus) and Oral Polio Vaccine are commonly used as a measure of health service availability.

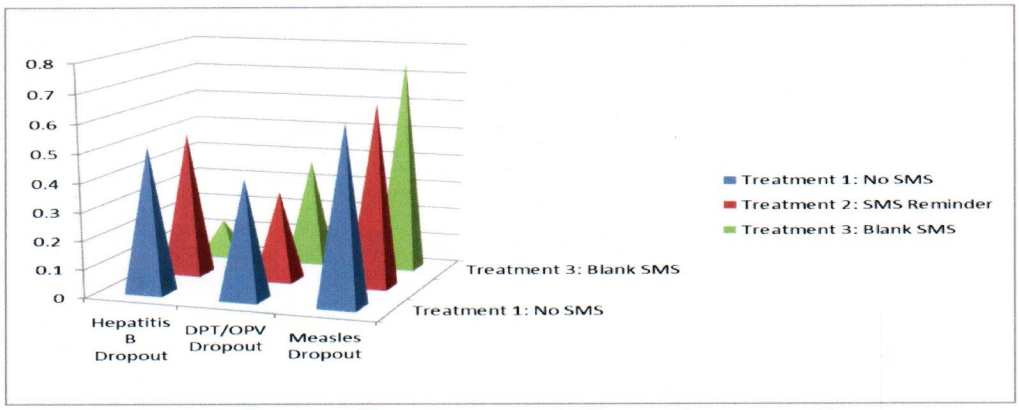


Figure 3: Dropout Trend between Different Treatment Groups

An analysis on the confounding factors that may explain the insignificant effect of SMS reminder on Hepatitis B and Measles Immunization was done. Hepatitis B immunization rate is significantly correlated with the income status of the family. Low income families, belonging to those with income less than less than 9,000 pesos, tend to dropout from the immunization program as compared to those belonging to high income families with income greater than 20,000 pesos (Figure 4).

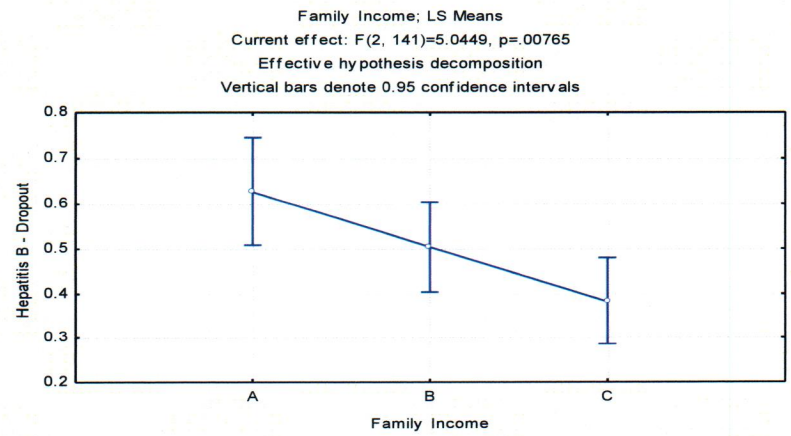


Figure 4: Correlation between Family Income and Hepatitis B Vaccine Dropout

The lack of Hepatitis B vaccine often results to erratic immunization schedule. Most of the time, the caregivers would inquire through text if there was already a vaccine. No answer was texted back since this was not part of the research.

The Philippines only procured 40% of its Hepatitis B vaccine needs in 2004. Coverage with the three doses of hepatitis B vaccine remained low or has been declining. Overall, the efforts to control hepatitis B in the Philippines remains suboptimal. Political commitment has been lacking to allocate resources for hepatitis B vaccination. Hence, there is a rise of out-of-pocket payments for health which has compromised the immunization compliance among the poor. This is evident by the significant effect of family income as a confounding factor to the dropout rate (p-value 0.008), delayed immunization rate (p-value 0.005) with a Pearson Correlation coefficient of 0.2.

Although most parents would opt to have their child immunized they end up disappointed with the unavailability of the vaccine. Parents who would rather spend their time for income generation and forego immunization schedules, eventually leading to dropouts.[23,24,25]

The lack of social fabric in urban slums often limits interpersonal interaction and information about services. Urban poor are often not able to muster enough confidence to access services even when services are proximal. [26]

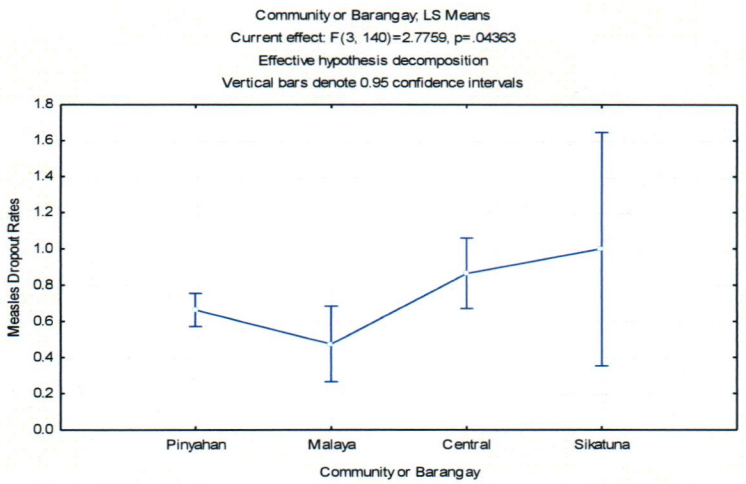


Figure 5: Correlation between the Barangay location and the Dropout Rate

The SMS reminder has no significant effect on the measles immunization rate. Confounding factors were identified of which barangay location has a significant correlation with Measles dropout rate. (Figure 5)

Distance to vaccination sites can affect utilization. The inter-barangay variation of immunization coverage in an urban area such as Quezon City, reveals a service coverage gap which calls to rethink on resource allocation and strengthening processes to improve immunization coverage among urban dwellers.[28, 29]

Considering the distance and high dropout rates in Central and Sikatuna, the health center may engage in supplemental immunization activity in these barangays. These areas are observed to have more dropouts as compared to Pinyahan and Malaya. The latter barangays are geographically nearer to the health center which may explain the more number of subjects and lesser dropout rate. Although national statistics claim a substantial increase in immunization coverage, large numbers of slum dwelling children remain incompletely immunized even in Quezon City.

Measles immunization also reflects the compounded dropout from previous immunizations of DPT, OPV and Hepatitis B. Caregivers who are reminded of the Measles immunization schedule tend to inquire instead for the availability of Hepatitis B vaccine. The inter-barangay difference may also be due to the tendency of health practitioners to wait for an optimum number of children in a certain area before opening a multi dose vial of measles vaccine.[27, 28, 29]

CONCLUSION

There is an increasing interest and funding for ICT facilitated health care. However, these projects need to be discussed and studied so as not to fall short of its objective in improving immunization rate. Some studies on SMS reminder have shown improved compliance to vaccination while some have no effect due to lack of community preparedness in terms of resources.

This study shows that SMS reminder in comparison to regular follow-up has no significant effect on the immunization rate for DPT, OPV, HPV and Measles in Barangay Pinyahan Health Center.

A dropout in any of the vaccines progresses cumulatively to an overall high incomplete immunization of 94% of the 144 subjects, a contrasting result to the national report of fully immunized child coverage.

An analysis on the dropout pattern showed Hepatitis B vaccine dropout to be similar to the DPT/OPV and Measles dropouts for Treatment Group 1 (Regular follow-up) and Treatment Group 2 (Blank SMS). However, in Treatment Group 3 (SMS Reminder), the dropout rates per vaccine are significantly different from each other. The dropout of DPT/OPV which is expected to be equal or higher than Hepatitis B and Measles vaccine is lower. SMS reminder show significant dropout of DPT and OPV in comparison to Hepatitis B and Measles dropout. SMS reminder may prevent dropouts given that the immunization is consistently available. DPT and OPV are consistently available since they are often used to monitor performance in health centers.

SMS reminder does not have an effect on dropout rates for Hepatitis B and Measles. Confounding factors were identified that may have affected these immunizations. The family income significantly affected Hepatitis B dropout rate. Hepatitis B vaccine is the least available in the health center with caregivers receiving erratic immunization schedules. The unavailability of vaccine and erratic scheduling in turn affected compliance, especially among those belonging to the low income group.

The community location or barangay was identified as a confounding factor for Measles vaccination. Barangays Sikatuna and Central were the farthest which had the highest dropout rates compared to Barangay Pinyahan and Malaya.

Variation in immunization rates among different income groups and geographic areas is particularly evident. SMS reminder may be most effective in a setting where the basic health services are available. The basic component of immunization, which is the vaccine, must be readily available. SMS reminder may be an adjunct tool in improving immunization coverage by breaking patterns of dropouts.

Information communications technologies such as SMS reminder are more likely to affect immunization rate when health systems offer a supportive environment and confounding barriers are minimal.

To improve the power of the study, a larger sample size may be utilized. It could also be implemented in hard to reach areas where this is more beneficial in terms of geographic diversity.

Manual texting to a large number of parents and caregivers is tedious and time consuming, hence it is recommended that a software program be developed to handle the inflow of immunization schedule and outflow of text messages. For the program to be successful, system maintenance must troubleshoot

problems. These include consistent availability of the vaccines and prompt updates to the system in case of change of scheduling owing to national and local holidays, unavailability of vaccine, unavailability of health provider and postponement of work due to catastrophic events such as typhoons and flooding.

Other monitoring schemes, aside from DPT and OPV coverage may be used as an indicator of health center performance. These may include exit interviews with mothers and providers to evaluate timeliness of vaccination, drop-out rates, and missed opportunities among children and mothers. Reasons for non-vaccination or incomplete vaccination should be investigated.

Mapping of areas with high dropout rates, with the help of the barangay health workers enables identification of endemically weak areas of immunization coverage. Regular outreach camps at a convenient, well publicized fixed location and day are essential in low coverage urban areas. This may be a form of supplemental immunization activity.

Given the high percentage of Hepatitis B dropout with correlation to family income, political will must be enforced in procuring the vaccine. With the limited stocks, low income families who are most likely to dropout must be prioritized.

REFERENCES

1. Global Alliance for Vaccines and Immunization. Scaling up immunization to meet the Millenium Development Goals (MDGs). 2005. February 15. Massachusetts.

2. S. Soegijoko, J. Hasugian, T.S. Barkah: "Internet and Mobile Phone – Based eHealth Systems for Outbreak Management and Safe Motherhood Program in Community Health Center Environment", Med-e-Tel 2009, Luxembourg, 1 – 3 April 2009.

3. S. Soegijoko, A.U. Jai Ganesh, I.F. Saligumba, S. Khoja: "Systematic Review of Current ICT Applications in Disasters – The Potentials

4. Department of Health (2008). "Racing Toward our Health Goals: Proposed Budget CY 2009." Manila.

5. Department of Health. National Objectives for Health Philippines 2005-2010. Reducing the Burden of Disease.2003;4:34-38.

6. Government Health Spending vs Income, 2004

7. Tolentino, Herman, Marcelo A, Marcelo P. Linking Primary Care Information Systems and Public Health Vertical Programs: An Open-Source Experience. Philippines. AMIA Annual Symposium. 2005; 2005:3111-315.

8. Villamor, Marites. Mobile Phones Key to Universal ICT Access – National Telecommunications. Official.Business World, 2008; 15:53-54.

9. World Health Organization. Adherence to long-term therapies: Evidence for Action. Paper presented to the World Health Organization. 2003 June 28. Geneva.

10. The Effectiveness of a Short Message Service (SMS) text message reminder system in decreasing Failure to Show Percentages among patients scheduled for consult at the Clinical Genetics Out-Patient Clinic of a Tertiary Hospital. Philippine Pediatric Society. Forthcoming.

11. World Health Organization. Immunization Update. Paper presented at: Fifty-Third World Heatlh Assembly. 2000 May 19.A53/40. Geneva.

12. Kharbanda, Elyse Olshen et. Al. Text for Health: A Qualitative Evaluation of Parental Readiness for Text Message Immunization Reminders. Columbia University. Division of General Pediatrics. The Medical Journal of Australia. 2008 May;26:8-3.

13. Sawyer SM, Zalan A, Bond LM. Telephone reminders improve Adolescent Clinic Attendance: a Randomized Controlled Trial. Journal of Paediatric Child Health 2002;38: 79-83.

14. Carmencita Uy. Letter to: Barangay Pinyahan Health Center. Quezon City. 2010. January 14. 2 leaves.

15. Dela Gente A. Midwifery in the Philippines: A Laudable Service but there are Issues and Challenges. International Midwifery. December 2008. 1-3.

16. Iglesias, M. Smart to Put Up 500 Cell Sites. Business Insight. October 2010. http://www.malaya.com.ph/10122010/busi6.html

17. Streatfield. K & Singarimbun, M 1988. Social factors affecting use of immunization in Indonesia, *Social Scienceand Medicine* 27, 11:1237-1245.

18. Snyder, LB & Hamilton, MA. 2001. Meta-analysis of US health campaign effects on behavior: Emphasize enforcement, exposure, and new information, and beware the secular trend. In R. Hornik Ed. *Public Health Communication: Evidence for Behavior Change*, 357-383. Hillsdale: Lawrence Erlbaum Associates.

19. Streefland, P., Chowdury, A. M. R. & Ramos-Jimenez, P. 1999. Patterns of vaccination acceptance, *Social Science& Medicine* 49: 1705-1716. UNICEF. 2003. A critical leap

20. Streatfield K, Singarimbun M. Social factors affecting the use of immunization in Indonesia. *Soc Sa Med* 1988; 27: 1237-45 Nations and Taft Avenues, Manila, the Philippines. Correspondence to Manju Rani.

21. Western Pacific Regional Office, World Health Organization, corner of United

22. Regional Variations in Living Conditions During the North Korean Food Crisis of the 1990s Asia Pac J Public Health October 1, 2010 22:460-476

23. Department of Medical Health and Family Welfare, Government of Uttaranchal. Five Year Urban Health Proposal (Under RCH II) for Dehradun. Dehradun: Government of Uttaranchal; 2003.

24. Health and Family Welfare Department, Government of West Bengal, Five Year Urban Health Proposal (Under RCH-II) for Bally, West Bengal. Kolkata : Government of West Bengal; 2004.

25. Hall AJ, Wild CP. Liver cancer in low and middle income countries. *BMJ* 2003;326:994-5. PMID:12742895 doi:10.1136/bmj.326.7397.9947. Teo EK, Fock KM. Hepatocellular carcinoma: an Asian perspective. *Dig Dis* 2001;19:263-8. PMID:11935085 doi:10.1159/000050692

26. Yao GB. Importance of perinatal versus horizontal transmission of hepatitis B virus infection in China. *Gut* 1996;38 suppl 2;S39-42. PMID:8786052 doi:10.1136/gut.38.Suppl_2.S39

27. Department of Family Welfare, Pulse Polio Immunization in India. Operational Guide 2003-04. New Delhi: Government of India; 2003.

28. Health Systems Program, Department of International Health, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Room E8132, Baltimore, MD 21205, USA. bweiss@jhsph.edu

VITAMIN D STATUS OF FILIPINO HIGH SCHOOL STUDENTS IN SELECTED SCHOOLS IN QUEZON CITY

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ABSTRACT

BACKGROUND: Vitamin D deficiency has been documented as a frequent problem in almost every region of the world even in the tropical countries and its health consequences are enormous. Infancy and adolescence are age groups particularly at risk of developing vitamin D deficiency. However, there are no data on the Vitamin D status of Filipino adolescents.

OBJECTIVES: To determine the serum 25-hydroxyvitamin D levels in Filipino high school students in selected schools in Quezon City

METHODS: A cross-sectional study of Filipino high school students was undertaken. A total of 97 boys and girls aged 11- 18 years old, attending selected private and public secondary schools in Quezon City participated in the study after parents' consent and students' assent were taken. Serum 25(OH)D levels were determined by electrochemiluminescence immunoassay (ECLIA) using Roche HITACHI Cobas e immunoassay analyzer.

RESULTS: The total serum 25-hydroxyvitamin D levels of the students ranged from 19.92 nmol/L to 88.63 nmol/L with a mean of 52.43 nmol/L. Among the 97 high school students, there was a prevalence of hypovitaminosis D (serum 25 (OH)D <50nmol/L) of 41.2% with 20.6% having deficient (<37.5 nmol/L) and 20.6% insufficient (37.5-<50 nmol/L) serum 25-hydroxyvitamin D levels. In a final multivariate model, low vitamin D intake, BMI Z-score outside the normal range of 0 to <1SD and upper socioeconomic status were significant independent predictors of hypovitaminosis D.

CONCLUSION: Hypovitaminosis D is highly prevalent among Filipino adolescents in secondary school despite abundance of sunlight. Appropriate interventions are needed to address the problem of poor vitamin D status in schoolchildren.

KEYWORDS: hypovitaminosis D, vitamin D deficiency/insufficiency, Filipino, adolescents, serum 25(OH)D levels

INTRODUCTION

Vitamin D, also known as the "Sun Vitamin", is not in a strict sense a vitamin but a pre-pro-hormone which acts via metabolites of which the most active is 1,25 $(OH)_2D$ or 1,25-dihydroxycholecalciferol or calcitriol; this binds to specific receptors, the vitamin D receptors (VDRs) functioning as nuclear transcription factors[1], and as such exerts transcriptional control over a large number of genes. Of great interest is the role it can play in decreasing the risk of many chronic illnesses, including common cancers, autoimmune diseases, infectious diseases, and cardiovascular disease. [2]

The principal functions of vitamin D are for augmentation of intestinal absorption of calcium and promotion of normal bone formation and mineralization.[3] In a vitamin D-sufficient state [25 9OH)D levels of >50 nmol/L (20 ng/mL)], net intestinal calcium absorption is up to 30%, although calcium absorption can reach 60%- 80% during periods of active

growth. In a vitamin D-deficient state, intestinal calcium absorption is only ~10%-15% and there is a decrease in the total reabsorption of phosphate.[4] Bone problems such as osteoporosis is a significant health problem today. One of every 3 Filipino women, as well as 1 in 5 Filipino men, is likely to suffer from osteoporosis.[7] These are said to be higher than the number of people succumbing to breast or prostate cancer, and should be given the attention they deserve. Thus strategies to maximize peak bone mass in children have been identified as a priority area for research.[8] This would be of great importance in the prevention of the common and costly public health problem of osteoporosis.

It is widely believed that osteoporosis prevention may be best accomplished during childhood and adolescence, when bones are growing rapidly. As adolescence is a critical period for bone health, the effect of vitamin D status on bone mineral density in adolescents could be of major importance.[9] At least 90% of peak bone mass is obtained by the age of 18 years.[10] A 10% increase in peak bone mass is estimated to reduce the risk of an osteoporotic fracture in adult life by 50%.[11]

Data from national surveys in the UK, USA and New Zealand show that the prevalence of low vitamin D status is less of a concern for children than for adolescents. The prevalence of low vitamin D status appears to increase with age in early life with adolescents being the life-stage with the highest prevalence of low vitamin D status.[9] Although most studies have demonstrated lower vitamin D levels among adolescents, such findings have been predominantly generated from studies conducted in temperate regions. There is limited data on this issue on adolescents in tropical countries. To date, studies on vitamin D status of Filipino children are scarce, and to our knowledge, there are no data on the vitamin D status of the adolescent population in the Philippines.

The best method for determining a person's vitamin D status is to measure the serum 25- hydroxyvitamin D (25 (OH)D).[2] Although a global consensus has not been reached with regard to the biochemical definition of VDD, for children it has been recommended that a serum 25 (OH)-D level of > 50 nmol/L (20 ng/mL) be considered indicative of sufficiency , serum 25-(OH)D concentration of <37.5 nmol/L,or 15ng/ml, as an indication of VDD, and 25-(OH)D level of 37.5- 50 nmol/L,or 15-20 ng/ml, be considered to indicate insufficiency. Severe deficiency is somewhat arbitrarily defined as 25 (OH)-D level of $\leq$ 12.5 nmol/L (5 ng/mL).[4] The cut-off of 50 nmol/L or 20 ng/mL is based on studies that show that levels below this value may be associated with rickets, and impaired bone accrual.

VDD has reemerged as a significant pediatric health issue worldwide. Adolescence is an age group particularly at risk of developing VDD because of the greater mineral demands of their growing skeleton. [18] Recent studies show that 16% to 54% of adolescents have serum 25(OH)D concentrations of $\leq$50 nmol/L.[15]This is why the adolescent period is a very important phase to catch VDD. VDD in adolescent females identifies an increased risk of future infants with VDD.[19] It could also progress to osteoporosis or osteomalacia in adulthood.

To address this concern, the American Academy of Pediatrics (AAP) issued new recommended intakes for healthy pediatric and adolescent population of a minimum of 400 IU/day of vitamin D that replaces the previous recommendation of a minimum daily intake of 200 IU/day of vitamin D supplementation.

In a Cochrane review on Vitamin D supplementation for improving mineral density in children published in 2010, the authors concluded that the available evidence does not support the use of vitamin D supplementation to improve bone health in healthy children with normal vitamin D levels, but does suggest that supplementation given to vitamin D deficient children may have clinically useful benefits for peak bone mass.[20]

With the studies on Vitamin D that are coming out at this time, there is a crucial need for nutritional public health awareness

campaigns about the importance of Vitamin D. Evidence from developed countries of the safety and efficacy of vitamin D supplementation for the improvement of musculoskeletal outcomes of those children with hypovitaminosis D renders the institution and implementation of national health policies in developing countries mandatory.[21] These policies however, require revisions based on evidence from locally conducted studies. To date, limited data on Vitamin D are available among Filipino children and no data are available on the vitamin D status in the adolescent group.

This study was done to determine the baseline vitamin D levels of apparently healthy Filipino school-going adolescents so as to determine if further recommendation on supplementation should be needed in order to improve bone health of our children that would impact later in life.

The general objective of this study is to determine the serum 25-hydroxyvitamin D levels of Filipino high school students, 10-18 years of age in selected schools in Quezon City. The specific objectives are: to determine the prevalence of hypovitaminosis D (serum 25-hydroxyvitamin D level of <50 nmol/L) in Filipino high school students in selected schools in Quezon City and to determine the relationship between serum 25-hydroxyvitamin D levels and the factors within the adolescent lifestyle that represent predictors of hypovitaminosis D such as: gender, socioeconomic status, body mass index (BMI), waist circumference (WC), waist-hip ratio (WHR), obesity, duration of sun exposure, skin color, clothing style, sunscreen use, and nutrient intake.

METHODOLOGY

This cross-sectional study was conducted in July 2012 in 97 boys and girls aged 11-18 years old living in Metro Manila and attending secondary schools in Quezon City. This age group was chosen based on the study done by CDC/NCHS, National Health and Nutrition Examination Survey (NHANES) from 2001-2006, which showed that the season-adjusted prevalence at risk of hypovitaminosis D by age was lowest in children aged 1-8 years and the risk of deficiency increased significantly with age, until age 30 in males and age 18 in females, after which it did not change significantly with age. The school children came from a government high school, the Flora Ylagan High School, that caters to children of lower socioeconomic status (LSES) and a private school – the St. Paul University of Quezon City, that enroll children of middle to upper socioeconomic status (USES). Socioeconomic stratification of the subjects was based on the type of school attended. Exclusion criteria were chronic disease such as asthma, tuberculosis, IBD, malabsorption, cardiac problem, chronic liver disease, neurologic or renal disease; intake of drugs that interfere with bone mineral metabolism such as glucocorticoids, anticonvulsants, and antituberculous drugs; intake of vitamin D supplement or multivitamins containing Vitamin D; skeletal disease/deformity and signs of vitamin D deficiency such as rachitic rosary rib cage, genu varum (bowlegs),genu valgum (knock-knees),frontal bossing, epiphyseal enlargement of the wrists and knees, etc. as noted on physical examination. An invitation to participate in the study was given to parents and guardians of the students selected by stratified sampling per level and per section of the selected schools. Out of approximately 830 invitation letters accompanied by consent and assent forms that were distributed, 110 consent forms returned, primarily because the parents refused to have blood extraction done on their children. Of those who assented and who obtained parents' written informed consent, a random sampling was done in the primary sampling unit per section. 97 questionnaires were then given to the participants. Using the reported prevalence of VDD of 20% among Filipinos in a study done by Kruger in 2010, a total sample size of 96 was deemed to be sufficient to estimate the prevalence within 8% of the true value, with 95% confidence level.

Data on age, gender, monthly family income, parents' occupation, class schedule, duration of direct sun exposure, style of school uniform, outdoor clothes worn during the day, use of sunscreen, and the type, frequency, and

amount of fish and milk intake, were asked through a self-administered pretested questionnaire supervised by the investigator. The questionnaire used was pretested to 40 high school students at San Francisco High School in Quezon City. Review of systems and physical examination was done by the investigator to all the subjects to include the skin color using the Fitzpatrick skin type scale. The estimation of the percentage of skin exposed to the sun was done using Wallace's rule of nines as determined by their clothing style. Weight and height were measured twice using a single calibrated weighing scale and tape measure fixed to the wall. Weight and height were measured with students in their school uniform and without shoes. Weight was measured to the nearest 0.1 kg while the height was measured to the nearest 0.1 cm. BMI was calculated as weight/height^2 by the investigator and classified based on the World Health Organization recommendation. Nutritional assessment and dietary assessment of total energy, protein, carbohydrate, fat, and Vitamin D intake was done by a dietician based on a 3-day (2 weekdays and 1 weekend) dietary recall record of their food intake which were done 3 days prior to the study. The daily dietary prescription for schoolchildren and teenagers adapted from the Recommended Energy & Nutrient Intake, FNRI/DOST,2002 was used as basis for the computation of percentage of caloric intake.

A single blood sample (6mL) was extracted from each of the 97 subjects by registered medical technologists at the school clinic on the same day. Samples were centrifuged at the end of the collection and stored on ice before transporting to the laboratory. All the specimens were processed on the same day by an ISO certified private laboratory not associated to the funding agencies. The serum 25 hydroxyvitamin D levels (both vitamin D_2 and D_3 derivatives) of all the subjects were determined by electrochemiluminescence immunoassay (ECLIA) using Roche HITACHI Cobas e immunoassay analyzer.

The study protocol was approved by the Institutional Review Board of the Office of Research Development of the Philippine Children's Medical Center.

The definitions of clinical entities (i.e. Hypovitaminosis D, VDI, VDD, etc.) used in the study are shown in Table 1.

Table 1. Definition of terms used in this study for laboratory test results.

Clinical entity	Test	Level
Vitamin D sufficiency (VDS)	Serum 25-OHD	50- 250 nmol/L[4]
Vitamin D insufficiency(VDI)	Serum 25-OHD	37.5-50 nmol/L
Vitamin D deficiency (VDD)	Serum 25-OHD	<37.5 nmol/L
Severe VDD	Serum 25-OHD	<12.5 nmol/L

*from World J Pediatr 2012; 8:2:145-150

Data were encoded and tallied in SPSS version 10 for Windows. Descriptive statistics were generated for all the variables. For nominal data, frequencies and percentages were computed. For numerical data, mean $\pm$ SD were generated. Analysis of the different variables was done using the following test statistics: ANOVA was used to compare more than two groups with numerical data, Kruskal Wallis test which is a non-parametric equivalent of the ANOVA and Chi-square test to compare or associate nominal data. P value <0.05 was considered significant. Multivariate regression analysis was done to determine independent predictors for hypovitaminosis D.

Table 2. Distribution of Subjects According to Serum 25-hydroxyvitamin D Levels

Vit.D status	Frequency N=97(%)	Vitamin D Level (nmol/L)	
		Range	Mean + 1SD
Sufficient	57 (58.8%)	50.25 – 88.63	63.31 ± 10.10
Insufficient	20 (20.6%)	37.72 – 49.75	43.10 ± 3.60
Deficient	20 (20.6%)	19.92 – 36.67	30.74 ± 4.46

RESULTS

A response rate of 100% was obtained (questionnaires returned and completed). A total of 97 subjects were included in the study including a twin. Table 2 shows the distribution of subjects according to serum 25-hydroxyvitamin D levels. Hypovitaminosis D was documented in 41.2% (40) of the subjects and there's equal percentage (20.6%) of those having vitamin D insufficiency and deficiency. There was no one with severe VDD. The serum 25-(OH)D levels of the twin sisters differ (48.41 nmol/L and 43.61 nmol/L) but still in the insufficient range. The serum 25-(OH)D levels ranged from 19.92 nmol/L to 88.63 nmol/L with a mean of 52.43 nmol/L. Clinical characteristics of the participants are shown in Table 3.

Table 3. Clinical Characteristics of the High School Students

	School		**Total**
	Private n=49 (%)	**Public** n=48 (%)	n=97 (%)
Sex			
Male	8 (16.3%)	13 (27.1%)	21 (21.6%)
Female	41 (83.7%)	35 (72.9%)	76 (78.4%)
Age, years			
Mean ± SD			14.53 ± 1.32
Range			11 - 18
Nutritional Status			
Obese	4 (8.2%)	0	4 (4.1%)
Overweight	4 (8.2%)	3 (6.3%)	7 (7.2%)
No wasting	39 (79.6%)	37 (77.1%)	76 (78.4%)
Wasted	2 (4.1%)	7 (14.6%)	9 (9.3%)
Severely wasted	0	1 (2.1%)	1 (1.0%)
Duration of Sun Exposure			
<15 mins	9 (18.4%)	21 (43.8%)	30 (30.9%)
15mins – 30mins	27 (55.1%)	21 (43.8%)	48 (49.5%)
>30mins	13 (26.5%)	6 (12.5%)	19 (19.6%)
Clothing style			
Clothes that cover> half of the body	47 (95.9%)	48 (100%)	95 (97.9%)
Clothes that cover the whole body	2 (4.1%)	0	2 (2.1%)
Uses sunblock			
Yes	13 (26.5%)	8 (16.7%)	21 (21.6%)
No	36 (73.5%)	40 (83.3%)	76 (78.4%)
Eat Fish			
Yes	48 (98.0%)	46 (95.8%)	94 (96.9%)
No	1 (2.0%)	2 (4.2%)	3 (3.1%)
Drink Milk			
Yes	44 (89.8%)	38 (79.2%)	82 (84.5%)
No	5 (10.2%)	10 (20.8%)	15 (15.5%)
Had Respiratory Tract Infection >6x/year			
Yes	3(6.1%)	2 (4.2%)	5 (5.2%)
No	46 (93.9%)	46 (95.8%)	92 (94.8%)
Caloric intake	830.04±331.58	1262.23±399.12	

A higher percentage (75%) of those with VDD and VDI have >15 minutes of daily sun exposure as compared to those with <15 minutes (25%) (Table 4). However, results showed that duration of sun exposure is not significantly associated with serum 25-(OH)D levels.

All of those with hypovitaminosis D wear clothes covering more than half of their body, but clothing style was not found to be significantly correlated with vitamin D levels.

Among the sunblock users, 36.4% have VDD and VDI and majority have sufficient vitamin D levels. Results showed that the use of sunblock has no significant association with serum 25-(OH)D levels.

Table 4. Univariate Analysis of Risk Factors for VDD and Association with Serum 25-(OH)D Levels

	Serum 25-hydroxyvitamin D Levels			**Total**	**P value**
	Deficient (n=20)	**Insufficient** (n=20)	**Sufficient** (n=57)		
SES					
Private (USES)	17 (34.7%)	11 (22.4%)	21 (42.9%)	49 (50.5%)	0.0009(S)
Public (LSES)	3 (6.3%)	9 (18.8%)	36 (75%)	48 (49.5%)	
Skin Color*					
Type I -III	4 (20%)	3 (15%)	7 (12.3%)	14(14.4%)	0.70 (NS)
Type IV-VI	16 (80%)	17(85%)	50 (87.7%)	83 (85.6%)	
Duration of Sun Exposure					
<15 mins	5 (25%)	5 (25%)	20 (35.1%)	30 (30.9%)	
>15 mins	15(75%)	15 (75%)	37(64.9%)	67 (69.1%)	0.57 (NS)
Clothing style					
Clothes that cover <50% of the body	0	0	0	0	
clothes that cover >50% of the body	20(100%)	20(100%)	57(100%)	97(100%)	1.00 (NS)
Uses sunblock					
Yes	5 (22.7%)	3 (13.6%)	14(63.6%)	22 (22.7%)	0.65 (NS)
No	15 (20%)	17 (22.7%)	43 (57.3%)	75 (77.3%)	

*based on Fitzpatrick skin type classification scale

On multivariate analysis of risk factors for hypovitaminosis D (Table 5), upper socioeconomic status, BMI Z-score out of the normal range of 0 to 1 SD, and vitamin D intake of less than 5 ug/day are found to be significant predictors of hypovitaminosis D (p values of 0.001,0.012 and 0.019,respectively). Skin color, sun exposure, clothing style, use of sunscreen, gender, obesity, waist circumference, waist-hip ratio, and fish and milk intake are not predictors of hypovitaminosis D. Higher socioeconomic status has 17x risk of having vitamin D insufficiency/deficiency, while those with high or low BMI score are 10x at risk of having VDD/VDI. Vitamin D intake of less than 5 ug/day increases the risk by 28x.

Table 5. Independent Predictors of Hypovitaminosis D

	OR	95 CI	P value
Skin Color (Type IV-VI)	2.531	0.571 – 11.208	0.221 (NS)
Sun exposure (<15 mins)	0.850	0.279 – 2.591	0.775 (NS)
Clothing Style	0.000	0.000	1.00 (NS)
Use sunscreen (Yes)	0.437	0.132 – 1.446	0.175 (NS)
Sex (Female)	1.425	0.382 – 5.318	0.598 (NS)
Socioeconomic status (USES)	17.285	3.327 – 89.810	0.001 (S)
BMI (0 to <1 SD- No)	10.381	1.660 – 64.900	0.012 (S)
Obesity	0.105	0.005 – 2.297	0.152 (NS)
Waist (cm)	1.092	0.837 – 1.423	0.518 (NS)
Waist Hip Ratio	0.554	0.152 – 2.024	0.372 (NS)
Eat fish (no)	1.033	0.045 – 23.677	0.984 (NS)
Drink Milk (No)	0.407	0.090 – 1.840	0.243 (NS)
Vitamin D intake <5ug/day	28.768	1.723 – 480.207	0.019 (S)

Abbreviations: OR,odds ratio; CI,confidence interval

DISCUSSION

This study is the first in the Philippines to investigate the vitamin D status among adolescents. The importance of this study is the unexpected finding of a high prevalence of hypovitaminosis D among apparently healthy Filipino high school students. None of the adolescents have symptoms nor clinical features of vitamin D deficiency as per questioning and physical examination. The presence of asymptomatic Vitamin D deficiency was also observed in 61% of female adolescents in Saudi Arabia [22] and 87% of East African adolescents living in Melbourne, Australia.[19] This finding is congruent with the 42% prevalence of hypovitaminosis D and 24.1% prevalence of VDD in US adolescents [24] and add to the growing data including studies done in the United States, Europe, Middle East, India, Australia, and Asia which suggest that upwards of 30-50% of children and adults are at risk of vitamin D deficiency.[25] Data on the prevalence of hypovitaminosis D among adolescents are scarce and a comparison of serum vitamin D data with other studies may not be entirely appropriate as different studies were conducted at different latitudes, different seasons and different population group. Nevertheless, prevalence this high was not expected to occur in our subjects given that the study was conducted immediately after the students' summer vacation, wherein children were expected to have had abundant sunlight exposure and outdoor activities.

The high prevalence of hypovitaminosis D among high school students may be attributed to several factors. This study found poor vitamin D intake as significantly correlated with prevalent hypovitaminosis D which maybe because generally, only few foods are naturally good sources of vitamin D and foods as well as milk products are usually not fortified with vitamin D in the Philippines because like other Asian and European countries, there are no governmental regulations mandating vitamin D fortification of food products in the country. Vitamin D-rich foods such as salmon, mackerel, herring, cod liver oil and sun dried mushrooms

do not belong to the regular staple food of Filipinos and most of the vitamin D-rich fatty fishes except for sardines are not that affordable to most of the population. In our study, we observed that majority of the high school students eat less than 50% of the required caloric requirement for age. This can be partially attributed to the psychological consequence of adolescent/pubertal stage wherein as adolescents pay close attention to their appearance and body image they tend to develop unhealthy behavior for weight control thus resorting to dieting. Lesser intake would lead to lesser vitamin D intake.

Having higher socioeconomic status is another factor found to be significantly correlated with VDD/VDI. This conforms with the study done in South India wherein high prevalence of hypovitaminosis D is found in population with upper socioeconomic status[30] and in contrast with the study in Northern India among adolescent schoolchildren which showed otherwise.[31] Another study in Brazilian children did not find any significant difference in mean vitamin D level between the 2 groups.[32] This could be due to the adolescents' lifestyle changes and poor overall intake, as majority of those in the USES group eat less with lower protein and vitamin D intake than those in the LSES group. Another reason could be inadequate sun exposure as the USES group's sun exposure usually occur before their classes start at 7:30 AM or after their classes at 5 PM. According to studies by Holick and Webb, the angle at which the sun reaches the earth has a dramatic effect on the number of ultraviolet B (UVB) photons that reach the earth's surface. This is why when the zenith angle is increased in the early morning and late afternoon, little if any vitamin D3 synthesis occurs.[33-34] This may partially explain the decreased 25(OH)D levels despite enough duration of sunlight exposure.

This study found BMI z-score outside the normal range of 0 to <1SD as significantly correlated with VDD/VDI. These are the overweight, obese, wasted and severely wasted. Studies have demonstrated inverse associations between body fat and circulating serum 25(OH)D levels, given that vitamin D, being fat soluble, is stored in the body fat, making it more difficult to be bioavailable, leading to deficient state. There are also studies which show reverse-causation- that inadequate vitamin D status could be a risk factor for childhood obesity due to vitamin D's effects on lipolysis and adipogenesis in human adipocytes through its role in regulating intracellular calcium concentrations.[35-36] This raises the possibility that vitamin D could influence body weight and energy expenditure through calcium regulation. However, result of obesity per se as a predictor did not show significant correlation in this study probably due to small number in the obese group. On the other hand, studies in India found VDD significantly associated with wasting.[37-38] The etiology of wasting is complex and includes the increased secretion of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interferon-gamma (INF-γ), and interleukins (IL) 1 and 6. Both TNF-α and INF-γ are known to inhibit myosin expression in muscle cells and TNF-α also induces anorexia. A potential explanation of the observed association between vitamin D and wasting may be the known anti-inflammatory role of vitamin D that includes decreasing the levels of TNF- α.[39]

Environmental factors such as air pollution may also have a contributory role in the low vitamin D status of the students. Existing studies have generally shown lower vitamin D reserves among urban population.[23,40-41] All of the participants in this study reside in big cities, 94% being in Metro Manila with 85% living in Quezon City area. A study in Bangkok Thailand (13°45'N) which has almost the same latitude as Quezon City, (14°38'N) found an association between lower vitamin D status and living in the urban where air pollution is high.[23] Quezon City is one of the most polluted areas in the Philippines due to its large population and rapid urbanization. Based on the latest Department of Environment and Natural Resources (DENR)-Environmental Management Bureau (EMB) data, the total suspended particulates (TSP),which is the standard measurement of pollution in Metro Manila is 117 Ug/Ncm, which is above the healthful Philippine standard of 90 Ug/Ncm. Tropospheric ozone is a common urban air pollutant and an

efficient absorber of UV radiation.[42] and would therefore interfere with the penetration of UVB radiation into the skin which will affect the cutaneous synthesis of vitamin D leading to inadequate vitamin D levels.

Other factors that could lead to decreased vitamin D synthesis thus hypovitaminosis D are lack of adequate sun exposure, skin pigmentation, sunscreen use and clothing style. Ultraviolet radiation (UVR) exposure to the skin is measured as the minimum erythema dose (MED) or the amount of UVR exposure that will cause minimal skin erythema. The amount of UVR exposure that is equivalent to 1 MED depends on skin pigmentation and duration of exposure. Exposure of 40% of the body to ¼ MED will result in generation of ~1000 IU of vit D per day, the minimum amount of vitamin D synthesis necessary to maintain concentrations in the reference range.[43] In our study, clothing style of the students expose just 25%-30% of the body to the sun, which is estimated to result in release of approximately 625-750 IU of vitamin D which is inadequate to maintain vitamin D concentrations at normal range. Another factor is skin pigmentation which is one of the reasons for VDD in Delhi despite abundant sunlight.[44] More UV-B is necessary to produce MED in darker-skinned people; therefore they require a longer duration (5-10x) of sun exposure than light-skinned people for a similar response.[15,45] Holick et al. have also shown that the time to maximum previtamin D formation is 15-30 minutes in type III skin, during exposure to equatorial amounts of UV energy. In our study, we observed that 85.6% of the students have type IV-VI skin type but only 19.6% have more than 30 minutes of sun exposure, which is inadequate for optimal vitamin D synthesis. In relation to this, increased time spent indoors at home or in school may lead to less sun exposure thus decrease vitamin D synthesis, even in light-skinned individuals as shade reduces the amount of solar radiation by 60%, and windowpane glass blocks UVR.[42] Added to this is the use of sunscreen. Sunscreen absorbs UV-B and some UV-A light and prevents it from penetrating the skin. A sunscreen with a sun protection factor (SPF) of 8 can decrease vitamin D_3 synthetic capacity by 95% and SPF 15 can decrease it by 98%.[46] Nonetheless, in our study, the association of the duration of sun exposure, sunscreen use, clothing style, and skin color with 25-(OH)D levels did not reach statistical significance. This is in agreement with previous studies done wherein no relationship was found between 25 (OH)D concentrations and skin color, sunscreen use, duration of sun exposure and kind of clothing.[47-49]

The association between hypovitaminosis D and poor vitamin D intake, BMI outside the normal range and upper socioeconomic status suggests that attention should be paid to optimize the adolescents' vitamin D intake. Given the dietary practices of many adolescents especially those belonging to the USES, a dietary intake of 400 IU of vitamin D is difficult to achieve therefore, vitamin D stores would depend on sunlight exposure and vitamin D fortification of food or vitamin D supplementation. In view of current guidelines of many pediatric organizations including the AAP of decreasing sunlight exposure due to the risks of various skin cancer, in addition to the concern of photo-aging, higher sunlight exposure may be resisted in this population. The timing of optimal sun exposure (between 10 AM and 3PM) would also pose a problem to school-going adolescents because it is during these times that classes are held. Nonetheless, awareness needs to be generated about the benefits derived from direct sunlight exposure. Nutritional education should be encouraged in schools focusing on the importance of increased intake of vitamin D-rich foods. The help of nutritionists and health professionals who can advise students on how to increase dietary intake of vitamin D-rich foods may be sought. Because of the lower dietary intake in this age group there may also be a public health need to fortify Filipino foods with vitamin D, however this would entail a huge cost. A daily multivitamin with vitamin D content or vitamin-D only preparation would also be necessary since it is easy and inexpensive.

We acknowledge some limitations in this study. First, the study is cross-sectional, and therefore, causality cannot be inferred. Only a

longitudinal study will be able to give greater validity to correlations observed in this study. Second, the many randomly chosen subjects who refused to participate may lead to non-response bias which may have an effect on representativeness of the sample and results, though it may have affected the results only marginally. Third, the limited number of subjects may have resulted in low variability in the different variables studied. However, this research will serve as a baseline for future studies on Vitamin D in Filipino adolescents. Lastly, as the original objective of the study is to determine the vitamin D status of adolescents, bone turnover markers were not measured due to high cost. These should be included in future studies to determine changes of bone metabolism and its relation with vitamin D. Because of the various consequences of VDD, long term skeletal and other consequences of these findings deserve further study.

CONCLUSION

This study has shown that hypovitaminosis D is highly prevalent among apparently healthy Filipino high school students despite the abundance of sunlight. Cases may be missed due to lack of symptoms. Poor vitamin D intake, BMI scores outside the normal range of 0 to <1SD and high socioeconomic status emerged as significant predictors of vitamin D deficiency/insufficiency. Gender, skin color, duration of sun exposure, clothing style, sunscreen use, waist measurement/WHR, obesity, and intake of milk and fish were not found to be significantly associated with serum 25(OH)D levels.

It is recommended that interventional studies to evaluate the role of nutrition in improving vitamin D status be done in future studies. Additional studies are also needed to evaluate what the optimal dose of vitamin D supplement should be to achieve the optimal vitamin D status for adolescents.

REFERENCES:

1. Gueli N,Verrusio W,et al. Vitamin D:drug of the future. A new therapeutic approach. Archives of Gerontology and Geriatrics 2012 54;222-227.

2. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.

3. Morris, HA. Vitamin D: a hormone for all seasons- how much is enough? Clin. Biochem. Rev. 2005 26;21-32.

4. Misra, M, Pacaud D,et al. Vitamin D Deficiency in Children and its Management: Review of Current Knowledge and Recommendations. Pediatrics 2008;122:398-417

5. Gullberg B, Johnell O, Kanis JA: World-wide projections for hip fracture. Osteoporos Int 1997, 7:407-413.

6. Lau EM, Lee JK, Suriwongpaisal P, Saw SM, Das DS, Khir A, et al.: The incidence of hip fracture in four Asian countries: the Asian Osteoporosis Study (AOS). Osteoporos Int 2001, 12:239-243

7. Aban,MM: Osteoporosis is a looming epidemic. Medical Observer: Organized Medicine 2012.

8. Osteoporosis Prevention, Diagnosis and Therapy. 2000 March 2000;17:1-45. NIH Consensus Statement 2000 Mar 27-29 2000;Vol 17,issue 1:1-45.

9. Cashman KD. Vitamin D in childhood and adolescence. Postgrad Med J 2007;83:230-235.

10. Bailey DA, McKay HA,et al. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the University of Saskatchewan bone mineral accrual study.J Bone and Min Res 1999;14:1672-9.

11. Cummings SR, BlackDM,et al. Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet 1993;341:72-5.

12. Peterlik,M, Boonen S,et al. Vitamin D and calcium insufficiency-related chronic diseases: an emerging world-wide public health problem. Int J Environmental Res.Public Health 2009;6:2585-2607.

13. Lu L, Yu Z,et al. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. Diabetes Care.2009 Jul;32:1278-83.

14. Zitterman A. Vitamin D in preventive medicine: are we ignoring the evidence? British Journal of Nutrition 2003;89:552-572.

15. Wagner CL,Greer FR,The Section on Breastfeeding and Committee on Nutrition. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics 2008;122:1142-1152.

16. Masud F. Vitamin D levels for optimum bone health. Singapore Med J 2007; 48(3):207-12 .

17. Jones G. Dwyer T.,et al. Vitamin D insufficiency in adolescent males in southern Tasmania: prevalence, determinants and relationship to bone turnover markers. Osteoporosis Int 2005;16:636-41.

18. Prentice A. Vitamin D deficiency: a global perspective. Nutrition Review 2008; 66:S153-S164

19. McGillivray G,Skull S,et al. High prevalence of asymptomatic vitamin D and iron deficiency in East African immigrant children and adolescents in a temperate climate. Arch Dis Child 2007;92:1088-1093

20. Winzenberg TM, Powell S, et al. Vitamin D supplementation for improving bone mineral density in children (Review). The Cochrane Library 2010, Issue 10.

21. Arabi A,et al. Hypovitaminosis D in developing countries-prevalence,risk factors and outcomes. Nat. Rev. Endocrinol. 2010;6:550-561

22. Siddiqui,AM,Kamfar HZ. Prevalence of vitamin D deficiency rickets in adolescent school girls in Western region,Saudi Arabia. Saudi Med J 2007;28:441-444

23. Chailurkit L. et al. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health 2011,11;853

24. Gordon,CM, DePeter KC,et al. Prevalence of Vitamin D deficiency among healthy adolescents. Arch Pediatr Adolesc Med 2004;158:531-537.

25. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequenses. Am J Clin Nutr 2008:87,1080S-1086S.

26. Holick MF. Evolution, biologic functions, and recommended dietary allowance for vitamin D. In: Holick MF(ed) Vitamin D: physiology,molecular biology and clinical applications. Humana Press, Totowa, New Jersey 1999,pp1-16.

27. Von Muhlen,Miller III,et al. Science Daily.American Heart Association. Low Vitamin D Levels Associated with Several Risk Factors in Teenagers 2009.

28. Hollis BW, Wagner CL. Nutritional vitamin D status during pregnancy: reasons for concern. CMAJ.2006;174:1287-1290

29. Mannion Ca,Gray-Donald K,et al. Association of low intake of ilk and vitamin D during pregnancy with decreased birth weight. CMAJ.2006;174:1273-1277

30. Chittari H,Joshi S,et al. Vitamin D status of upper socioeconomic status subjects of South Indians living in high altitude (Bengaluru. Endocr Rev 2012;33:352

31. Marwaha R., Tandon Nikhil,et al. Vitamin D and bone mineral density status of healthy schoolchildren in northern India. Am J Clin Nutr 2005;82:477-82'

32. Linhares ER,Jones DA,et al. Effect of nutrition on vitamin D status:studies on healthy and poorly nourished Brazilian children. Am J Clin Nutr 1984;39:625-30

33. Holick MF. Vitamin D: A millennium perspective. J Cell Biochem 2003;88:296-387

34. Webb AR,et al. Influence of season and latitude on the cutaneous synthesis of vitamin D3:exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocr Metab 1988:67:373-8

35. Zemel MB,Shi H,et al. Regulation of adiposity by dietary calcium. FASEB J 2000:14;1132-8.

36. Xue B,Greenberg AG,et al. Mechanism of intracellular calcium inhibition of lipolysis in human adipocytes. FASEB J 2001;15:2527-9.

37. Sachdeva S, Amir A, et al. Potentially modifiable microenvironmental and comorbid factors associated with severe wasting and stunting in children below 3 years of age in Aligarh District. Indian J Community Med 2010;35:353-355.

38. Raghuramulu N and Reddy V. Serum 25-hydroxyvitamin D levels in malnourished children with rickets. Archives of Disease in Childhood 1980;55:285-287.

39. Mehta S,Mugusi F,et al. Vitamin D status and its association with morbidity including wasting and opportunistic illnesses in HIV-infected women in Tanzania. AIDS Patient Care and STDs 2011.25;579-585.

40. Heere C.,Skeaff CM,et al. Serum 25-hydroxyvitamin D concentration of indigenous-Fijian and Fijian-Indian women. Aisa Pac J Clin Nutr 2010.19:43-48.

41. Manicourt DH,Devogelaer JP: Urban tropospheric ozone increases the prevalence of of VDD among Belgian postmenopausal women with outdoor activities during summer. J Clin Endocrinol Metab 2008:93:3893-3899.

42. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr. 1995;1:638S-645S

43. Holick MF. Sunlight and Vitamin D for bone health and prevention of autoimmune diseases, cancers, and CV disease. Am J Clin Nutr 2004;80:1678-1688

44. Goswami R,Gupta N,et al. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. Am J Clin Nutr 2000;72:472-75

45. Lo CW,Paris PW,et al. Indian and Pakistani immigrants have the same capacity as Caucasians to produce vitamin D in response to UV irradiation. Am J Clin Nutr 1986;44:683-685.

46. Matsuoka LY, Ide L,Wortsman J,et al. Sunscreens suppress cutaneous vitamin D3 synthesis. J Clin Endocrinol Metab 1987;64:1165-1168.

47. Nicolaidou P,Kakourou T,et al. Low vitamin D status in preschool children in Greece. Nutrition Research 2006;620-625

48. Ataie-Jafari A,Hossein-nezhad A,et al. The influence of sunlight exposure on serum vitamin D concentration and bone turnover;a controlled clinical trial. Iranian J Publ Health,2008;1:41-48.

49. Hashemipour S,Larijani B,et al. Vitamin D deficiency and causative factors in the population of Tehran. BMC Public Health 2004;25:38

SAFETY AND EFFICACY OF ORAL VERSUS INHALED CORTICOSTEROID FOR MODERATE PERSISTENT ASTHMA IN CHILDREN 6 TO 15 YEARS OLD: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT

BACKGROUND: One out of 10 Filipino children suffer from bronchial asthma. Its increasing prevalence over the past decades causes significant morbidity to the patients and their families, especially when it is uncontrolled. In developing countries, the form of medicine, cost and access to medications are significant factors in achieving good control of symptoms. No study has been done to determine if the use of low dose oral corticosteroid is a safe and effective alternative treatment for children with asthma.

Objective: To compare the safety and efficacy of oral versus inhaled corticosteroid in moderate persistent asthma.

METHODS: An open label randomized controlled trial was carried out in 40 patients 6 to 15 years old with newly diagnosed moderate persistent asthma. They were assigned to either inhaled or oral corticosteroid group. Efficacy of treatment based on daytime and nighttime coughing, limitation of activity, use of bronchodilators, FEV1, PEFR and level of asthma control were assessed. Safety of both treatment options were evaluated by the occurrence of corticosteroid induced hypertension, adrenal insufficiency and diabetes.

RESULTS: The use of inhaled and oral corticosteroid showed improvement in daytime and nighttime cough, need for bronchodilators, limitation of activity, FEV1, PEFR and level of control. Furthermore, both treatment options had comparable safety profile.

CONCLUSION: There was no significant difference on the safety and efficacy of oral versus inhaled corticosteroid in the longterm treatment of moderate persistent bronchial asthma.

INTRODUCTION

Childhood asthma is a global health care problem. In the Philippines, 1 out of 10 children suffer from asthma.[1] Its increasing prevalence over the past decades causes significant morbidity to the patients, especially during periods when it is insufficiently controlled. This is reflected in frequent symptoms, high rates of unscheduled emergency department visits and hospitalisation, as well as absence from school. These combine to interfere with normal daily activities and education and result in a considerable clinical and economic burden for the whole family of an asthmatic child.[2]

Inhaled corticosteroid therapy has emerged as the treatment of choice for persistent asthma in children.[3] It effectively reduces airway inflammation, improves lung function and reduces asthma symptoms. It is taken daily on long-term basis to achieve control chiefly through their anti-inflammatory effects and to prevent secondary airway remodelling. Despite its direct delivery into the airways by inhalation, a substantial portion may be deposited in the oropharynx, subsequently swallowed and absorbed from the gastrointestinal tract, hence are not without adverse systemic corticosteroid effects.[4,5] However, they are generally known to have lesser risk for serious adverse effects than the oral or systemic corticosteroids.

Despite the presence of a recommended and effective treatment for asthma, majority of patients still have poor control of symptoms. Data shows that 2 out of 5 asthmatic children cannot engage in sports and recreation and are deprived of good night's sleep.[6] Two out of three grow up to have uncontrolled asthma and that every hour, 28 asthmatic patients die of an asthma attack.[4,7] This poor asthma control in children is not usually due to a lack of efficacy of the medication, but is more often related to sub-optimal use of medication or aggravating factors, comorbidities, poor inhaler technique, poor environmental control or a lack of continuity of care.[8] In developing countries, the form of medicine, cost and access to medications are significant factors in achieving treatment goals. [9] In effect, this creates a vicious cycle of clinical and economic problems to the children, families and the society, including days off from school, time off from work and loss of productivity.

As health care providers, we are faced with the task to offer our asthmatic patients an affordable and effective alternative. Corticosteroids may be administered in a variety of ways other than the inhaled route, including orally or systemically. In severe cases of asthma, oral or systemic administration of corticosteroids is preferred. In clinical practice, patients with poor control of symptoms, due to poor adherence to medications because of its high cost, are given oral corticosteroid regardless of severity as maintenance medication. As with ICS, it has anti-inflammatory effect that may provide good control of symptoms for bronchial asthma, however, its use is marred by their potential systemic side effects.

Chernick indicates that a dose of 0.25 to 2mg/kg daily in single dose in am may be given to achieve control.[5] On the other hand, a Cochrane study comparing inhaled versus oral steroids for adults with chronic asthma, concluded that a daily dose of prednisolone 7.5 to 10 mg/day appears to be equivalent to moderate-high dose inhaled corticosteroids.[10]

No study has been done to provide support for the use of oral steroid among children with chronic asthma. Hence, this study will determine the safety and efficacy of oral corticosteroid in children with chronic asthma.

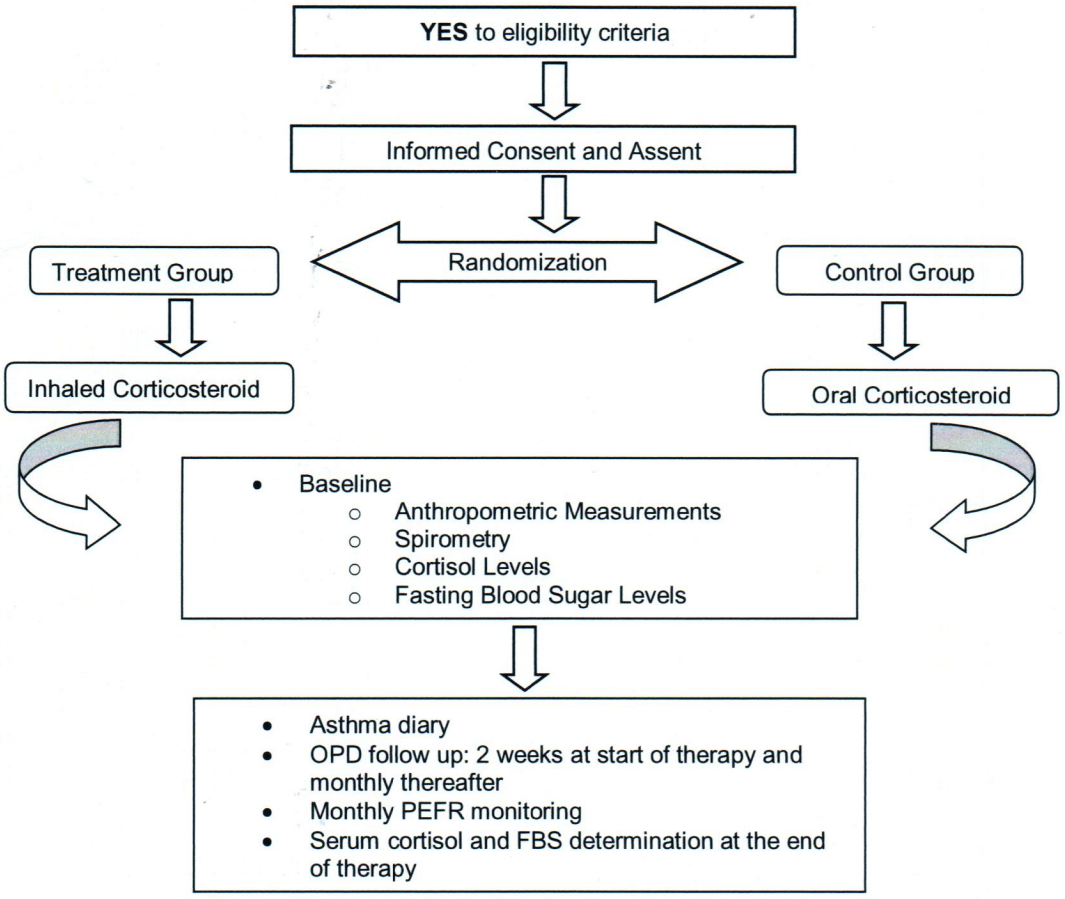
Specific Objectives:

1. To compare efficacy of oral versus inhaled corticosteroids in terms of: Frequency of daytime symptoms, Frequency of nighttime symptoms, Frequency of use of short acting bronchodilators, Limitation of activity, FEV1, PEFR, Level of asthma control;

2. To compare the safety of oral versus inhaled corticosteroids in terms of effect on: blood pressure, FBS, Serum cortisol levels

METHODOLOGY

This was an open Label Randomized Controlled Trial conducted in patients aged 6 to 15 years old with newly diagnosed moderate persistent asthma and those previously diagnosed patients, but off inhaled steroids for the past three months. They were recruited from the outpatient clinics of a tertiary hospital as well as those admitted in the ward. Excluded were (a) those with physician diagnosed allergic rhinitis on intranasal steroids or anti-leukotrienes, (b) children maintained on corticosteroids (c) those with previously diagnosed hypertension, growth disorder or history of glaucoma and cataract and (d) those with known hypersensitivity to prednisone. The study flow is depicted below:



The protocol for this study was reviewed and approved by the Hospital's Institutional Review Board and Ethics Committee. The subjects were randomly assigned to either control or treatment group. Randomization was prepared prior to recruitment using a table of random numbers. The same numbers were sequentially placed on sealed opaque envelopes and were given to a research assistant together with the control and study drugs. Group A, the control group, were given the standard treatment of inhaled corticosteroid (Budesonide) 200mcg twice a day for 3 months. Study participants and their parents where instructed and shown how to properly administer the drug at the beginning of the study period. On the other hand, group B or the treatment group was given a single morning dose of oral corticosteroid (Prednisone) at 0.5mg/kg with a maximum dose of 10 mg/day, for 3 months. This was based on a Cochrane review conclusion that a daily dose of prednisolone 7.5 to 10 mg/day is equivalent to moderate-high dose inhaled corticosteroids among adults with chronic asthma.[10]

Upon enrollment, cardiac and respiratory rate were taken one full minute and blood pressure was measured, using an appropriate size of sphygmomanometer cuff, on the right arm with the study participant seated and arm resting on a table. Height in centimeters and weight in kilograms were measured by a standard calibrated stadiometer and a calibrated weighing scale, respectively. Baseline spirometry was performed on each patient, measuring Forced Expiratory Volume in 1 second (FEV1). Each subject was provided with an asthma diary to be accomplished by the patient or patient's guardian. Prior to start of therapy, study participants fasting blood sugar and early morning cortisol levels were determined.

All study participants were evaluated by a pulmonology fellow during each follow up, initially two weeks after onset of treatment and monthly thereafter for 3 months. Each were assessed using the asthma diary as to the (a) frequency of daytime and nighttime symptoms, (b) need for short acting beta agonist and the (c) presence of limitation of activity. Level of asthma control based on the GINA guidelines, was determined for each participant.

Peak Expiratory Flow Rate (PEFR) was recorded and vital signs, such as CR, RR and BP, and the participant's height and weight were taken during each follow up visit. Possible drug reactions, adverse effects and symptoms of adrenal insufficiency such as nausea, vomiting, abdominal pain, excessive sweating and hypotension were observed and noted during each follow up. (Appendix G).

After 3 months of therapy, spirometry was again performed and serum cortisol and fasting blood sugar measured. For study participants with controlled asthma by the 3rd month of treatment tapering of steroid dose was done by decreasing dose by 25% weekly until discontinued. However, if asthma is still partly controlled or uncontrolled, they were managed appropriately. Monthly follow up of participants continued until 6 months from start of treatment.

A patient was considered as a treatment failure if he/she has 2 or more exacerbations necessitating the need for a short course of steroid. Drop outs are patients who were lost to follow up or those who withdrew from the study. Intention to treat analysis was done. Data were described using means, standard deviation for quantitative variables while frequency intervals and percentages were used for qualitative variables. Comparison of 2 groups of continuous variables was done using the t-test for independent samples and chi-square test for qualitative values. When chi square test failed due to the presence of expected value less than 5, the Fischer exact test was used. A 95% confidence interval will be considered significant.

The Epi Info version 3.5.3 was used in the analysis of data.

Table 1. Baseline Profile of the Study Participants by Group

Profile	Inhaled Corticosteroid (IC) Group N = 20	Oral Corticosteroid (OC) Group N = 20	p value
Age in years (mean ± SD)	8.1 ± 2.4	8.3 ± 2.2	0.732
Sex, n (%)			0.507
Male	14 (70.0)	12 (60.0)	
Female	6 (30.0)	8 (40.0)	
Daytime Symptom (mean ± SD)	4.7 ± 1.9	5.0 ± 1.8	0.611
Nighttime symptom (mean ± SD)	3.1 ± 0.9	3.1 ± 0.8	1.000
Weight in kg (mean ± SD)	24.0 ± 8.0	24.3 ± 5.4	0.890
Height in cm (mean ± SD)	121.7 ± 11.8	126.8 ± 10.9	0.165
Systolic BP (mean ± SD)	96.5 ± 4.9	94.5 ± 5.1	0.214
Diastolic BP (mean ± SD)	63.0 ± 4.7	60.5 ± 6.0	0.153
FEV1, (mean ± SD)	139.9 ± 38.5	137.1 ± 39.5	0.822

RESULTS

A total of 40 study participants were assigned randomly into two groups namely, control or inhaled corticosteroid (IC) group and the treament or the oral corticosteroid (OC) group.

The socio-demographic and clinical profiles of the study participants are summarized in Table 1. There was no significant difference in the distribution of the baseline profile of the participants, as to age, sex, daytime and nighttime symptoms, height, weight, blood pressure and baseline FEV1 levels. Hence both groups were comparable.

Efficacy of inhaled and oral corticosteroids were compared in terms of daytime and nighttime symptoms, need for short acting beta agonist (SABA) and presence of limitation of activity, mean PEFR, and level of asthma control were assessed at week 2, month 1, month 2 and month 3. Almost all parameters showed no significant difference in both groups. This indicates that inhaled and oral corticosteroids are equally effective. Peak Expiratory Flow Rate (PEFR) measured on the 2nd month of treatment, is the only parameter that showed a significant difference between IC and OC groups.

However, one out of 20 participants from the inhaled corticosteroid (IC) group had two exacerbations during the 3 month study period hence there was a need to increase the IC dose and he was considered as a treatment failure while one from the OC group was lost to follow up on the 3rd month.

By the first month of treatment, 90% of study participants from both inhaled (IC) and oral (OC) corticosteroid groups had daytime cough less than twice per week and further increased to 95% by the third month. (Figure 1)

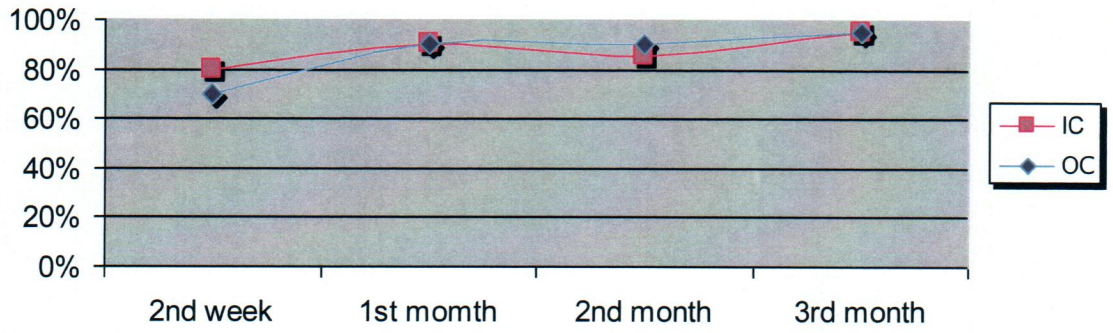


Figure 1: Percentage of Patients with Daytime Symptoms <2x/week

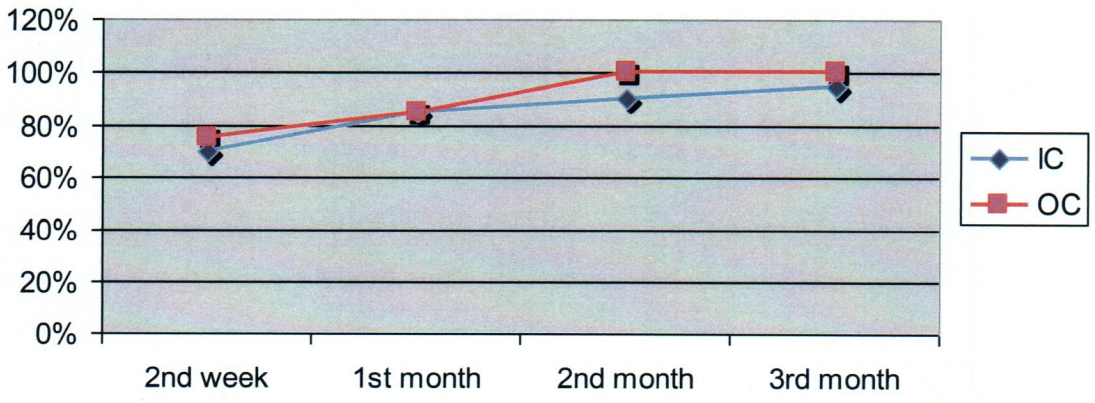


Figure 2: Percentage of Patients with No Nightime Symptoms

Eighty-five (85%) of subjects from both groups had no nocturnal symptoms after the 1st month of therapy. On the 3rd month, 100% of study participants from the OC group as compared to 95% of the IC group were asymptomatic at nighttime. (Figure 2)

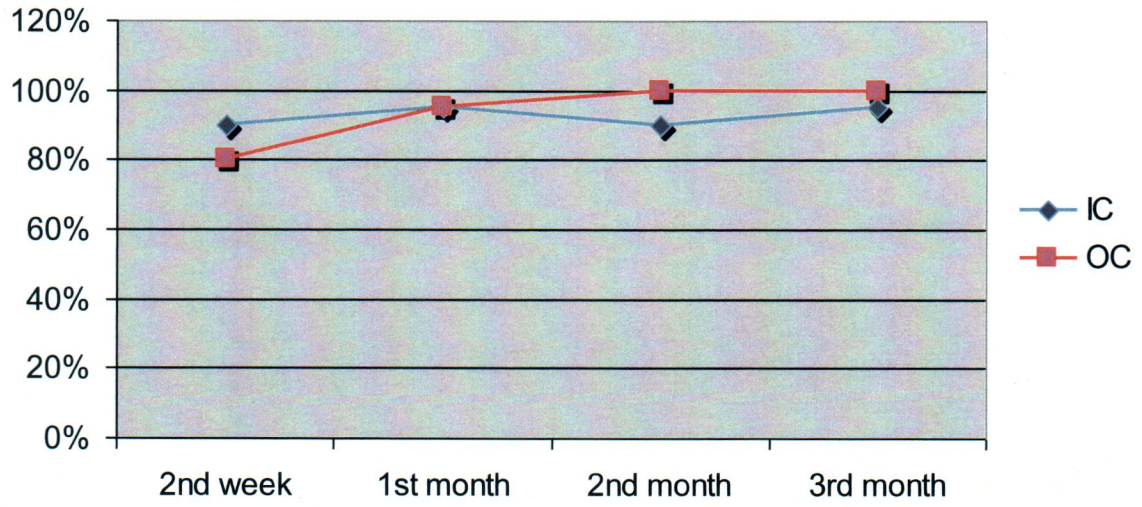


Figure 3: Percentage of Patients with Use of Short Acting Bronchodilator (SABA) <2x/week

All study participants belonging to the OC group used short acting bronchodilators less than 2x/week by the 2nd month whereas a small percentage, 5 to 10%, of subjects from the IC arm had to use SABA for more than 2x/week from the 1st to the 3rd month of the study period. (Figure 3)

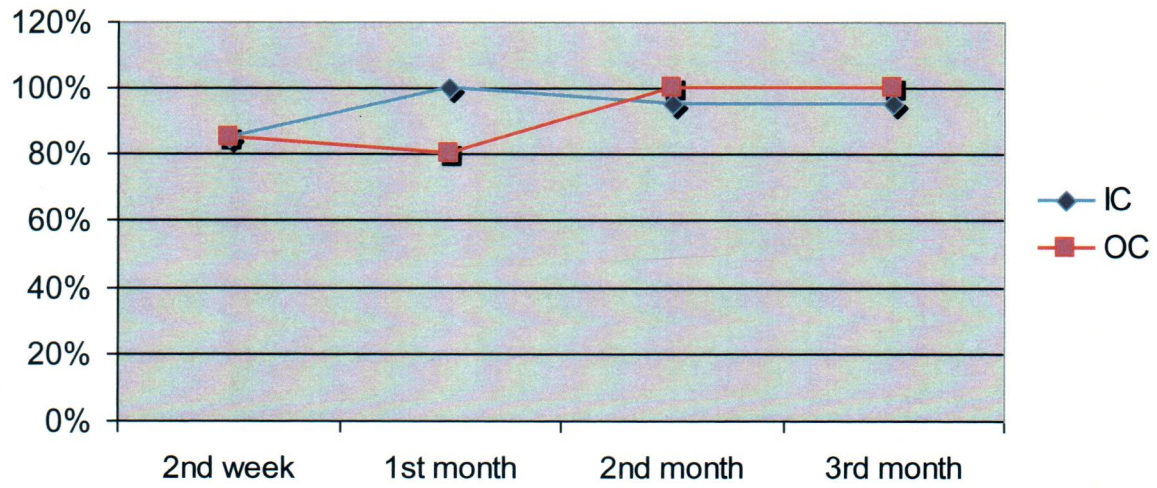


Figure 4: Percentage of Patients with No Limitation of Activity

One hundred percent of subjects for the IC group as compared to 80% from the OC group no longer had limitation of activity by the 1st month of treatment. However, by the 2nd month, one out of the 20 participants from the IC group had limitation of activity. (Figure 4)

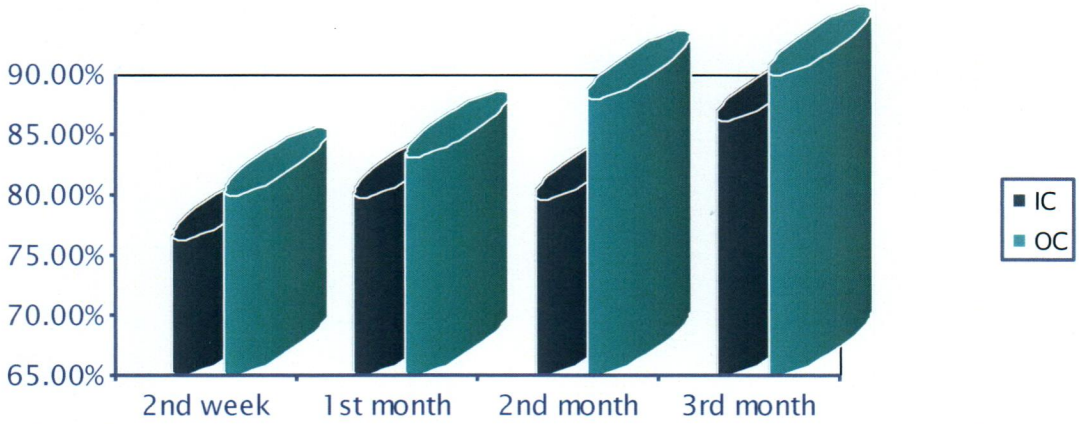


Figure 5: Comparison of Peak Expiratory Flow Rates between groups

Peak Expiratory Flow Rate (PEFR) is the only parameter that showed a significant difference. Study participants under the IC group showed lower PEFR values than the IC group throughout the entire study period. There was a proportional increase in the values in both groups as the duration of the treatment increases. (Figure 5)

FEV1 results of patients showed almost the same values. In both groups, there was no significant difference in FEV1 measured at the start and end of the treatment period.

For both groups, control of asthma was attained in majority of the subjects by the 2nd month of treatment. Table 2 shows the summary of the level of control of the study participants.

Table 2: Level of Asthma Control

Follow up	Levels of Control	IC Group n (%)	OC Group n (%)	p-value[a]
2nd week	Controlled	5 (25)	9 (45)	0.185
	Partly Controlled	13 (65)	8 (40)	
	Uncontrolled	2 (10)	3 (15)	
1st month	Controlled	12 (60)	13 (65)	0.744
	Partly Controlled	7 (35)	6 (30)	
	Uncontrolled	1 (5)	1 (5)	
2nd month	Controlled	15 (75)	18 (90)	0.407
	Partly Controlled	4 (20)	2 (10)	
	Uncontrolled	1 (5)	0 (0)	
3rd month	Controlled	18 (90)	20 (100)	0.487
	Partly Controlled	1 (5)	0 (0)	
	Uncontrolled	1 (5)	0 (0)	

Aside from efficacy, safety profile of both treatment options were also considered in the study. These are systolic blood pressure (SBP), diastolic blood pressure (DBP), serum cortisol and fasting blood sugar levels. Blood pressure measurements were taken during each OPD follow up while the FBS and cortisol measurements were taken at baseline and month

3. All safety parameters showed no significant difference (p-value>0.05). There was no note of significant increase in the blood pressure of the subjects throughout the entire study period. FBS levels of study participants in both groups showed no increase while early morning serum cortisol levels of subjects on IC and OC for 3 months showed a decrease in the values.

DISCUSSION

Inhaled corticosteroids are the first choice for preventive treatment of asthma but they remain expensive for health economies in developing countries. Other than the inhaled route, corticosteroids can be given orally or systemically but several controversies exist in the its prolonged use or when given at high doses, particularly because of its documented adverse effects. This is the first study that investigated the safety and efficacy of oral corticosteroids as treatment for persistent asthma in children. Study participants were randomly divided into two groups, group A received the standard treatment of inhaled corticosteroid while the group B received oral corticosteroid.

Assessment of efficacy parameters, such as frequency of daytime and nighttime symptoms, limitation of activity and use of short acting bronchodilators, showed that there was no significant difference between both treatment groups. The functional effects of corticosteroids on airways, administered systemically or by inhalation, have been well established mainly among asthmatic patients.[11] Its use can reduce the severity of asthma symptoms, improve airflow limitation, reduce airway hyperresponsiveness, and decrease the risk and severity of exacerbations.[12] This study showed that corticosteroids in asthma given orally or by inhalation has similar efficacy.

Objective measurement of efficacy of both treatment options was also done. Spirometry was done on each participant, before and after the treatment period, to measure Forced Expiratory Volume in one second (FEV1) while Peak Expiratory Flow Rate (PEFR) was measured during each follow up. FEV1 showed no significant difference between both groups. However, PEFR on the 2nd month of treatment revealed significant difference *(pvalue: 0.001)*.

FEV1 measurement is the gold standard for functional airway obstruction assessment due to its less intrasubject variability. FEV1 and PEFR are both excellent methods in pursuing the course of asthma in patients, but it has not been proven if PEFR can be an acceptable alternative for FEV1.[13] Some authors also found that PEFR is a relatively insensitive and unreliable measure of airway caliber and found discrepancies between PEFR and "true lung function". [13 14]

Further statistical analysis was done to investigate PEFR findings in this study. The mean PEFR for each group was compared across time using the repeated measures analysis of variance. For the inhaled group, there was a significant change of PEFR across time particularly noted on month 1 to month 3 (p-value=0.013). While the mean PEFR among the patients given oral corticosteroids increased from baseline at 79.9% to 90.1% during the third month. Significant mean difference was noted from baseline to 2 month (p-value=0.001), baseline to third month (p=value-0.000), first month to second month (p-value=0.037), first month to third month (p-value=0.012). Based on this, PEFR on both treatment groups had a significant difference which their FEV1 results failed to document. (See Appendix J)

The second and more important focus of the study is the comparison of the safety of oral versus inhaled corticosteroids in the long term treatment of bronchial asthma in children. The use of inhaled corticosteroids over the past decades has improved the benefit-risk ratio for the preventive treatment of asthma.[15] However, IC are not without adverse effects, in a systematic review and meta analysis by Lipworth revealed that all inhaled corticosteroids, especially fluticasone, exhibit dose-related systemic adverse effects. Marked adrenal suppression was documented to occur at doses above 0.8mg/d.[16]

In this study, subjects belonging to the OC group were given low dose Prednisone in

the morning at 0.5mg/kg/day with a maximum dose of 10 mg daily. This was based on a Cochrane review conclusion that a daily dose of prednisolone 7.5 to 10 mg/day appears to be equivalent to moderate-high dose inhaled corticosteroids among adults with chronic asthma.[10]

Among the various side effects of prolonged corticosteroid use, three were evaluated in this study namely; drug induced diabetes, hypertension and adrenal insufficiency. There was no significant difference between both treatment groups after 3 months of observation based on the aforementioned safety parameters.

Hyperglycemia can be one of the troubling consequences of both short- and long term steroid use. Steroids elevate blood glucose levels by increasing hepatic glucose production and inhibiting glucose uptake into the muscles. They also have a complex effect on beta cell function.[17] The risk factors and impact of developing steroid induced diabetes are poorly characterised or quantified but based on review of related literature suggests that old age, weight, family history of diabetes, increasing dose, duration of therapy and presence of underlying disease may be risk factors for its development.[17 18]

The association of adrenal insufficiency and treatment with oral corticosteroids has long been recognized but the magnitude of risk has not been determined. Its relation to inhaled corticosteroids has come to attention more recently with several case reports.[19 20] It was noted that 90% of study participants from both groups had decreased serum cortisol levels. However statistically, there was no significant difference between the IC and OC groups. At the same time, no signs and symptoms of adrenal crisis were observed. However since adrenal insufficiency is rare in the general population and may present nonspecifically, the diagnosis needs to be considered in children with suspicious symptoms who are on prolonged use of inhaled or oral corticosteroid, particularly when taken at higher doses.

In a study comparing the effects of inhaled with those of oral corticosteroids, asthmatic patients given fluticasone propionate produced dose-related suppression of 8AM cortisol levels was comparable to that of prednisolone. Those given fluticasone had a maximal suppression amounting to 56% versus that 67% with oral prednisolone.[16]
The main clinical finding in this study is that the use of low dose oral corticosteroid has comparable safety and efficacy profile to inhaled corticosteroid. Our analysis still suggests that patients on long term treatment of bronchial asthma should be given the lowest effective dose of inhaled or oral corticosteroid to avoid the occurrence of debilitating side effects. In effect, this study does not aim to discourage the use of steroids, orally or by inhalation, in bronchial asthma nor to replace inhaled corticosteroid as the treatment of choice for persistent asthma. It only hopes to discover a suitable, effective and safe alternative for those patients with uncontrolled asthma in developing countries.

CONCLUSION

There was no significant difference in efficacy and safety of inhaled and oral corticosteroid after three months of treatment in children with moderate persistent asthma.

RECOMMENDATIONS

- A study on the effective lowest dose of oral corticosteroid can be done to minimize side effects.
- A longer period of observation and a bigger sample size may be conducted to confirm the observed consistent improvement of signs and symptoms early during treatment.
- Objective measurement of limitation of activity can also be done.

REFERENCES:

1. Asher, IM. Worldwide trend in the prevalence of symptoms of asthma (ISAAC Phase 3).

2. Gustaffon PM, Watson L, Davis KJ, Rabe KF. Poor asthma control in children: evidence from epidemiological surveys and implications for clinical practice. Int J Clin Pract, March 2006, 60, 3, 321–334.

3. E Verona, D Petrov, E Cserhati, J Hofman, N Geppe, H Medley, S Hughes. Fluticasone propionate in asthma: a long term dose comparison study. Arch Dis Child 2003;88:503–509.

4. Global strategy for asthma management and prevention, Global Initiative for Asthma (GINA) guidelines, 2008, pp 2-8.

5. Chernick, V., Boat, T., Disorders of the respiratory tract in children, 7th ed., pp 808-810

6. Neffen H et al. Pan American J Public Health 2005;17:191-197.

7. Branan SS. Chest 2006;130:4S-12S.

8. Canadian Pediatric Asthma Consensus Guidelines 2004. Canadian Medical Association Journal. 13 September, 2005; 173.

9. Dawood OT, Ibrahim MIM and Palaian S. Medication Compliance among Children. World J Pediatr, Vol 6 No 3 · August 15, 2010.

10. Mash BRJ, Bheekie A, Jones P. Inhaled versus oral steroids for adults with chronic asthma. *Cochrane Database of Systematic Reviews* 2001, Issue 1. Art. No.: CD002160. DOI: 10.1002/14651858.CD002160

11. De Benedictis FM and Bush, A. Corticosteroids in Respiratory Diseases in Children. Am J Respir Crit Care Med. Jan 2012;185:12–23.

12. National Institutes of Health, National Heart, Lung, and Blood Institute. National Asthma Education and Prevention Program Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma. Bethesda, MD: National Institutes of Health; July 1997. Publication 97–4051.

13. Gharagozlou M, Kompani F and Morahedi M. Comparison between PEFR and FEV1 in the Evaluation of Children Suspected to have Asthma. Iranian Journal of Allergy, Asthma and Immunology. March 2004;3:21-24

14. Meltzer A, Smolensky MH, Alonzo GE, Harrist RB and Scott PH. An Assessment of Peak Expiratory Flow as a Surrogate Measurement of FEV1 in Stable Asthmatic Children. Chest 1989;96:329-333.

15. The British guidelines on asthma management:1995 review and position statement. Thorax. 1997;52:S1-S21.

16. Lipworth BJ. Systemic Adverse Effects of Inhaled Corticosteroid Therapy: A Systematic Review and Meta-analysis. Arch Intern Med 1999;159:941-955

17. Simmons LR, Molyneaux L, Yue DK and Chua EL. Steroid-Induced Diabetes: Is It Just Unmasking of Type 2 Diabetes?. ISRN Endocrinology. April 2012.

18. Trence DL. Management of patients on chronic glucocorticoid therapy: an endocrine perspective. Prim Care Clin Office Pract. 2003:30;593–605.

19. Santiago AH and Ratzan S. Acute Adrenal Crisis in an Asthmatic Child Treated with Inhaled Fluticasone Proprionate. International Journal of Pediatric Endocrinology Volume 2010, 4 pages.

20. Zollner EW. Hypothalamic-pituitary-adrenal axis suppression in asthmatic children on inhaled corticosteroids (Part 2) – the risk as determined by gold standard adrenal function tests: A systematic review. Pediatr Allergy Immunol 2007: 18: 469–474.

21. Ursulla, C., Maccartner, D., and Pollart, S. American Family Physician 2005 May Vol 71 No 10.

EFFICACY OF FLASH HEAT TREATMENT VS HOLDER PASTEURIZATION ON ANTIMICROBIAL ACTIVITY AND IMMUNOGLOBULIN: A PRESERVATION IN DONOR BREAST MILK

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ABSTRACT

BACKGROUND: Human milk is considered the optimal nutritional source for infants.Due to the possibility of microbial contamination during collection and handling, milk is pasteurized to prevent transmission of pathogens. In low income areas where pasteurization is inaccessible, the pursuit for the best alternative in rendering donor milk safe remains.

OBJECTIVE: We aimed to determine and compare the efficacy of flash heat treatment and holder pasteurization in preserving human milk IgA while reducing bacterial contamination ofdonor breast milk.

METHODOLOGY: This is an experimental study which utilized pooled donor breastmilk from healthy mothers later subjected to bacterial analysis and immunoglobulin A level determination prior to and post flash heat treatment and pasteurization. Standardized scores were used to normalize population with unknown parameters. T-test comparison of means and Levene's test for equality of variances were used.

RESULTS: Twenty samples of aliquoted breastmilk were subjected to pasteurization and flash heat treatment, both yielding a statistically significant reduction in colony forming units using Blood agar and MacConkey plates. These sample groups also underwent IgA level determination using Bindarid Kit IgATM and there was no significant decline in IgA levels.

CONCLUSIONS: Flash heat treatment may be an alternative for holder pasteurization in providing safe and effective breastmilk.

KEYWORDS: Breastmilk, flash heat, immunoglobulin A, antimicrobial, pasteurization

INTRODUCTION

Human milk is considered the optimal nutritional source for infants. The World Health Organization recommends exclusive breastfeeding for the first six months of life. Breast milk is safe and contains antibodies that protect infants from common childhood illnesses. Immunoglobulin A (IgA), present in the colostrum and milk offers passive protection for the gastrointestinal system. When breastfeeding is not possible, pasteurized human donor milk is the best alternative.

Due to the possibility of microbial contamination during collection and handling, milk is pasteurized to prevent transmission of pathogens to the infant. Holder pasteurizationuseslow temperature long time pasteurization (LTLT), rendering the milk at 62.5 C for 30 minutes, by which most pathogenic organisms are inactivated.[1]Flash-heat mimics high-temperature, short-time (HTST) pasteurization, wherein it imitates the intense heat of a fire, until the water reaches 100°C and is at a rolling boil. Milk is immediately removed from water bath and immersed in crushed ice. [2]Since contents of immunoglobulin in milk are known to be thermolabile compounds, heat denaturation has been observed in both pasteurization methods.

We aimed to determine andcompare the efficacy of flash heat treatment and holder pasteurization in preserving human milk IgA with the ability to reduce bacterial

contamination of the donor breast milk.The data gathered from this paper will be developed to support the use of flash heat treatment in low income areas where pasteurization is inaccessible.

If breastfeeding is not possible, pasteurized donor human milk is considered the best alternative. However, human milk is a perfect culture media for microorganisms due to its relatively easy contamination. Microbial contamination can occur during collection and handling of the human milk, which paved the way for pasteurization for the prevention of transmission of pathogens to the infant.

Milk processing in North America follows guidelines set out by the Human Milk Banking Association of North America (HMBANA) [3]. HMBANA guidelines for operation of a donor milk bank uses Holder method of pasteurization for 30 minutes at 62.5°C, then immediately cooled down by immersing in crushed ice. [1,3] Similar guidelines from the Human Milk Banking Association of South Africa suggests the same manner of pasteurization since it inactivates Cytomegalovirus, Human Immunodeficiency Virus, Human Lymphotropic virus and kills most pathogenic bacteria found in breastmilk. [5]

In certain areas which lack facilities for pasteurization, flash heat method may be utilized. The former was described as ''low tech'' method of pasteurization appropriate for home use. [8] Flash-heat mimics high-temperature, short-time (HTST) pasteurization, wherein it imitates the intense heat of a fire, until the water reached 100°C and was at a rolling boil. Milk is immediately removed from the water bath and rapidly cooled by immersing in crushed ice. [2]

Prior to pasteurization of breast milk, it is first stored properly. Fresh raw milk can be kept safely at room temperature (25 ° C) for up to 6 hours. Fresh raw milk can be stored in a refrigerator at 4 ° C for 48 hours. Donor milk should be refrigerated as soon as possible after it has been expressed, this prevents bacteria multiplication and lipolysis. Milk thawed can be frozen immediately and then more added to the same container over 24 hours, provided that the fresh milk is well chilled. Donor milk should be chilled then frozen as soon as possible. Raw donor milk should be stored in a freezer (-18 ℃) for a maximum of 3 months while waiting for pasteurization. At the Milk Bank, human milk must be stored in a separate freezer where the temperature is rigorously controlled. [5]

METHODOLOGY

This was an experimental study using pooled donor breast milk from healthy reproductive aged women.Breast milk samples were obtained by donation of healthy reproductive aged group women. Donors should be mothers who have established lactation and are meeting their own infant's needs and have volunteered to donate surplus breast milk. Donor milk was chilled then frozen in a chest freezer as soon as possible at a temperature of -23 to -18 ℃ for a maximum of 48 hoursbefore it is pasteurized. At the Milk Bank, donor milk wasstored in a separate freezer where the temperature is rigorously controlled.These milk samples were thawed and pooled together. The pooled expressed milk wasequallydivided to make 10 bottles per group, with 50ml of milk sample per bottle. Each sample bottle was randomly designatedinto 2 groups: 10 bottles for the flash heat treatment group, and another 10 bottles for the holder pasteurization. An initial bacteriologic culture (using blood agar and McConkey agar) and Immunoglobulin A (IgA) level determination were done prior to intervention.

Aliquot milk samples, 0.5ml each from the two groups were subjected to microbiologic analysis before and after interventions (flash heat treatment and holder pasteurization). Milk samples will were inoculated on Blood agar and McConkey agar, to facilitate growth of gram positive and gram negative organisms respectively. Samples were stored for at least 48 hours in the incubator and thereafter subjected to colony count.Aliquot of milk samples, 0.005ml (5µl) per well from the different groups were subjected to IgA level determination by radio-immunodiffusion assay (RIA) using Bindarid

Kit IgA™ before and after the interventions (flash heat treatment and holder pasteurization). In the study done by Vergara, identical IgA kits and reagents were used. A liquid control serum and high calibrators were run in each plate to ensure all kits are performing correctly. A 'double fill' of the sample well were done to ensure adequate sampling as the kits are not designed for measuring low concentrations of IgA in breastmilk was but designed to measure serum IgA levels. After an incubation period of 48 hours at room temperature, samples were analyzed. Ring diameters were measured to the nearest 0.1mm using an RID plate reader. Images of ring diameters in each kit were sent verified by the manufacturer as positive diffusion. Measurements of ring diameter corresponds to a specific IgA concentration reported in milligram per liter (mg/L) as seen in Appendix A.Mean difference between the experimental groups were determined.

The remaining aliquot of pooled donor milk (49ml) were placed in a 2oz plastic bottle, sealed, and immersed in a water bath. The water bath, including the 2oz plastic bottle were heated over a butane stove burner to replicate the intense heat of a fire until the temperature reaches 100C.Upon reaching 100C the plastic bottles were immediately removed from the water bath and immersed in crushed ice until the temperature reaches37C. The sample, upon reaching a temperature of 37C, were brought to the laboratory for bacteriologic analysis and IgA level determination.

The remaining aliquot of pooled donor milk (49ml) werepasteurized using the existing method of pasteurization at the Philippine Children's Medical Center milk bank, using the ASTI table top microprocessor pasteurizer. Temperature was set at 62.5C for 30 minutes. After which, the milk samples were immersed in crushed ice. The milk samples were brought to the laboratory for microbiologic analysis and IgA level determination once cooled.

T-test comparison of means for two independent samples were used in comparing the sample groups.Levene's test for equality of variances was used to test if the null hypothesis that population variances are equal or homogenous. Standardized scores were used to normalize population with unknown parameters. This study expresses a confidence interval of 95%.

Results:

	Pre Holder Pasteurization (Raw Milk)	Post Holder Pasteurization
1	3,700 CFU/ml	No growth
2	6,200 CFU/ml	No growth
3	9,800 CFU/ml	No growth
4	8,400 CFU/ml	No growth
5	7,900 CFU/ml	No growth
6	10,400 CFU/ml	No growth
7	6,500 CFU/ml	No growth
8	6,500 CFU/ml	No growth
9	4,200 CFU/ml	No growth
10	600 CFU/ml	No growth

Table 1. Bacteriologic analysis on holder pasteurization group reported in colony forming units per milliliter (CFU/ml).

	Pre Flash Heat (Raw Milk)	Post Flash Heat
1	4,900 CFU/ml	300 CFU/ml
2	6,600 CFU/ml	No growth
3	8,300 CFU/ml	100 CFU/ml
4	11,000 CFU/ml	100 CFU/ml
5	6,800 CFU/ml	300 CFU/ml
6	5,000 CFU/ml	300 CFU/ml
7	1,700 CFU/ml	100 CFU/ml
8	1,700 CFU/ml	300 CFU/ml
9	7,300 CFU/ml	300 CFU/ml
10	900 CFU/ml	300 CFU/ml

Table 2. Bacteriologic analysis on flash heat treatment reported in colony forming units per milliliter (CFU/ml).

Using MacConkey and Blood agar plates, bacteriologic analysis was done. Blood agar plates were used to isolate gram positive organisms which may also be derived from normal bacterial flora, while MacConkey agar plates were used to isolate gram negative microorganisms. Some colonies that grew out of the inoculum were considered as contaminants and were therefore not counted. Tables 1 and 2 showthat in the pretreatmentdetermination (raw milk), there is a significant amount of bacterial growth as compared to post treatment (holder pasteurization and flash heat treatment).

	Raw Milk	Post Holder Pasteurization	Post Flash Heat Treatment
Control	3,930 mg/L	3,930 mg/L	3,930 mg/L
High concentration	9,080 mg/L	9,080 mg/L	9,080 mg/L
1	2,620 mg/L	605 mg/L	605 mg/L
2	605 mg/L	1,510 mg/L	2,620 mg/L
3	1,510 mg/L	1,510 mg/L	605 mg/L
4	1,510 mg/L	2,620 mg/L	605 mg/L
5	1,510 mg/L	1,510 mg/L	2,620 mg/L
6	1,510 mg/L	605 mg/L	1,510 mg/L
7	2,620 mg/L	605 mg/L	1,510 mg/L
8	605 mg/L	5,450 mg/L	1,510 mg/L
9	2,620 mg/L	605 mg/L	605 mg/L
10	605 mg/L	1,510 mg/L	2,620 mg/L

Table 3. Immunoglobulin A level determination in raw milk (pre treatment) and post treatment (holder pasteurization and flash heat treatment) reported in milligram per liter (mg/L).

Immunoglobulin A level measurement was based on ring diffusion in the agarose gel which corresponds to a specific IgA concentration reported in milligram per liter (mg/L). IgA levels showed minimal decrease in mean concentrations. Table 3 shows that IgA levels of raw milk were lower as compared to its respective IgA level concentration post treatment. In this experiment, aliquoted milk samples were used and treated (holder pasteurization and flash heat). Before and after undergoing treatment process, milk samples are re-pooled into a bigger container to ensure homogeneity and thereafter re-distributed to smaller containers for experimentation. Hence *mean* Immunoglobulin A concentrations were determined.

Both holder pasteurization and flash heat treatment, with p-values of 0.00 and 0.001 respectively, have significantly decreased bacterial load therefore suggests that both were effective in decreasing bacterial contamination of breastmilk after each treatment. The

immunoglobulin A concentration post treatment, with p-values of 0.881 and 0.815, for holder pasteurization and flash heat treatment respectively, despite a notable drop in IgA concentrations, are not statistically significant.

Levene's test for equality of varianceswas used to test if the null hypothesis that population variances are equal or homogenous. If the resulting p-value of Levene's test is less than some significance level, the obtained differences in sample variances are unlikely to have occurred based on random sampling from a population with equal variances.[10]Holder pasteurization and flash heat treatment have no significant difference and therefore suggests that both are effectivein decreasing bacterial load on donor breast milk. Both treatment groups are effective in maintaining immunoglobulin A levels.

DISCUSSION

This study aimed to determine whether flash heat treatment is as effective as holder pasteurization in decreasing bacterial load while preserving immunoglobulin A levels in donor breastmilk. It is most beneficial in low income and resource limited areas wherein pasteurization is not available. Young et al advocated the use of flash heat treated milk as an infant feeding option as recommended by the World Health Organization as a strategy to reduce vertical transmission.[12]Flash heat treated breast milk is recommended as temporary feeding strategy during mastitis, or when prophylactic antiretroviral drugs are unavailable.[12]Promoters included successful breastmilk expression, infant health after initiation of flash heat, and inability to pay for milk, while barriers included doubt about the safety or importance of pasteurized breastmilk, and difficulties with expressing milk.

Anotherstudy done by Israel-Ballard et al entitled Bacterial Safety of Flash Heated and Unheated Expressed Breastmilk During Storage, compared bacterial growth between flash heat treated samples against unheated samples during storage at room temperature for 0, 2, 6, and 8 hours. Total colony counts were performed and identified Escherichia coli, Staphylocuccus aureus and Group A and Group B streptococci. Unheated samples had a significantly higherbacterial growth at each time point. It was also evident in the same study that unheated samples had a significantly higher rate of bacterial propagation over time than flash-heated samples. No pathogenic growth was observed in the flash-heated samples, while the unheated samples showed growth of Escherichia coli and Staphylococcus aureus, therefore suggesting that storage of flash-heated breastmilk is safe at room temperature for up to 8hours. [13] In the study of RT Vergara, both flash-heat treated and pasteurized samples showed growth of Pseudomonas aeruginosa, Escherichia coli, Enterobacter and Klebsiella pneumonia prior to treatment. In the same study, there were no growth noted from 0 to 6 hours of incubation. After 8 hours post flash heat treatment, there were notable growth of Enterobacter spp at 100 CFU/ml. After 24 hours of incubation, growth of Diptheroids and coagulase negative Staphylococcus amounted to 100 CFU/ml. After 48 hours of incubation, growth of Enterobacter spp amounted to 100 CFU/ml. For pasteurization group, Diptheroids grew after 24 hours and at 48 hours Enterobacterspp and Diptheroids were noted amounting to 100 CFU/ml.[11]This paper proposes a parallel outcome. As seen on Table 1 and 2, we can observe that prior to treatment, there are approximately 600 – 11,000 colony forming units per ml (CFU/ml) bacterial growth on MacConkey or Blood Agar. Also on the same table, we noted that for the gold standard, holder pasteurization, there were no noted significant growth of bacterial pathogens after treatment. Prior to colony count, the investigators noted very minimal growth of colonies post holder pasteurization on the Blood agar which were away from inoculating site. To verify truepresence of pathogenic organisms, inspection of MacConkey agars were done. The absence of colonies on MacConkey agar signifies absence of gram negative organisms and therefore are considered contaminants only. Bacteriologic analysis of donor milk post flash heat treatment group showed a significant decrease in bacterial count.

A similar study by RT Vergara mentioned earlier in this study, concluded that flash heat treatment is capable of rendering breast milk bacteriologically safe, therefore suggesting that it can be used as an alternative to holder pasteurization. [11] Immunoglobulin A levels werealso measured but were undetected due to adaptation in measurement. The IgA kits were not specifically designed for breastmilk. In that said study, storage of milk ranged from 3 – 6 months prior to experimentation. The investigators in this studyparticularly used freshly collected expressed breast milk samples stored up to 48 hours only. Undetectable levels of immunoglobulin A may be attributed to prolonged storage. It may also be indicative of poor sample application technique or moving the plates too quickly before the samples have diffused in properly. Sample application was done differently in this study. Secretory IgA are noted to be very high in colostrum for the first few days, and decline rapidly. Double filling of sample wells was done as the levels of IgA in breast milk can be estimated as low as approximately 364mg/L on the first five days and 142 mg/L from 30 days and beyond.[14] The measuring range of the IgA kit used is 545 – 5450 mg/L,therefore asingle fill of the sample rings, could be inadequate in detecting IgA levels.Chantry et al also suggested that our "low tech" version of pasteurization, namely flash heat treatment did not essentially denature immunoglobulins as compared with the gold standard, holder pasteurization.[2]One of theirobjectives was to evaluate the effects of each treatment on the concentrations of breast milk IgA. In the 20 samples analyzed, as shown in Table 3, both holder pasteurization and flash heat treatment group induced no statistically significant decrease in IgA concentration. These results suggest that both heat treated milk samples would still contain considerable passive protection.

CONCLUSION

Flash heat treatment is a simple, "low tech" method which may be comparable to the gold standard, holder pasteurization in decreasing microbial contamination of donor breast milk while preserving essential immunoglobulins such as immunoglobulin A. This study was successful despite using IgA kits not specifically designed for breast milk. Better immunoglobulin A level determination will result from breastmilk specific IgA kits. Prolonged storage of milk may result in significant reduction of immunoglobulin A levels.

Thus, flash heat treatment may be utilized in far flung areas and low income communities as alternative for holder pasteurization in providing safe and effective breast milk. Werecommend future researchers to use immunoglobulin A specific kit in measuring IgA levels. Future researches can also use colostrum for in measuring immunoglobulin A levels. Studies can be done comparing bacterial growth and IgA concentrations in freshly collected milk, stored milk, and frozen milk in different periods of time.

BIBLIOGRAPHY:

1. Human Milk Banking Association of North America. Guidelines for the Establishment and Operation of a Donor Human Milk Bank. 2013.

2. Chantry CJ, Israel-Ballard K, Moldoveanu Z, Peerson J, Coustoudis A, Sibeko L, et al. Effect of Flash Heat Treatment on Immunoglobulins in Breast Milk. J Acquir Immune DeficSyndr 2009; 51:264-267.

3. Kim JH, Unger S, Canadian Paediatric Society, Nutrition and Gastroenterology Committee. Human Milk Banking. 2010; 15(9): 595-598.

4. Jenness R. The composition of human milk. 1979 July; 3(3): 225-239.

5. Human Milk Banking Association of South Africa. Guidelines for the Operation of a Donor Human Milk Bank in South Africa. 2008.

6. Israel-Ballard KA, Abrams B, Coutsoudis A, Sibeko LN, Cheryk LA, Chantry CJ. Vitamin content of Breast milk from HIV-1 infected mothers before and after flash-heat treatment. J Acquir Immune DeficSyndr. 2008 August 1; 48(4): 444-449.

7. American Academy of Pediatrics Policy Statement. Breastfeeding and the use of human milk. Pediatrics 2005: 115; 146.

8. Chantry CJ, Wiedeman J, Buehring G, Peerson JM, HayfronKweku, L'Aluoch O, et al. Effect of Flash Heat on Antimicrobial Activity of Breastmilk. Breastfeeding Medicine. Volume 6, Number 3, 2011.

9. Terpstra F, Rechtman D, et al. Antimicrobial and Antiviral Effect of High Temperature Short Time (HTST) Pasteurization Applied to Human Milk Bank. Breastfeeding Medicine. Volume 2, Number1, 2007.

10. https://en.wikipedia.org/wiki/Levene%27s_test

11. Vergara R, Ramirez G. Bacteriologic Safety of Flash Heat Treated Breast Milk. December 2012.

12. Young, S. Leshabari, S et al. Barriers and Promoters of Home-Based Pasteurization of Breastmilk Among HIV-Infected Mothers in Greater Dar esSalaam,Tanzania. Breastfeeding Medicine. Volume 8, Number 3, 2013.

13. Israel-Ballard, K. Coutsoudis, A et al. Bacterial Safety of Flash Heated and Unheated Expressed Breastmilk during Storage. Journal of Tropical Pediatrics. December 2006.

14. Lawrence, R. Lawrence R. Breastfeeding: A Guide for the Medical Profession. 6th edition.1999.

CLINICO-RADIOLOGIC, LABORATORY, AND HISTOPATHOLOGIC PROFILE OF PATIENTS DIAGNOSED WITH NEONATAL CHOLESTASIS AT PHILIPPINE CHILDREN'S MEDICAL CENTER

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ABSTRACT

BACKGROUND: Neonatal Cholestasiswarrants early, accurate and prompt intervention and comprises a wide spectrum of differential diagnosis which present with overlapping features, thus making a diagnosis difficult.

OBJECTIVE: To evaluate the clinical and laboratory parameters that could aid to differentiate between intrahepatic and extrahepatic neonatal cholestasis.

METHODS: Retrospective and Descriptive study of Neonatal Cholestasis patients who underwent Liver Biopsy and admitted at the Philippine Children's Medical Center from January 2007 to December 2011.

RESULTS: Factors that favor an intrahepatic cause of Cholestasis are ultrasound finding of a normal gallbladder, marked degree of giant cell transformation and presence of extramedullary hematopoiesis. Factors that favor an Extrahepatic cause of Cholestasis are presence of Splenomegaly, markedly elevated GGT, and histopathology findings of Portal and Periportal Ductal proliferation, bile plugs, lesser degree of giant cell transformation, septal fibrosis and cirrhosis, portal and neoductular cholestasis, and Portal-Portal bridges.

CONCLUSION: In this study, meticulous history and physical examination aid in the diagnosis of Neonatal Cholestasis. Splenomegaly and markedly elevated serum GGT are suggestive of Biliary Atresia, and a normal Gallbladder by Ultrasound favors Neonatal Hepatitis. Although there is significant overlap of histopathologic findings of patients with neonatal cholestasis, certain parameters favor an extrahepatic over an intrahepatic process.

KEYWORDS: Clinical and Histopathologic features, Extrahepatic Cholestasis, Intrahepatic Cholestasis, Neonatal Hepatitis, Biliary Atresia.

INTRODUCTION

Neonatal cholestatic jaundice is defined as jaundice with elevated conjugated bilirubin due to an underlying hepatobiliary dysfunction. This usually occurs during the first month of life and persists for more than 10 days, and may present with choluria, hypocholic or acholic stools, hepatomegaly, and/or splenomegaly. It is the most common liver disease in infancy and affects about 1 in every 2500-5000 live newborns [3]. Neonatal cholestasis can be due to extrahepatic causes such as biliary atresia, common bile duct obstruction, choledochal cyst with biliary sludge, and an inspissated bile/mucous plug. Intrahepatic causes include bacterial and viral infections, metabolic diseases, genetic diseases, toxic, vascular, immune and idiopathic intrahepatic bile duct paucity, cirrhosis, total parenteral nutrition, drugs, and idiopathic neonatal hepatitis[3].A delay in the diagnosis of Neonatal Cholestasis may cause irreversible damage to the liver leading to end stage liver failure or cirrhosis [2]. Approach to management differs for each etiology. Intrahepatic cholestasis is managed mostly by medical intervention alone, while extrahepatic cholestasis, particularly Biliary Atresia, warrants an early surgical intervention or Kasai procedure at not more than 8 weeks of life for a favorable outcome[3]. To make an accurate and definitive diagnosis, numerous laboratory tests, radiologic work-ups, and liver biopsy are necessary.

However, due to a significant overlap in clinical and histopathologic presentation, differentiating neonatal hepatitis from biliary atresia is difficult especially in the early stages of the disease. A delay in the diagnosis would mean a delay in the management, which is very crucial particularly in cases of biliary atresia.

Numerous studies have tried to differentiate intrahepatic cholestasis from extrahepatic cholestasis using clinical and laboratory parameters and histopathologic variables.In Brazil, Brandao and colleagues evaluated 168 patients, using both clinical and laboratory parameters to differentiate extrahepatic causes from intrahepatic causes. In these study, birth weight and lengths, fecal hypocholia/acholia, hepatomegaly, and a 10.8 times increase in GGT were the only variables that would point to extrahepatic cholestasis. Over-all, both clinical and laboratory parameters have failed to identify the etiology of neonatal cholestasis.

In another study by Santos and colleagues, 46 liver biopsy specimens were evaluated using a discriminant analysis test. In the discrimination between intrahepatic cholestasis from extrahepatic cholestasis, the following histopathological variables were found to be most significant in decreasing order of the coefficient value of the canonical discriminant function: periportal ductal proliferation, portal ductal proliferation, portal expansion, cholestasis in neoductules, portal cholestasis, foci of myeloid metaplasia, portal-portal bridges, focal necrosis, cholestasis in canaliculi, periductal fibrosis and portal-central bridges. Of these, only foci of myeloid metaplasia would indicate intrahepatic cholestasis.

In a study done by Alagille and colleagues, histopathologic features that would indicate intrahepatic cholestasis are lobular disarray, giant cell proliferation, and hepatocellular necrosis, and minimal fibrosis, rare formation of neoductules, steatosis, and extramedullary hematopoiesis. Conversely, data that would indicate Extrahepatic cholestasis include neoductule proliferation, portal and perilobular fibrosis, bile plugs, normal lobular architecture, and little inflammatory response[3].

Lee and colleagues studied 102 infants with neonatal cholestasis using clinical manifestations, laboratory data and histopathologic features as parameters. Of these, 66 were biliary atresia cases, 21 were neonatal hepatitis cases and 15 were cases of intrahepatic bile duct paucity. The outcome of the study showed bile ductular proliferation, bile duct loss, and advanced fibrosis as useful parameters for the differential diagnosis of neonatal cholestasis. Giant cell transformation, ballooning degeneration, lobular disarray, portal inflammation and extramedullary hematopoiesis are among the features of neonatal hepatitis; however, these are also seen in other disease entities, even in biliary atresia. GGT concentration of more than 300IU/L had a diagnostic accuracy of 85% for biliary atresia in patients less than 10 weeks old. In this study, GGT was the only reliable laboratory marker for making the diagnosis of biliary atresia.

Urganci and colleagues did a retrospective study of 70 infants diagnosed with cholestasis, with ages ranging from 15 days to 8 months, using clinical and laboratory parameters. This study showed that Biliary Atresia has an earlier onset of jaundice and acholic stools. Furthermore, total bilirubin levels, GGT and ALP 0were remarkably higher than in the groups with intrahepatic biliary hypoplasia or hepatocellular disease. They emphasized the role of scintigraphy in the diagnosis of biliary atresia as compared to other groups.

Lee and colleagues, in their prospective observational study of 146 patients with neonatal cholestasis, reported that the two most common causes of neonatal cholestasis were Biliary Atresia and Idiopathic Neonatal Hepatitis, accounting for 29% and 38% respectively. Thirty-nine patients died at the time of study, 35 of which succumbed to end-stage liver disease and 4 expired after liver transplant. Six of the 107 survivors had liver cirrhosis. The overall four year survival rate for patients with neonatal cholestasis with and without transplant were 73% and 72% respectively, while in patients with biliary atresia, the four year survival rate with and without liver transplant were 36% and 38% respectively.

This study aims to identify and determine the frequency of the different etiologies causing neonatal cholestasis among patients who underwent Liver Biopsy. Clinical presentation, Laboratory data and Histopathologic features will

be assessed to classify neonatal cholestasis in two groups, intrahepatic and extrahepatic. Knowing which among the parameters would favor a diagnosis on each group will help both the clinicians and pathologists make a prompt definite diagnosis.

METHODOLOGY

This is a retrospective descriptive study of patients who underwent liver biopsy at the Philippine Children's Medical Center from January 2007 to December 2011 and diagnosed as a case of Neonatal Cholestasis. Diagnosis was based on the Final Histopathologic Diagnosis and Explorative Laparotomy Findings. According to the diagnosis, the patients were classified into two groups: Group 1 or the Intrahepatic group; and Group 2 or the Extrahepatic group. Pertinent clinical, radiologic and laboratory data were gathered. Histopathology slides were reviewed and certain histopathologic parameters were reevaluated and reassessed by a pathologist based on the presence and/or severity of these parameters. Chosen Clinical, Laboratory Data and Histopathologic parameters from each group were assessed to determine which among the parameters would differentiate both groups.

All patients clinically diagnosed with Neonatal Cholestasis who underwent liver biopsy at Philippine Children's Medical Center from January 2007 to December 2011 were the subjects of the study. The Hematoxylin and Eosin slides, Immunohistochemistry (IHC) slides with Cytokeratin and Histopathology results of each patient were gathered. IHC with Cytokeratin were done on cases where the portal tracts are obscured by inflammation. Inpatient and Outpatients charts were also reviewed. Patients were divided into two groups, under Group 1 are patients classified under Intrahepatic Cholestasis and Group 2 are patients classified under Extrahepatic Cholestasis. The slides were reevaluated by an anatomic pathologist based on the following histopathologic parameters: bile duct proliferation, site of cholestasis (portal, neoductules, canalicular and intrahepatic), fibrosis (absent, portal, periportal, septal and cirrhosis), necrosis, ballooning degeneration, giant cell transformation, portal and lobular inflammation, and extramedullary hematopoiesis. The degree of lobular inflammation and giant cell transformation were graded based on the severity (absent to mild or moderate to severe). The pathologist who reevaluated the slides was blinded of the official result. For the clinical data, the patients age, gender, onset of jaundice, birthweight, absence and presence of pertinent signs and symptoms such as hypocholia or acholia, dark urine, hepatomegaly, splenomegaly and ascites noted on each patient were tabulated for each group. Pretreatment Clinical Laboratory Test results of AST, ALT, Total Bilirubin, Conjugated Bilirubin, ALP, GGT, INR, and Albumin were gathered for each patient and tabulated for each group. Radiologic findings of each patient were considered. Ultrasound Results and Hepatobiliary iminodiacetic scan (HIDA) results if done on the patient were noted and compared to the final result. All Clinical data, Laboratory Results, Histopathologic and Radiologic findings gathered were used as parameters differentiating extrahepatic cholestasis and intrahepatic cholestasis.

The clinical parameters, laboratory, and radiologic results were reviewed using the patient's inpatient and outpatient charts. Clinical data not indicated in the history and physical examination findings were excluded in the analysis. Likewise, laboratory results, ultrasound results, and other ancillary findings either not done or were missing from the charts were also excluded in the analysis. Therefore, the total number of patients evaluated in this study varied for each clinical, radiologic, and laboratory parameter considered.

RESULTS

Table 1. Patient Clinical Characteristic, according to group

Variable	N –px with available data	Intrahepatic (n=32)	Extrahepatic (n=39)	P	Interpretation
Gender	71				
Male		17	24	0.475	Not Significant
Female		15	15		
Age of Patient at time of biopsy (months)	71	2 (2-3)	2 (2-3)	0.450	Not Significant
Onset of Jaundice	52				
Within 24 hours after birth		3	5	0.648	Not Significant
1-2 days of Life		2	2	1.000	Not Significant
3-7 days of Life		6	12	0.247	Not Significant
1 to 4 weeks of life		8	14	0.323	Not Significant
More than 1 month of life		4	4	1.000	Not Significant
Birthweight (g)	17				
<2500 g		2	0	0.113	Not Significant
>2500 g		8	7		
Pale/Acholicstools	54				
Absent		9	3	0.469	Not Significant
Present		15	27		
Dark Urine	53				
Absent		7	5	0.057	Not Significant
Present		16	25		
Hepatomegaly	63				
Absent		15	10	0.231	Not Significant
Present		12	25		
Splenomegaly	62				
Absent		19	15	0.026	Significant
Present		5	11		
Ascites	51				
Absent		19	30	0.207	Not Significant
Present		2	2		

Table 1 shows that there are no significant differences on Patient Clinical Characteristic in terms of Gender, Onset of Jaundice, Birthweight, Pale/Acholic, Dark Urine, Hepatomegaly and Ascites. On the other hand, a significant difference between group 1 and 2 was observed with splenomegaly, a finding present in Biliary Atresia.

Table 2. Laboratory Test Results at the beginning of investigation, expressed as the median (interquartile range)

Variable	N	Intrahepatic	Extrahepatic	P	Interpretation
ALT IU/L	42	(n=19) 228 (176.5-542)	(n=23) 201 (163-658)	0.144	Not Significant
AST IU/L	29	(n=13) 331 (284-718)	(n-16) 270.5 (190.25-850)	0.448	Not Significant
ALP IU/L	30	(n=15) 598 (403.6-1412)	(n=15) 678 (512.28-1110)	0.412	Not Significant
GGT IU/L	8	(n=5)308 (296-679)	(n=3)882 (517.5-1054)	0.043	Significant
Albumin (g/dl)	17	(n=8) 29 (26.25-41)	(n=9) 32 (27-49)	0.606	Not Significant
Globulin (g/dl)	17	(n=8) 31 (26-38)	(n=9) 28 (23-53)	0.815	Not Significant
INR	55	(n=21) 1.05 (1.01-1.8)	(n=34) 1.01 (0.96-1.86)	0.052	Not Significant
TBIL (mg/dl)	44	(n=20) 205.81 (143.5-315)	(n=34) 186 (144-476.79)	0.075	Not Significant
DBIL (mg/dl)	44	(n=20) 159.335 (112.5-282)	(n=24) 148 (110.5-356.63)	0.944	Not Significant
Total Protein (g/dl)	16	(n=8) 64 (53.75-72)	(n=9) 61 (54-85)	0.956	Not Significant

Analysis of Table 2 shows that there is no significant differences between Group 1 and 2, except for GGT, being consistently elevated in Group 2.

Table 3. Histopathologic Features of Neonatal Cholestasis

Variable	Intrahepatic (n=32)	Extrahepatic (n=39)	P	Interpretation
Portal Ductal Proliferation				
Absent	31	5	0.000	Significant
Present	1	34		
Periportal Ductal Proliferation				
Absent	31	5	0.000	Significant
Present	1	34		
Bile Plugs				
Absent	29	11	0.029	Significant
Present	3	28		
Lobular Inflammation				
Absent to Mild	5	22	0.394	Not Significant
Moderate to Severe	27	17		
Giant Cell Transformation				
Absent to Mild	4	14	0.029	Significant
Moderate to Severe	28	25		
Fibrosis				
Absent	17	5	0.001	Significant

Portal	8	5	0.200	Not Significant
Periportal	5	7	0.205	Not Significant
Septal	1	5	0.000	Significant
Cirrhosis	1	17	0.000	Significant
Cholestasis				
Portal				
Absent	21	13	0.001	Significant
Present	11	26		
Neoductules				
Absent	28	13	0.000	Significant
Present	4	26		
Canalicular				
Absent	4	2	0.153	Not Significant
Present	28	37		
Intrahepatic				
Absent	1	2	0.065	Not Significant
Present	31	37		
Portal-Portal Bridges				
Absent	25	8	0.047	Significant
Present	7	31		
Portal Inflamation				
Absent	0	0	0.239	Not Significant
Present	32	39		
Extramedullary Hematopoiesis				
Absent	17	30	0.010	Significant
Present	15	9		
Ballooning Degeneration				
Absent	0	0	0.389	Not Significant
Present	32	39		
Necrosis				
Absent	20	20	0.477	Not Significant
Present/Focal	12	19		

Table 3 depicts the histopathologic parameters considered in adjudicating neonatal cholestasis. There was no significant difference between two groups on these parameters: Lobular Inflammation, Portal and Peri-portal fibrosis, Canalicular and Intrahepatic Cholestasis, Portal Inflammation, Ballooning Degeneration and Necrosis. On the other hand, significant differences between Group 1 and 2 were observed on these parameters: Presence of

bile plugs, Portal Ductal Proliferation, Periportal Ductal Proliferation, Giant Cell Transformation, Absence of fibrosis, Septal FIbrosis and Cirrhosis, Portal and Neoductular Cholestasis, Portal-Portal Bridges and extramedullary hematopoiesis.

For ultrasound and HIDA scan findings, only the presence of a normal gall bladder for intrahepatic cholestasis achieved statistical significance.

Overall, 81 patients underwent liver biopsy with a clinical diagnosis of Neonatal Cholestasis. Of these, only 71 were included in the study, because 5 of the cases have no slides and block to retrieve for review (these were probably borrowed and were not returned), while the remaining 5 have absent or scanty portal tracts to be adequately evaluated.

Seventy – one cases included in the study were finally grouped into two. Thirty-two cases (45%) were classified as Group 1 or intrahepatic group, and 39 cases (55%) as Group 2 or extrahepatic group. The etiologies of hepatitis for group 1 are Cytomegalovirus Hepatitis(6 cases, 16%), Herpes Simplex II Hepatitis(1 case, 3%), and Unidentified or Possibly Idiopathic (25 cases, 81%). All viral-induced Hepatitis for group 1 cases was diagnosed based on positivity on TORCH IgM. For the etiologies of Cholestasis for Group 2, these are Biliary Atresia (33 cases, 85%), Inspissated Bile/Bile Sludge (4 cases,10%), Choledochal Cyst (1 case,2.5%) and Common Bile Duct Stone (1 case, 2.5%).

Final Diagnosis was based on Intraoperative Cholangiography findings, Hepatobiliary exploration findings, and Histopathology findings. In our study, 12 biliary atresia cases and all Inspissated Bile/Bile Sludge cases were confirmed by Intraoperative Cholangiography.The case of Choledochal Cyst and Common Bile duct obstruction were confirmed by surgical exploration. The 21 cases of Biliary atresia were confirmed by Histopathology findings. Six cases of biliary atresia are Cytomegalovirus-associated as confirmed by Urine CMV Culture(2 cases) and TORCH IgM(4 cases). One patient included in Group 2 is a case of Trisomy 21(Down Syndrome). Three postmortem liver biopsy were included in the study, two belonging to Group 2 with final diagnosis of Extrahepatic Biliary Atresia with cirrhosis and Extrahepatic Biliary Atresia with Cirrhosis with Cytomegalovirus Hepatitis(IgM Positive) and one belonging to Group 1 with final diagnosis of Hepatitis with Cirrhosis.

In this study, we evaluated 25 cases of core biopsy and 7 cases of wedge biopsy for group 1. For Group 2, there were 21 cases of core biopsy, 12 cases of wedge biopsy, and 6 cases with both core and wedge biopsy. No difference was noted on cases having both core and wedge biopsy on evaluation, hence the histologic findings were considered as one.

Review of the histopathology reports showed that 3 of the 32 cases of group 1 would need additional Cytokeratin IHC for confirmation of the diagnosis, and 16 of 39 cases of group 2 would need Cytokeratin for confirmation of diagnosis. IHC with Cytokeratin were all done on these cases and were evaluated with the H&E slides. Comparing the provisional diagnosis without Cytokeratin with the final diagnosis with Cytokeratin, only 2 of 19 cases in which Cytokeratin was requested resulted in revision of diagnosis, and these cases were initially diagnosed in favor of Hepatitis but after doing Cytokeratin the final diagnosis showed Bile Duct proliferation consistent with a diagnosis of Biliary Atresia. For these two cases, the proliferating bile ducts were obscured by severe portal and periportal inflammation.

Included in the study are 41 males (58%) and 30 females (42%) patients. There were 17 males and 15 females comprising 53 % and 47% of group 1 respectively, and 24 males and 14 females comprising 61% and 39% of group 2 respectively. No significant differences were noted as to sex distribution in both groups. We also found no significant differences on the age of patient at time of biopsy. All presented with jaundice and most presented within 3 to 7 days of life and one to four weeks of life. Almost all patients have birthweight >2500

grams. While most patients presented with pale/acholic stools (78%), choluria (77%), and hepatomegaly (59%), no significant differences were noted on these 3 parameters. Few patients presented with splenomegaly(26%) and ascites(7%). Of these two parameters, a significant difference is noted among patients presenting with splenomegaly which is more likely found on patients with Biliary Atresia. No significant difference was obtained on patients presenting with ascites on both groups.

On all the Laboratory parameters evaluated, using the median of all the laboratory results per parameter, Only GGT showed significant differences between the two groups.(Table 2)

The histopathologic features for each group are summarized in Table 3. The characteristic features belonging to Group 1 include absence of fibrosis which is noted on 53% of patients in Group 1 but only 13% of patients in Group 2, and presence of Extramedullary Hematopoiesis which is seen in 47% of patients belonging to group 1 but only in 23% of patients belonging to Group 2. Also quite prominent is the degree of giant cell transformation in which moderate to severe giant cell transformation is seen in 88% of group 1 and only in 64% of patients in group 2 while absent to mild giant cell transformation is noted only in 12 % of group 1 and 36% of group 2. Thus, a significant difference is noted on both groups with regards to the degree of giant cell transformation.

The characteristic histopathology features of patients belonging in group 2 includes: portal and periportal ductal proliferation which are seen in 87% of patients in group 2 and only in 3% of patients in group 1; presence of bile plugs which are seen in 72% of patients belonging to group 2 and only in 9% of patient in Group 1; presence of septal fibrosis seen in 13% of patients in group 2 and only in 3% of patients in group 1; presence of cirrhosis which is seen in 44% of patients in group 2 and only in 3% of patient in group 1, presence of Cholestasis in the portal tract seen in 67% of patients in group 2 and only in 33% of patients in group 1; presence of cholestasis in the neoductules seen in 67% of patients in group 2 and only in 13% of patients in group 1; and, lastly, presence of portal-portal bridges which is seen in 79% of patients in Group 2 and only in 29% of patient in group 1. Other histopathologic variables evaluated but were found not significant are Degree of lobular Inflammation, Portal and Periportal Fibrosis, Canalicular and Intrahepatic Cholestasis, portal inflammation , ballooning degeneration, and necrosis.

Only 28 Ultrasound results were present on the patient's charts, 15 for group 1 and 13 for group 2. Results show that the finding of a normal gallbladder predominated group 1 while finding of atretic/small gallbladder is more common in Group 2. Findings of an absent or nonvisualized gallbladder and contracted gallbladder do not significantly differ on both groups.

Intraoperative Cholangiography (IOC) is the gold standard in the diagnosis of biliary atresia. Of the 23 cases with IOC, 12 had findings of Biliary Atresia, 4 had Inspissated Bile/ Bile Sludge, and 7 had diagnosis of Cholestasis with negative exploration. Eleven cases of Biliary Atresia had similar IOC findings with histopathology results, however 2 cases have differing diagnosis. Of these two cases, one case has an IOC finding of Biliary Atresia while the Liver Biopsy finding showed Neonatal Hepatitis and for the second case IOC showed negative exploration while the liver biopsy showed findings consistent with Biliary Atresia. On review of both cases, we found consistent findings with the histopathology report. These could happen for several reasons as follows: ductular reaction may occur in patients with Neonatal Hepatitis which may not seem conspicuous in a core biopsy specimen, in early cases of biliary atresia bile duct proliferation is not prominent, thus a repeat biopsy is recommended, and some diseases may mimic biliary atresia histologically like Neonatal Sclerosing Cholangitis, CMV Hepatitis, alpha-1 antitrypsin deficiency and Total Parenteral nutrition[10]. Four cases have IOC findings of Inspissated bile/Bile Sludge but a diagnosis of Neonatal Hepatitis on Histopathology report.

Histopathologic changes in inspissated bile is nonspecific and can mimic changes consistent with Neonatal Hepatitis thus it cannot be diagnose by Liver Biopsy. Clinical Correlation is warranted in such cases. Comparing the Liver Biopsy findings among cases with IOC findings of Biliary Atresia, Liver Biopsy yielded sensitivity of 92%, specificity of 86%, positive predictive value of 92%, negative predictive value of 86%, and accuracy of 89%.

DISCUSSION

The focus of initial approach in this study is to differentiate between Intrahepatic Cause and Extrahepatic Cause, since an Extrahepatic cause of obstruction would warrant surgical intervention while only medical intervention is needed for the Intrahepatic group. Idiopathic Neonatal Hepatitis represents 15% of patients presenting with Neonatal cholestasis[9]. It is diagnosed after a thorough history, physical examination and laboratory evaluation fail to identify an underlying cause of Neonatal Hepatitis[5]. Cytomegalovirus Hepatitis is second to idiopathic neonatal Hepatitis, representing 3-5% of Neonatal Hepatitis.

Among the extrahepatic causes of cholestasis, Biliary Atresia represents 25 to 30% of patients with neonatal cholestasis[9]. Biliary atresia is the most important cause of severe neonatal liver disease and the major indication for liver transplant, since Intrahepatic Bile Duct Damage continues to occur which leads to loss of intrahepatic bile ducts and recurrent Cholestasis due to Bile Duct Paucity even after a successful Kasai procedure [10].

Diagnosis of Cholestasis is a matter of urgency since biliary atresia is a common cause of Cholestasis. The goal of management is to complete the diagnostic evaluation, or at least exclude biliary atresia by 45 to 60 days of life [7]. The prognosis for a successful Kasai or portoenterostomy procedure depends primarily on operation before 60 days of age and absence of cholangitis[9]. CMV infection in combination with a genetic predisposition may play a role in the development of perinatal pattern of biliary atresia. In our studywe found 6 out of 33 cases of Biliary Atresia positive for CMV as confirmed by serology and Urine CMV. One of the six cases is a postmortem liver biopsy which may entail worse prognosis as compared to biliary atresia alone. This is similar to the study done by Tarr and colleagues, wherein 5 of 23 patients with Biliary Atresia had evidence of CMV hepatitis based on serology, culture and histopathological evidence[9].

In our study idiopathic neonatal hepatitis and biliary atresia accounted for 46% and 37% respectively of Neonatal Cholestasis among patients who underwent Liver Biopsy. This is almost similar to studies done in Brazil [2] and Malaysia [7] in which both diseases accounted for 54% and 67% of cases of Neonatal Cholestasis respectively.

Cytomegalovirus Hepatitis is the most common perinatal infection seen in 40 to 50% of infants delivered to mothers with primary CMV. Six out of 32(16%) cases in group 1 are positive for CMV IgM. Liver diseases range from mild portal inflammation to severe giant cell hepatitis to cirrhosis. Another congenital infection seen in one case in group 1 (3%) is Herpes Simplex Virus type 2, a virus responsible for almost 80% of perinatal infections. Disseminated infection which results in fulminant hepatic failure occur in approximately 20% of newborns. Hepatocyte necrosis devoid of inflammatory cells and viral inclusions are the microscopic features of this entity[9].

Inspissated bile syndrome(IBS) is obstructive jaundice caused by intraluminal bile plugs, sludge or gallstones and is uncommon in infancy. In a study by Redkar and colleagues, possible predisposing factors include gastrointestinal pathology, prematurity, total parenteral nutrition, sepsis, and other miscellaneous causes like IUGR, birth asphyxia, and hemolysis. Biliary ultrasound was the most useful primary investigation and diagnostic tool. Inspissated bile and dilated biliary tree are common findings. Underlying structural anomalies of the bile ducts identified in the same study by Redkar were choledochal malformation, stricture, abnormal ductal anatomy, and a long common channel. Thus,

Inspissated bile syndrome is a cause of surgical jaundice in this age group and is of heterogenous etiology. Majority will require intervention, either radiological or surgical, but with excellent long-term outcome[8].

One cause of neonatal cholestasis is Choledochal Cyst, representing 2.5% of the extrahepatic causes of cholestasis in the study. Choledochal cyst is a segmental dilatation of the biliary ductal system. It is usually diagnosed by ultrasound, however in neonates it is important to distinguish this lesion from biliary atresia.

Another cause of cholestasis in our study is obstruction by common bile duct stones, and it represents 2.5% of extrahepatic causes of cholestasis. It is very rare in neonates and infants and occurs only in approximately 0.13% to 0.22% in ultrasound-based studies[9]. It can be associated with prematurity, inspissated bile/stasis, correctable biliary atresia, and choledochal cyst.

Among the clinical parameters evaluated, only the finding of splenomegaly would favor a diagnosis of an extrahepatic cause of cholestasis. This could be very well correlated with the presence of cirrhosis predominantly seen in the same group causing increase in portal pressure secondary to blockage of blood flow in the portal vein and obstruction of bile flow. Splenomegaly represents a late sequelae of a disease particularly in Biliary Atresia. This would indicate either the severity of obstruction, late presentation of the disease and/or possible delay in arriving at the diagnosis.

Laboratory parameters evaluated in this study include: Bilirubin, which is a test for metabolic function of the liver; albumin and INR, which are tests for the synthetic function of the liver; AST and ALT, which are tests for liver injury; and ALP and gamma glutamyl transferase (GGT), which are tests for canalicular injury. In this study, high GGT levels would favor a diagnosis of Biliary Atresia. GGT has 78-86% sensitivity and 67-100% specificity for obstruction. GGT has been used in the past to distinguish biliary atresia from neonatal hepatitis; however a wide variability in levels makes interpretation difficult[5]. Normal values of GGT varies with age, gender and diagnostic methods. It is recommended that GGT results be expressed as the number of times the upper limit of normaland a cut-off value be set to differentiate Biliary Atresia from other causes of Cholestasis[2].

Giant Cell transformation of hepatocytes is seen in 15% of Extrahepatic Biliary Atresia and may occasionally be prominent [10]. In this study, a less degree of giant cell transformation is noted in favor of Biliary Atresiaand a marked degree of Giant Cell transformation favors Neonatal Hepatitis. Our findings are Similar to the study by Santos and Alagille which include extramedullary hematopoiesis among the variables that favor an intrahepatic cause of cholestasis.

In our study, portal and periportal Ductal Proliferation were findings indicating an extrahepatic cause of cholestasis as also demonstrated by Brough and Bernstein in 1974 and Santos and colleagues in 1998. There are two conflicting studies on the findings of cholestasis in Neoductules; Brough and Bernstein considered it as a non specific finding, while Shiraki and colleagues considered it as the most specific discriminatory element. Similar to the findings of Santos, which showed that Portal Cholestasis and Cholestasis in Neoductules are directly correlated and are discriminatory variables indicating an extrahepatic cause of cholestasis. Portal-Portal bridges or fibrosing piecemeal necrosis is a reversible phenomenon; in our study, similar to that of Zerbini and Santos, we found this variable as indicative of an extrahepatic cause of cholestasis[3]. Similar to the study by Lee and colleagues, the occurrence of septal fibrosis and cirrhosis were significantly greater in patients with Extrahepatic Cholestasis[1].

Ultrasonography is use to identify anatomic abnormalities such as choledochal cyst. Findings of a small or absent gallbladder may suggest extrahepatic biliary atresia but with low sensitivity of 73%. Combination of three gallbladder features (gallbladder ghost triad) namelylength less than 19 mm,irregular wall,

and an indistinct mucosal lining for experienced operators yielded >90% sensitivities and specificities[11].No data regarding these findings were noted on the results evaluated. In our study, only the finding of a normal gallbladder is significant in differentiating intrahepatic from extrahepatic cholestasis. Presence of triangular cord sign on ultrasound has been considered a significant diagnostic feature of biliary atresia with high sensitivity and specificity[7]. Triangular cord sign is a focal area of increased echogenicity anterior to the bifurcation of the portal vein representing the fibrotic remnant of the extrahepatic biliary tree[11]. However, these appears to be operator dependent since in our study none of the ultrasound results of patients with Biliary Atresia have findings of triangular cord sign. We emphasized that multiple ultrasound parameters should be analyzed to have an accurate diagnosis of biliary atresia.

CONCLUSION AND RECOMMENDATION

Biliary Atresia and Idiopathic Neonatal Hepatitis represent the majority of the causes of Neonatal Cholestasis comprising 46% and 37% respectively.

Findings of splenomegaly and markedly elevated GGT favor an extrahepatic cause of cholestasis while ultrasound finding of a normal gallbladder favors an intrahepatic cause of cholestasis.

Liver biopsy is essential in making an accurate diagnosis with a high Sensitivity (92%), Specificity (86%) and Accuracy (89%). We recommend percutaneous Liver Biopsy to all patients with cholestasis particularly in cases with diagnostic uncertainty and even in CMV positive cases.

Histopathology findings of Portal and Periportal Ductal proliferation, bile plugs, lesser degree of giant cell transformation, septal fibrosis and cirrhosis, portal and neoductular cholestasis, and Portal-Portal bridges indicate an extrahepatic cause of cholestasis. A marked degree of giant cell transformation and presence of extramedullary hematopoiesis favor an intrahepatic cause of cholestasis. These Histopathologic parameters would help the pathologist in making a definitive diagnosis. We recommend the use of Cytokeratin IHC on all tissues showing obliteration of portal tracts due to fibrosis or inflammation for proper evaluation.

The biggest drawback of the study is the incomplete clinical and laboratory data for evaluation. This is due to incomplete documentation of relevant history and physical examination findings, the failure of incorporating the results of different laboratory tests and ancillary procedures in the charts, and the non-performance of some critical laboratory tests and procedures. Significantly limiting the number of cases and parameters for evaluation in this study are unrecovered slides, paraffin blocks, and missing charts of patients. A prospective study is recommended since it would better document and archive the data needed. It is likewise beyond the scope of this study to determine and follow-up the clinical outcome of the patients. Determining the patients' outcome would support the diagnosis and determine the prognosis and disease process.

BIBLIOGRAPHY/ REFERENCES:

1. Heejin Lee, Kim KM,Jang JY, Jang S, Yu S. The Clinopathological Parameters for making the differential diagnosis of Neonatal Cholestasis. Korean Journal of Pathology,2009.

2. BrandaoMA,Arnaut LT, De Tommaso AM, Hessel G. Differential Diagnosis of Neonatal Cholestasis:Clinical and Laboratory Parameters. J Pediatr (Rio J).2010;86(1):40-41: Neonatal Cholestasis,children,hepatitis.

3. Santos JL,Almeida H, Cerski CTS, Silveira TR. Histopathological diagnosis of intrahepatic and extrahepatic neonatal cholestasis. Brazilian Journal of Medical and Biological Research (1998) 31:911-919.

4. Wang JS, Wang ZL, Wang XH, Zhu QR, Zheng S. The Prognostic Value of Serum Gamma Glutamyl Transferase Activity in Chinese Infants with Previously diagnosed Idipathic Neonatal Hepatitis. HK J Pediatr 2008;13:39-45.

5. Moyer V,Freese D, Whitington P, Olson A, Brewer F,Colleti R, Heyman, M. Guideline for the Evaluation of Cholestatic Jaundice in Infants: Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition.Journal of Pediatric Gastroenterology and Nutrition 39:115-128.

6. Urganci N. ,Çetinkaya F. , Kalyoncu D. , Cakir E.P. , Yilmaz B. Infants with Cholestasis: Diagnosis, Management and Outcome Marmara Medical Journal 2012;25:83-

7. Lee W.S., Chai P.F. Clinical Features Differentiating Biliary Atresia from other Causes of Neonatal Cholestasis. Ann Acad Med Singapore 2010;39:648-654.

8. Redkar RG, Masarweh M, Howard ER, Karani J, Miele-Vergani G, Davenport M. Inspissated Bile in Infants: Recent Advances and Current Concepts. Department of Paediatric Surgery, Radiology & Institute of Liver Studies, King's College Hospital, London. Ped Gastro Conf 2005.

9. Gilbert-Barnes E. (2007). Pathology of the Fetus, Infant and Child.2nd Edition. Mosby Elsevier.

10. Burt AD,Portmann B.C. Ferrell L.D. (2002). MacSweens Pathology of the Liver.4th edition. Churchill Livingstone, Elsevier.

11. Humphrey T.M. Stringer M.D. et al , Biliary Atresia: US Diagnosis. Radiological Society of North America. September 2007 Radiology,244, 845-851.

CERVICAL CANCER SCREENING, HPV VACCINE: KNOWLEDGE AND ATTITUDES OF FEMALE ADOLESCENTS SEEKING CONSULT AT PHILIPPINE CHILDREN'S MEDICAL CENTER

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ABSTRACT

OBJECTIVE: This study aims to assess the knowledge and attitudes of female adolescents aged 14-19 years old seeking consult at Philippine Children's Medical Center regarding cervical cancer screening and HPV vaccine.

DESIGN: Cross-sectional survey of mid to late adolescents.

METHODOLOGY: The survey questionnaire consists of 18 items in the domain of knowledge, attitudes, practice behaviors, barriers and demographic information. Survey forms in a sealed envelope were given to adolescents 14 to 19 years old after obtaining their assent and parents/guardians' consent.

RESULTS: There were a total of 107 respondents; majority belonged to the mid adolescents (14-16 years old), high school level and non sexually active 57%, 64% and 62% respectively. Most of the respondents (60%) were in a relationship or had a boyfriend, 61% engaged in a monogamous sexual relationship with their partners, 10% had 2 partners and 15% had more than 3 partners. There were significantly more late adolescents who are sexually active compared to mid adolescents (67.4% and 14.8%). Awareness of cervical cancer screening is only 23% of the surveyed adolescents (mean age 16.36). Majority of the sexually active respondents knew that HPV infection is through vaginal intercourse, and that pap smear is necessary even if no history of cancer in the family. More respondents with high education knew that avoidance of sexual intercourse, using condom and vaccination are all effective methods of HPV infection prevention. 53% of the respondents heard about HPV vaccine. Sources of information were mostly from the mass media. Majority were willing to receive the vaccine especially if given for free.

CONCLUSION: Knowledge on HPV and Cervical cancer prevention especially through pap smear is poor. Health education and improvement of personal practices should be emphasized as necessary factors for preventing HPV infection acquisition.

Keywords: adolescents, cervical cancer screening, Pap smear, HPV vaccination

INTRODUCTION

Human Papilloma Virus (HPV) is a group of more than 150 related viruses, of which more than 40 types can be sexually transmitted. This DNA- containing viruses causes conditions that range from the common benign genital warts to malignancy such as cervical cancer. At least 25 different subtypes have been involved in the genital region. The most common subtypes, HPV 16 and 18, causes premalignant or malignant lesions of the female genital tract. While types 6 and 11, are more commonly associated with benign lesions. Sexual intercourse is the easiest way to spread HPV infection or HPV-related conditions, which are not easily prevented with condoms, since any skin to skin contact can spread the virus. As with other STD, peak incidence occurs between the ages of 15 and 25 years. [1] Persistent infection with the high risk or

oncogenic types of HPV causes cervical, vulvar, vaginal and anal cancer.

The incidence of cervical cancer remained stable from 1980 to 2005 according to the Filipino cancer registry annual report, with an annual age- standardized incidence rate of 22.5 cases per 100,000 women. The overall 5-year survival rate was 44% and mortality rate was 1 per 10,000 women. [2] The recorded incidence has increased from an annual average of 4,536 new cases in 1998 to 7,277 new cases in 2005. The specific reason behind this rise is unclear; it may simply be due to improved reporting, or to increasingly unhealthy lifestyle, or to the rise in carcinogens in an industrializing environment, or to an increase in the sexually transmitted spread of carcinogenic types of HPV warts.[3] Most HPV infections go away on their own without causing any type of abnormality. However, persistent infection with cancer-associated HPV types along with other factors such as smoking and having many children increases the risk that mild abnormalities will progress to cervical cancer. Early coitarche, multiple sexual partners, sexual partners who have other partners were also implicated, however the greatest risk for cervical cancer is the lack of regular Pap smear screening. [4]

Pap smear or Pap test is a way to examine cells collected from the cervix (the lower, narrow end of the uterus). The main purpose is to detect cancer or abnormal cells that may lead to cancer. It can also find non cancerous conditions, such as infection and inflammation. Pap tests continue to be essential to detect cervical cancers and precancerous changes, even in women who have been vaccinated against HPVs, because current HPV vaccines do not protect against all HPV types that cause cervical cancer. Therefore, it is important for vaccinated women to continue to undergo cervical cancer screening in accord with recommendations for women who have not been vaccinated.[5]

The American College of Obstetricians and Gynecologists announced on November 20, 2009 that women should have their first cancer screening at age 21 and can be rescreened every two years, instead of annually as previously recommended. Moving the baseline cervical screening to age 21 is a conservative approach to avoid unnecessary treatment of adolescents which can have economic, emotional, and future childbearing implications. Although the rate of HPV infection is high among sexually active adolescents, invasive cervical cancer is very rare in women under age 21; but because of the immaturity of the adolescent cervix, there is a higher incidence of HPV-related precancerous lesions (called dysplasia) in this age group.[6] The surest way to eliminate risk for genital HPV infection is to refrain from any genital contact with another individual. For those who are sexually active, a long-term, mutually monogamous relationship with an uninfected partner is the strategy most likely to prevent HPV infection. However, it is difficult to determine whether a partner who has been sexually active in the past is currently infected. Correct and consistent condom use does not offer complete protection because there are areas not covered by a condom. The food and Drug Administration (FDA) has approved two vaccines to prevent HPV infection: Gardasil (quadrivalent) and Cervarix (bivalent), which are both effective in preventing infections with HPV types 16 and 18, two oncogenic HPVs that causes 70% of cervical cancers. Ideally, girls should be vaccinated before the onset of sexual activity and together with screening program (Pap smear) can reduce the lifetime risk of acquiring cervical cancer by up to 94%. [7]

To date, there is no known local study focusing on the adolescents and young adult's knowledge and beliefs on cervical cancer and attitude towards HPV vaccination. This study will provide the baseline local data; aid health practitioners to develop effective strategies to communicate with the adolescents; give relevant information on HPV, cervical cancer, and its prevention; and recommend HPV vaccination to lower if not completely eradicate cervical cancer cases. This will also determine the content of lectures to be given to this age group for recommendation to school programs for proper information dissemination thus lower incidence of HPV morbidity and mortality.

Cervical cancer remains the number one cause of reproductive tract mortality among females worldwide; and HPV being the major risk factor. There is a need for women and young girls to understand the link between HPV infection and development of cervical cancer and the need for regular Pap smear.

General Objective:

To determine the knowledge and attitudes on cervical cancer screening and HPV vaccination among female adolescents ages 14-19 years.

Specific Objectives:

1. To determine the profile of Adolescents in terms of age, education and sexual activity.

2. To determine the number of adolescents who are aware of cervical cancer screening and HPV vaccination

3. To identify the sources of information among female adolescents who have heard of HPV vaccine

4. To compare the knowledge and attitude on cervical cancer screening and HPV vaccination based on the educational attainment and age of the adolescents.

5. To compare the knowledge and attitude towards cervical cancer screening and HPV vaccination by sexual activity.

6. To determine the proportion of adolescents who are willing to receive the HPV vaccine.

METHODOLOGY

This study is a cross sectional study. It employed a sealed envelope technique. A self administered survey questionnaires were given to all Female Adolescents (ages 14-19) upon consult at the Adolescent clinic of Philippine Children's Medical Center for any reason. Consent forms to parents/guardians, as well as assent form by the adolescents were given prior to answering the 18- question survey form.

The questionnaire was formulated specifically for the young respondents. Survey questions as well as research proposal were reviewed by UP Population Institute Social Scientist. Questionnaire was pre-tested among 10 adolescent girls who sought consult at Philippine Children's Medical Center - Adolescent Clinic, after explaining to the parents and subjects the purpose and content of the questionnaire. Parental consent as well as adolescents' assent was obtained. There were no problems encountered and all respondents answered all the questions leaving no blanks. It was validated and croonbachs alpha yielded a value of 0.81 denoting that the questionnaire was reliable.

The survey included questions regarding knowledge of cervical cancer, role of HPV infection in cervical cancer, HPV vaccination, the purpose of Pap smears, and demographics. Female adolescents were considered sexually active when they answered the questions pertaining to age of 1st sexual contact, number of sexual partners and affirmation of sexual intercourse with partner (heterosexual/homosexual) regardless of her status of sexual activity at the present.

All 107 questionnaires had an introduction that explained the purpose of the study and emphasized the anonymity and confidentiality of the results. The questionnaire elicited both quantitative and qualitative data. Non-response bias was addressed by the supervision of respondents by the researcher during the administration of the questionnaire. Upon return of an accomplished questionnaire, the researcher immediately reviewed the survey to look for any errors or non-response. In such cases, questionnaires were given back to the respondents to be completed.

Excluded from the sample were males, patients with severe reading impediment, those with serious physical or mental handicap and the early adolescents (10-13years old). Middle to late Adolescents (14-19 years old) were focused

mainly because of their ability to respond to study questions pertaining to sex, sexuality and sexually transmitted infection (STI), its prevention and outcome. These age groups are also characterized as having increased involvement in partnering relations, manifested by dating activity, sexual experimentation, and intercourse. Their feeling of omnipotence and immortality leads them to risk-taking behaviour, and communities' and churches' conservative beliefs.[9]

This choice of age group is similar to the findings of Red et al in her study in 2008, conducted on 4 public high schools in Quezon City; the mean age of coital debut among females is at 15.67±1.15. The youngest age of coital debut was 15 years old. [10] A decreased age of coital debut was significant as compared with the Young Adult Fertility and Sexuality Study 3 in 2002 (YAFS 3) in which the Mean age of first sexual experience in females is 18 years old. [11]

All information gathered from the research was held in high confidentiality. The respondent's names were not collected or appeared anywhere on the survey and complete anonymity was guaranteed. The participants after answering the survey questions received an information sheet containing data regarding HPV, cervical cancer, its risks and mode of transmission and prevention.

The number of subjects included in the study was computed using a 95% level of confidence. With an estimated proportion of 38% of respondents having knowledge of HPV which is a factor in the high rates of accidents, suicide, drug use, pregnancy, and sexually transmitted diseases.[8] Including younger adolescents ages 10 to 13 would be difficult given the socio-cultural context in the Philippines. Impediments to studying the younger group also include: parents' objection; service providers' bias;

based from a previous study done at St. Lukes Medical Center, Philippines (Gratil et al, 2011; n=164)[12], at least 91 respondents is needed.

All data were encoded using Excel spreadsheet and SPSS analysis. Data were analyzed using SPSS version 10 for windows. Descriptive statistics were generated for all variables. For nominal data, frequencies and percentages were computed. For numerical data, mean ± SD were generated. Analysis of the different variables under study was done using the following test statistics: Chi- square test and Fisher Exact test when necessary used to compare/associate nominal data.

To test the significant relationship between the respondents' age, educational attainment and sexual status to the level of knowledge on HPV, cervical cancer, HPV Vaccine and Pap smear, the chi square test was used.

A high level of knowledge is evident if 75% of the total respondents answered the questions correctly; moderate knowledge if 50 to 74% answered correctly; and poor knowledge if less than 50% answered correctly.

TABLE 1. Distribution of Respondents According to demographic profile

	Frequency n=107	(%)
Age distribution		
14-16yo	61	57
17-19yo	46	43
Mean ± SD = 16.36 ± 1.71		
Level of Education		
Elementary level	15	14
High school level	68	64
College level	24	22
Heard of the Sexually Transmitted Disease		
Yes	86	80
No	21	20

RESULTS

There were a total of 107 respondents of the study distributed to 61 (57%) mid adolescents and 46 (43%) late adolescents; 15 (14%) elementary level, 68 (64%) high school level and 24 (22%) college level; 40 (37%) are sexually active (of which 42.5% belonged to ages 13-16 years old and 57.5% were aged 17-19 years old) while 67 (63%) were non sexually active. Majority of the respondents (mean age of 16.36) 80% have heard about sexually transmitted diseases.

TABLE 2. Practice of Respondents regarding Sexual Relationship

Parameter	Freq	%
Relationship status		
With boyfriend	64	*60*
Without boyfriend	43	*40*
Total	107	*100*
Sexual intercourse activity		
With boyfriend:		
With sexual intercourse	39	*61*
Without sexual intercourse	25	*39*
Total	64	*100*
Without boyfriend:		
With sexual intercourse	1	*2*
Without sexual intercourse	42	*98*
Total	43	*100*
Sex partners frequency & age at first sex		
Number of sex partners		
1	30	*75*
2	4	*10*
3 and more	6	*15*
Total	40	*100*
Mean ± SD = 1.45 ± 0.90		
Age at first sex		
13 - 16	17	*42.5*
17 - 19	23	*57.5*
Total	40	*100*
Mean ± SD = 16.35 ± 1.46		

Table 2 shows the practices of respondents regarding sexual relationship. 40 (37%) engaged in sexual intercourse. Of this 40 with sexual intercourse, 39 (98%) had it with their boyfriend and there was 1 without a boyfriend but had sexual intercourse. Among these sexually active adolescents, there were 10 (25%) who had >1 sexual partners; the age at first sex started at 13 years with an average of 16.35 years.

TABLE 3. Proportion of Sexually active versus Non Sexually active Adolescents according to Age

	Sexually Active Frequency	(%)	Non Sexually Active Frequency	(%)	Total
Mid Adolescents 14-16yo	9	(14.8%)	52	(85.2%)	61
Late Adolescents 17-19yo	31	(67.4%)	15	(32.6%)	46
Total	**40**		**67**		**107**

P<0.00001 (S)

Table 3 shows the comparison of sexually active and non sexually active adolescents according to age. The results showed that there were more late adolescents that were sexually active than the mid adolescents with 67.4% and 14.8% respectively.

TABLE 4. Distribution of Respondents with correct responses to questions related to knowledge of HPV, cervical cancer, HPV vaccine and papsmear according to Age and Level of Education

	all	Mid Ado (n=61) (%)	Late Adol (n=46) (%)	P value	Low Educ (n=83) (%)	High Educ (n=24) (%)	P value
HPV QUESTIONS							
HPV means							
A Bacterium *(false)*	5 (5%)	4 (7%)	1 (2%)		4 (5%)	1 (4%)	
A Virus *(true)*	39 (36%)	23 (38%)	16 (35%)	0.71	29 (35%)	10 (42%)	0.94 (NS)
A Cancer *(false)*	13 (12%)	7 (11%)	6 (13%)	(NS)	10 (12%)	3 (12%)	
I Don't Know	50 (47%)	27 (44%)	23 (50%)		40 (48%)	10 (42%)	
Part of the Body That HPV affects							
Uterus *(false)*							
Cervix *(true)*	44 (41%)	20 (33%)	24 (52%)	0.04 (S)	32 (39%)	12 (50%)	0.44 (NS)
Breast *(false)*	48 (45%)	29 (47%)	19 (41%)	(uterus	38 (46%)	10 (42%)	
Ovary *(false)*	7 (6%)	6 (10%)	1 (2%)	only)	7 (8%)	0	
	8 (7%)	6 (10%)	2 (4%)		6 (7%)	2 (8%)	
Methods one can become infected with HPV*							
Vaginal intercourse *(true)*	90(84%)	49 (80%)	41 (89%)	0.21(NS)	69 (38%)	21 (88%)	0.76(NS)
Kissing *(false)*	6 (6%)	4 (7%)	2 (4%)	0.70(NS)	6 (7%)	0	0.33(NS)
Toilet seats *(false)*	21(20%)	11 (18%)	10 (22%)	0.63(NS)	15 (18%)	6 (25%)	0.56(NS)
None of the above	6 (6%)	4 (7%)	2 (4%)	0.70(NS)	5 (6%)	1 (4%)	1.00(NS)
Methods of preventing HPV *							
Avoidance of sexual intercourse	22 (21%)	12 (20%)	10 (22%)	0.79(NS)	20 (24%)	2 (8%)	0.14(NS)
Using Condom *(true)*	9 (8%)	5 (8%)	4 (9%)	1.00(NS)	8 (10%)	1 (4%)	0.68(NS)
HPV vaccination *(true)*	26 (24%)	15 (25%)	11 (24%)	0.94(NS)	24 (29%)	2 (8%)	0.03 (S)
All of the above	38 (36%)	22 (36%)	16 (35%)	0.89(NS)	23 (28%)	15 (62%)	0.001 (S)
I Don't Know	22 (21%)	22 (36%)	10 (22%)	0.10(NS)	18 (22%)	4 (17%)	0.78(NS)
*multiple response							
CERVICAL CANCER QUESTIONS							
	all	**Mid Ado (n=61) (%)**	**Late Adol (n=46) (%)**	**P Value**	**Low Educ (n=83) (%)**	**High Educ (n=24) (%)**	**P Value**
Cervical Cancer occurs only in Older women							
True	12(11%)	10 (16%)	2 (4%)	0.10 (NS)	10 (12%)	2 (8%)	0.88 (NS)
False	39(36%)	19 (31%)	20 (44%)		30 (36%)	9 (38%)	
I Don't Know	56(52%)	32 (53%)	24 (52%)		43 (52%)	13 (54%)	
Cervical Cancer is caused by HPV virus							
True	50 (47%)	29 (48%)	21 (46%)	0.52 (NS)	38 (46%)	12 (50%)	0.93 (NS)
False	5 (5%)	4 (6%)	1 (2%)		4 (5%)	1 (4%)	
I Don't Know	52 (49%)	28 (46%)	24 (52%)		41 (49%)	11 (46%)	
Cervical Cancer caused by HPV virus can be prevented							
True	76 (71%)	39 (64%)	37 (80%)		56 (68%)	20 (83%)	
False	3 (3%)	3 (5%)	0	0.09 (NS)	3 (4%)	0	0.27 (NS)
I Don't Know	28 (26%)	19 (31%)	9 (20%)		24 (28%)	4 (17%)	

HPV VACCINE							
Did you hear about HPV vaccination?							
Yes, thru Media	32 (30%)	17 (28%)	15 (33%)		20 (24%)	12 (50%)	0.01 (S)
Yes, thru Doctor	6 (6%)	2 (3%)	4 (9%)	0.50 (NS)	6 (7%)	0	(comparis
Yes, Teacher	18 (17%)	12 (20%)	6 (13%)		14 (17%)	4 (17%)	on of thru
Friends/Classmates	0	0	0		0	0	media
Not yet	51(48%)	30 (49%)	21 (46%)		43 (52%)	8 (33%)	only)
	all	**Mid Ado** (n=61) (%)	**Late Adol** (n=46) (%)	**P Value**	**Low Educ** (n=83) (%)	**High Educ** (n=24) (%)	**P Value**
PAPSMEAR							
Pap smear Means							
Exam to detect early signs of cancer	25 (23%)	13 (21%)	12 (26%)		14 (17%)	11 (46%)	
Tests to determine if a girl is pregnant	1 (1%)	0	1 (2%)	0.20(NS)	0	1 (4%)	0.003 (S)
Test to know if one is with AIDS	4 (4%)	4 (7%)	0		4 (5%)	0	
No, I don't know	77(72%)	44 (72%)	33 (72%)		65 (78%)	12 (50%)	
Only Women who had many sexual partners need to have papsmear							
True	14(13%)	8 (13%)	6 (13%)	0.82 (NS)	11 (13%)	3 (12%)	0.80 (NS)
False	43(40%)	26 (43%)	17 (37%)		32 (39%)	11 (46%)	
I Don't Know	50(48%)	27 (44%)	23 (50%)		40 (48%)	10 (42%)	
Pap smear are necessary even if there is no family history of cancer							
True	49(46%)	20 (33%)	29 (63%)	0.005 (S)	34 (41%)	15 (62%)	0.15 (NS)
False	2 (2%)	2 (3%)	0		2 (2%)	0	
I Don't Know	56(52%)	39 (64%)	17 (37%)		47 (57%)	9 (38%)	

According to level of education, there was no significant difference in the responses of those with low and high education respondents. However when asked about the methods of preventing HPV, the higher the educational level of the adolescents the greater is their awareness that HPV vaccination, using condom and avoidance of sexual intercourse were all methods of preventing infection with HPV. More respondents with low education thought that HPV vaccination alone is the method of preventing HPV. There were more respondents with high education who heard about HPV vaccination mostly thru media; and knew that pap smears are exam to detect early signs of cancer. Majority (62%) of those with high education knew that even if there is no family history of cancer in the family, Pap smear needs to be done to all women.

TABLE 5. Distribution of Respondents with correct responses to questions related to knowledge of HPV, cervix cancer, HPV vaccine and papsmear according to Sexual Activity

	Sexually Active (n=40) (%)	**Non-Sexually Active (n=67) (%)**	**Total**	**P value**
HPV QUESTIONS				
HPV means				
A Bacterium *(false)*	1 (2%)	4 (6%)	5	
A Virus *(true)*	12 (30%)	27 (40%)	39	0.52 (NS)
A Cancer *(false)*	6 (15%)	7 (10%)	13	
Don't Know	21 (53%)	29 (43%)	50	
Part of the Body That HPV affects				
Uterus *(false)*	22 (55%)	22 (33%)	44	
Cervix *(true)*	15 (38%)	33 (49%)	48	0.02 (S)
Breast *(false)*	1 (2%)	6 (9%)	7	(comparison of
Ovary *(false)*	2 (5%)	6 (9%)	8	uterus only)

Methods one can become infected with HPV *						
Vaginal intercourse *(true)*	38	(95%)	52	(78%)	90	0.01 (S)
Kissing *(false)*	1	(2%)	5	(7%)	6	0.40 (NS)
Toilet seats *(false)*	5	(12%)	16	(24%)	21	0.15 (NS)
None of the above	1	(2%)	5	(7%)	6	0.40 (NS)
Methods of preventing HPV *						
Avoidance of sexual intercourse *(true)*	7	(18%)	15	(22%)	22	0.54 (NS)
Using Condom *(true)*	3	(8%)	6	(9%)	9	1.00 (NS)
HPV vaccination *(true)*	9	(22%)	17	(25%)	26	0.74 (NS)
All of the above	16	(40%)	22	(33%)	38	0.45 (NS)
I Don't Know	8	(20%)	14	(21%)	22	0.91 (NS)
CERVICAL CANCER QUESTIONS						
Cervical Cancer occurs only in Older women						
True	2	(5%)	10	(15%)	12	
False	17	(43%)	22	(33%)	39	0.24 (NS)
I Don't Know	21	(52%)	35	(52%)	56	
Cervical Cancer is caused by HPV virus						
True	17	(43%)	33	(49%)	50	
False	1	(2%)	4	(6%)	5	0.48 (NS)
I Don't Know	22	(55%)	30	(45%)	52	
Cervical Cancer caused by HPV virus can be prevented						
True	30	(75%)	46	(69%)	76	
False	0		3	(4%)	3	0.37 (NS)
I Don't Know	10	(25%)	20	(27%)	28	
HPV VACCINE						
Did you hear about HPV vaccination?						
Yes, thru Media	15	(38%)	17	(25%)	32	
Yes, thru Doctor	3	(8%)	3	(5%)	6	
Yes, Teacher	4	(10%)	14	(21%)	18	
Friends/Classmates	0		0		0	0.32 (NS)
No, I Don't Know	18	(45%)	33	(49%)	51	
PAPSMEAR						
Pap smear Means						
Exam to detect early signs of cancer	10	(25%)	15	(22%)	25	
Tests to determine if a girl is pregnant	1	(2%)	0		1	0.24 (NS)
Test to know if one is with AIDS	0		4	(6%)	4	
No, I don't know	29	(73%)	48	(72%)	77	
Only Women who had many sexual partners need to have papsmear						
True	7	(18%)	7	(10%)	14	
False	13	(32%)	30	(45%)	43	0.36 (NS)
I Don't Know	20	(50%)	30	(45%)	50	
Pap smear are necessary even if there is no family history of cancer						
True	24	(60%)	25	(37%)	49	
False	0		2	(3%)	2	0.05 (S)
I Don't Know	16	(40%)	40	(60%)	56	

Table 5 shows the comparison of the distribution of respondents with correct responses to questions related to knowledge of HPV, cervix cancer, HPV vaccine and papsmear according to sexual activity. The results showed that , there was no significant difference in the responses of sexually active and non-sexually active respondents except when asked on which part of the body HPV affects, how one can become infected with HPV, and whether pap smears are necessary even if there is no family history of cancer . When asked which part of the body HPV affects, significantly more proportion of sexually active respondents knew that the uterus is the one affected by HPV compared to the non-sexually active respondents (p=0.02). Most sexually active respondents knew that vaginal intercourse will cause infection with HPV and also knew that pap smears are necessary even if there is no family history of cancer. Awareness on HPV by non sexually active adolescents was less than that of sexually active adolescents.

TABLE 6. Distribution of Respondents Willingness to use HPV vaccine according to Age

	All	Mid Adol	Late Adol	P value	Sexually Active	Non Sexually Active	P value	Low Ed	High Ed	P Value
Use the vaccine if free										
Yes	76	40 (66%)	36 (78%)	0.30	32 (80%)	44 (66%)	0.12	60 (72%)	16 (67%)	0.28 (NS)
No	5	4 (7%)	1 (2%)	(NS)	0	5 (8%)	(NS)	5 (6%)	0	
Maybe	26	17 (28%)	9 (20%)		8 (20%)	18 (27%)		18 (22%)	8 (33%)	

Table 6 shows the comparison of the respondent's willingness to use HPV vaccine according to age and sexual activity. The results showed that according to age, there was no significant difference in the willingness of the mid adolescents and late adolescents. Also, there was was no significant difference in the willingness of the sexually and non-sexually active adolescents and same for those with low and high level of education (p=0.28). 71% of the surveyed adolescents were willing to receive the vaccine if given for free. More than 20% of the respondents cannot give a concrete answer whether they want to use the vaccine or not.

TABLE 7. Reasons for not Using HPV Vaccine

	N	Sexually Active (n=40) (%)	Non Sexually Active (n=67) (%)	P value
I Dont need the vaccine	13	3 (8%)	10 (15%)	0.36 (NS)
Currently not sexually active	13	1 (2%)	12 (18%)	0.02 (S)
Having sex only with Boyfriend	5	5 (13%)	0	0.006 (S)
Always use condom	0	0	0	---

Table 7 shows the reasons for not using HPV vaccine of sexually and non-sexually active adolescent. Significantly more proportion of those not sexually active answered that they do not need the vaccine because they are currently not sexually active. For those sexually active, they answered that they think they do not need the HPV vaccine because they have sex only with their boyfriend. There is no significant difference with the reasons provided based on educational level and age bracket.

DISCUSSION

The result of this study indicates that female adolescent's knowledge with regards to meaning of HPV and part of the body affected is poor, only 36% identified HPV as a virus and 45% identified cervix as the one affected. However, with regards to mode of transmission and method of prevention, level of knowledge was high at 84% and 89% consequetively. Sexually active adolescents were significantly more knowledgeable than the non sexually active regarding mode of transmission. Meaning as a woman engaged in sexual contact she becomes more knowledgeable that HPV infection is through vaginal intercourse. Out of the 89% who were aware of preventing HPV infection, the study shows that the higher the educational attainment the higher is their knowledge that avoidance of sexual intercourse, using condom and HPV vaccination were all methods of prevention; more low educational attainment group thought it was only HPV vaccination.

This finding is slightly higher than those observed in earlier surveys among 1348 adolescents and young women aged 14-24 years

in Italy where 30% have heard about HPV infection. 13 and significantly higher compared to study in 2010 at Kolkota India where 15% of 630 students have heard about HPV. 14 Low knowledge was also depicted in the study by Hoover et al of 60 women (15 to 28 years old) in southern New Jersey Shore, only 23.3% had heard of HPV; 34.5% of women 18 years or older had heard of HPV versus 8.3% of those 17 years and under ($P<0.01$). Most of those who heard of HPV , 14.8% had done so at school; 9% had heard from a doctor and 9% from television.15 Poor knowledge can be attributed to their low level of understanding with regards to importance and implication of Human papilloma virus infection which can be due to lack of public health education or inadequacy of existing campaigns. However, the slight increase in this study compared to previous ones may be due to the heightened interest growing in the youths of today, brought about by exposure to media (television, magazines) and internet regarding HPV vaccination and the need for self protection.

There is a strong association between HPV and cervical cancer. In the Philippines the reported prevalence of all HPV types is 93.8% in squamous cell carcinoma and 90.9% in adenocarcinoma/ adenosquamous carcinoma. 16

Cervical cancer is not a disease of old age. For patients with squamous cell carcinoma the mean age was 47.2 years; for those with adenocarcinoma/adenosquamous carcinoma it was 48.4 years. 16 These women are at the peak of their biologically and economically productive ages. Therefore when a woman dies of this cancer, a life is not simply lost; rather, a husband loses a wife, the children lose a mother, and the family is destabilized psychologically, financially and socially. The economy loses a productive pair of hands but more than a pair of productive hands is lost to the state, which inevitably pays a big part of the cost of treating this disease. 3 Depending on the stage of the disease, the cost of treatment per patient ranges from P35 thousand to more than P703 thousand. These amounts are prohibitive even to those with income, considering that the national annual average savings per family is only P24 thousand. One way or another, the government subsidizes a considerable portion of the cost for these families. But for a big part of the population who lives below the poverty level, and who must be treated as charity patients, the government must shoulder all these costs.3 The cost of preventing cervical cancer can be as low as P400 through a conventional Pap smear test which detects pre malignant condition, thus prevents progression to frank cervical cancer by early treatment or as high as P 21 thousand through HPV vaccination.

Educational programs must be structured to address the gaps in knowledge between HPV infection and cervical cancer. This was the findings in this study where only 47% knew that HPV causes cervical cancer, 49% do not know if HPV can cause cervical cancer and 5% do not believe that HPV can cause cervical cancer. Same findings in a descriptive cross sectional analysis of 449 females in Colombia (age range 18- 69 years old, mean of 38.7 years), 72.4% never heard of HPV, 86.2% were unaware of the role of HPV in the development of cervical cancer and 80.2% did not know that HPV is sexually transmitted.17

Knowledge about HPV among U.S. women ages 18 to 75 years old was relatively low (Tiro et al, 2005); 40% of women (n=1,248) reported that they had ever heard of HPV. Among those who have heard of it, less than half knew that HPV causes cervical cancer.18 Better understanding of HPV infection and role of HPV vaccination for the prevention of cervical cancer may motivate women to participate in HPV primary prevention through vaccination.

Two vaccines (Quadrivalent, Bivalent) are available to protect females against the types of HPV that causes most cervical cancers. The Quadrivalent HPV vaccine (Gardasil, MSD) was found to have 97% efficacy at preventing HPV 16 and/ or 18 related cervical intraepithelial neoplasia (CIN) 2 or 3, adenocarcinoma in situ and cervical cancer. It was approaved for use in males and females between 9 and 26 years of age. It also protects against most genital warts (caused by HPV 6 and 11). The Bivalent vaccine

(Cervarix, GSK) which also prevents cervical cancer and precancerous lesions caused by HPV types 16 and 18 was approaved for use in females between 10 and 25 years of age. 19 It is recommended that females get the same vaccine brand for all three doses, whenever possible. The best way a person can be sure to get the most benefit from HPV vaccination is to complete all three doses before beginning sexual activity.20

The result of this study with regards to knowledge of HPV vaccine does not differ much from the foreign researches, 53% of adolescents who sought consult at PCMC have heard about HPV vaccine, mostly from the mass media (30%), 17% from their teachers and only 6% through their doctors. Findings of Donders et al in Belgium of 381 women (mean age was 35.8 years SD 11.0) 69% were informed about the vaccine by the mass media (newspaper, television, radio). About 1.3% of all women were informed by the general practitioner, 3.8% by the Gynecologist, and 4.3% by peers.21 Similar to the findings of Caron et al in a cross sectional study of 361 female college students in New Hampshire, where 85% of the respondents have heard of HPV vaccine, 64.9% was from television commercial, Friends (41.8%) and 31.9% from family. This only shows that adolescents of today are exposed to technologies where they get most of the informations regarding topics which they deemed too personal to ask their parents. Therefore efforts should be made with science based information dessimination, so as not to mislead viewers of TV commercials and advertisements with regards to what is scientifically correct.

In line with the guidelines on giving of HPV vaccines to girls prior to onset of sexual activity, the Italian Ministry in March 2008 started the free vaccination for 12 years old girls. A Cross sectional survey was done on 863 high school students which showed more females than males were aware that HPV could concern themselves (45% vs 26% respectively) and would undergo vaccination against HPV (68% vs 40% respectively) of which $p < 0.001$.22 Majority of the surveyed adolescents in this study (71%) were willing to receive the vaccine especially if given for free.

Recommendation for women with HPV vaccination to undergo cervical cancer screening through Pap smear Test is the same as for the general population. Pap smear screening is the gold standard method for detecting pre-invasive cervical disease. Without screening, cancer usually presents in advanced stages when cure rates are low. In Canada, half of the 1,300 women diagnosed per year with cervical cancer occur in women who have not had regular Papanicolaou (PAP) smear screening. However, knowledge levels remain low that there is cervical cancer screening that would help detect early changes in the cervix. This was shown in a study done by Diane Blake et al in which only 2.7% of the 111 Female patients (aged 14 years or older) provided an accurate definition of the term Pap smear; 68% even mistakenly believed that it is the same as a pelvic examination.23 In this current study of 107 adolescent patients (aged 13 to 19 years old), 23% provided the correct definition of pap smear (to detect early signs of cervical cancer); 72% do not know the purpose of papsmear; and 4% mistakenly believed that it is a test to detect AIDS while 1% thought it is the same as a pregnancy test.

CONCLUSION AND RECOMMENDATIONS

This study clearly demonstrated substantial voids in knowledge- only 36-45% knew about HPV, almost nothing about cervical cancer screening at 23%. Given the limited knowledge about HPV, it is not surprising that low level of understanding exists that HPV causes cervical cancer.

Not much difference in the knowledge and attitudes of adolescents except with regards to sexual activity status, as adolescents became sexually active their knowledge that HPV is transmitted through vaginal intercourse increases as well as their awareness of the importance of pap smear.

Based on level of education: high educational attainment positively correlated to increase knowledge with regards to HPV, methods of prevention, and purpose of pap smear. This only shows that age does not

contribute much to ones knowledge in contrast to educational attainment and sexual status.

Based from the foregoing findings, there is an urgent need to design school and community-based interventions programs focusing on HPV, perceived risks, mode of transmission and prevention and HPV vaccine efficiency. Moreover, as teachers can play an important role in educating these adolescents, their knowledge should also be evaluated and improved if necessary. Physicians (Pediatrician, family medicine, OB-GYN) must be encouraged to do counseling and health education teachings that will empower these adolescents in preventing unwanted pregnancy and more importantly to delay their coital debut; which would render them at greatest risk for sexually transmited infection, cervical dysplasia and later on cervical cancer. Abstinence should be emphasized as the most reliable method of HPV infection prevention, next only to HPV vaccination.

HPV vaccination should be highly recommended. Parents, guardians and caregivers must also be made aware of the importance of giving HPV vaccine prior to exposure to the virus to attain its full advantage.

Dialogue with government officials and those concerned must be done for HPV vaccination to be included in the expanded program of immunization so that even the less fortunate would be protected.

Regular cervical cancer screening using Pap smear must be done to all women 3 years after sexual contact but not earlier than 21 years old.

REFERENCES:

1. Katz VL, Lentz GM, Lobo RA, Gershenson DM, Comprehensive Gynecology, 5th ed.

2. Domingo EJ, Dy Echo AV; Epidemiology, prevention and treatment of cervical cancer in the Philippines; J Gynecol Oncol Vol. 20, No. 1:11-16, March 2009 DOI:10.3802/jgo.2009.20.1.11

3. Ladines-Llave CA, MD; The Burden of Cervical Cancer in the Philippines and Efforts to Combat the Disease; http://www.ellentordesillas.com/?p=436

4. Williams Gynecology; Section 4 Gynecologic Oncology, Chapter 30. Cervical Cancer

5. National Cancer Institute; U.S. National Institutes of Health. www.cancer.gov

6. The American Congress of Obstetricians and Gynecologist; New Pap Smear and Cancer Screening Guidelines. www.acog.org

7. Waller J, Marlow LAV, and Wardle J; Mothers' Attitudes towards Preventing Cervical Cancer through Human Papillomavirus Vaccination: A Qualitative Study; Cancer Epidemiol Biomarkers Prev 2006; 15:1257-1261.

8. Radzik M, Sherer S, Neinstein LS. Adolescent Health Care: A Practical Guide 4th Ed, Chapter 2 Psychosocial Development in Normal Adolescents

9. Sexual and Reproductive Health of Adolescents and Youths in the Philippines http://www.wpro.who.int/NR/rdonlyres/C39735C6-2817-4BC0-B527-3F22FBA7F2B9/0/ASRHphilippines.pdf

10. Red D, ; Relationship of Religiosity and Sexual attitudes on the Coital debut among public high school students in Quezon City; Philippine Children's Medical Center, Philippines, 2008

11. Raymundo, C.M., Young Adult Fertility and Sexuality Study 3 (2002), University of the Philippines Population Institute, Manila, Philippines, 2002.

12. Gratil, CA, Ignacio, MJ et al; Knowledge, Attitudes, Perceptions, and Practice on Human Papilloma Virus (HPV) and HPV Vaccine, Of Female College Students of Trinity University Of Asia; St. Lukes College of Medicine; February 2011

13. Di Giuseppe G, Abbate R, Liguori G, Albano L, Angelillo IF; Human Papilloma Virus and Vaccination: Knowledge, attitudes and behaviour intention in Adolescents and Young women in Italy. British Journal of Cancer (2008) 99, 225-229

14. Saha A, Chaudhury AN, Bhowmik P, Chatterjee R ; Awareness of Cervical Cancer Among Female Students of Premier Colleges in Kolkata, India Asian Pacific Journal of Cancer Prevention, Vol 11, 2010

15. Hoover DR, Oli BC and Moench EA; Attitudes of Adolescent/Young adult Women toward Human Papillomavirus Vaccination and clinical trials; Health Care for Women International , 21:375–391, 2000

16. Ngelangel CA, Munoz N, Bosch FX, Limson GM, Festin MR, Deacon J et al. Causes of Cervical Cancer in the Philippines: A Case-Control Study. J Natl Cancer Inst 1998; 90: 43-9.

17. Hanisch R, Gustat J, Hagenseey ME, Baena A, Salazar JE , Castro MV, Gaviria AM, & Sanchez GI; Knowledge of Pap screening and human papillomavirus among women attending clinics in Medellın, Colombia Int J Gynecol Cancer 2007

18. Tiro JA, Meissner HI, Kobrin S, and Chollette V . What Do Women in the U.S. Know about Human Papillomavirus and Cervical Cancer? Cancer Epidemiol Biomarkers Prev 2007;16(2). February 2007

19. Blumenthal J, Frey MK, Warley MJ et al. Adolescents Understanding and Acceptance of the HPV Vaccination in an Underserved Population in New York City. Journal of Oncology, Vol. 2012, 1-8

20. HPV Vaccines; http://www.cdc.gov/hpv/vaccine.html

21. Donders GG , Gabrovska M , Bellen G, Van Keirsbilck J, Van Den Bosch T, Riphagen I, Verjans M; Knowledge of cervix cancer, human papilloma virus (HPV) and HPV vaccination at the moment of introduction of the vaccine in women in Belgium; Arch Gynecol Obstet (2008) 277:291–298

22. Pelucchi C, Esposito S, Galeone C, Semino M, Sabatini C, Picciolli I, Consolo S, Milani G and Principi N ; Knowledge of human papillomavirus infection and its prevention among adolescents and parents in the greater Milan area, Northern Italy; BMC Public Health 2010, 10:378

23. Blake DR, Weber BM, Fletcher KE. Adolescent and Young Adult Women's Misunderstanding of the Term Pap Smear arch pediatr adolesc med/vol 158, oct 2004 www.archpediatrics.com

A COMPARISON BETWEEN 9 MONTH VERSUS 6 MONTH CYCLOPHOSPHAMIDE INDUCTION CHEMOTHERAPY IN THE MANAGEMENT OF LUPUS NEPHRITIS IN A GOVERNMENT TERTIARY PEDIATRIC HOSPITAL IN THE PHILIPPINES

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ABSTRACT

BACKGROUND: Lupus nephritis is very common complications in SLE, with clinical symptoms of renal involvement occurring in 30%–70% of patients. Outcomes in children with proliferative lupus nephritis (PLN) show 9–15% progress to end-stage renal disease (ESRD) at 5 years.

OBJECTIVES: This study compared the outcome of children and adolescent patients with lupus nephritis treated with 9 month versus 6 month induction of cyclophosphamide therapy. Renal frequency and adverse effects of IV cyclophosphamide during and after induction therapy were described and determined.

DESIGN: Retrospective Cohort Study

SETTING: Tertiary Hospital

METHODS: Retrospective cohort study comparing 6 and 9 month protocol of IV cyclophosphamide for lupus nephritis were conducted in a government tertiary pediatric hospital in the Philippines. A total of 39 patients with lupus nephritis were gathered, 23 patients underwent 6 months and 16 patients underwent 9 months protocol.

RESULTS: The comparison of two protocols in the administration of intravenous cyclophosphamide (IVCY) did not show significant difference between the two in terms of changes in GFR levels, but some evidence of a greater percent increase from baseline with the 6 months protocol post treatment were observed. Among 39 subjects, creatinine, albumin and urinalysis profile did not also differ between the two groups and levels within each group changed insignificantly over time up to 24 months. Proportion of subjects with renal flare ups, adverse effects and who expired during the study period were also essentially similar between the two groups.

CONCLUSION: IV Cyclophosphamide seems efficacious if given at the very beginning of the flare and at the start after patient was diagnosed with lupus nephritis. No statistically difference between the duration of the protocol. Renal flare ups and adverse effects of cyclophosphamide such as nausea, vomiting and headache were observed similarly between two protocols. Diligent follow up is needed for further studies and specificity of the results.

KEYWORD: systemic lupus erythematosus (SLE), IV cyclophosphamide (CYP), lupus nephritis (LN), pediatric

INTRODUCTION

Systemic Lupus Erythematosus can occur in children as young as 4 years of age, with the majority of cases occurring after 12 years of age. Lupus nephritis is very common complications in SLE, with clinical symptoms of renal involvement occurring in 30%–70% of patients. Outcomes in children with proliferate lupusnephritis (PLN) show 9–15% progress to

end-stage renal disease (ESRD) at 5 years. Thus early diagnosis and prompt treatment is required to prevent the progression of lupus nephritis to end stage renal disease. One of the recommended treatment is induction therapy with cyclophosphamide for 6 months. Unfortunately, there are no large, well-designed studies of induction therapy duration and its side effects and outcome for pediatric patient with lupus nephritis.

Monthly intravenous cyclophosphamide (IVCY) has been a recommended therapy for severe lupus nephritis or neurological flare-ups in lupus patients. But the optimal treatment regimen and duration remains unknown. DeBandt M, et.al report their experience in 1994, an open study of 37 patients treated with monthly IVCY, after six months of IVCY, a significant improvement was noticed, in terms of reduced serum creatinine and proteinuria. Serum creatinine was the best predictor of long-term renal outcome. Its usefulness was obvious at six months among clinical and biological data in patients with severe lupus nephritis or neurological flare.(1)

Based on the randomized control trials on adults published by the National Institutes of Health (NIH), most clinicians advocate the use of intravenous (i.v.) cyclophosphamide (CYP) for induction of lupus nephritis, as it has been shown to improve longterm renal outcomes (3,4). Long-term immunosuppression has been shown to improve renal survival and reduce the risk of renal flares (6,7). On another study by Niadet,P. three regimens were compared at the NIH to 65 patients with severe lupus nephritis, defined by an impairment of renal function and/or a high activity index on renal biopsy: (1) six monthly pulses of cyclophosphamide, (2) the same regimen followed cyclophosphamide pulses every 3 months for 2 additional years, and (3) six monthly pulses of methylprednisolone without cyclophosphamide. The probabilityof relapse of lupus nephritis was significantly higher in patients receiving cyclophosphamide pulses for 6 months compared with those receiving the long-course cyclophosphamide regimen (55% versus 10% after 5 years of observation).(10)

Azkenazi et.al had a retrospective review of charts of 25 patients between 1990 and 2004 who had received 9 monthly doses of cyclophosphamide induction treatment. Clinical and biopsy results greatly improved after 9 monthly intravenously administered cyclophosphamide pulses in most children with class IV Proliferative Lupus Nephritis. Those who did not improve were at risk for flares and progression of disease. The tailoring of therapies based on findings from a biopsy after induction may improve outcomes.(2)

From the study of Tangnararatchakit K, thirty one children with severe lupus nephritis who received intravenous pulse cyclophosphamide for six months have been followed-up for at least 6 months. After 3 months of treatment, most patients were clinically improved as evidenced by significant improvements in 24-hour urine protein, creatinine clearance, serum creatinine, BUN, serum albumin and C3 level. These improvements were sustained up to 18 months and renal outcome at the last follow-up (range = 6-76 months) demonstrated that twelve patients (38.7%) had complete remission, 18 patients (58.0%) still had significant proteinuria and only one had serum creatinine of 1.6 mg/dl at 42 months.(8)

This study compared the outcome of children and adolescent patients with lupus nephritis treated with 9 month induction of cyclophosphamide therapy versus 6 month induction of cyclophosphamide therapy.The frequency of occurrence of renal flare of patients with lupus nephritis treated with 6 months induction of cyclophosphamide therapy compared to 9 months therapy at the end of induction and 24 months after induction were determined.The adverse effects in induction of cyclophosphamide therapy for patients with lupus nephritis were described and determined.

METHODOLOGY

This was a retrospective cohort study conducted in a government tertiary pediatric hospital in the Philippines. Included in the study were patients less than 18 years old diagnosed

with proliferative lupus nephritis based on the World Health Organization Criteria and confirmed by Renal Biopsy and treated with either 6 months or 9 months induction of cyclophosphamide therapy from January 2002 up to December 2004 for 6 months therapy and January 2006 to Dec 2008 for 9 months therapy.

A list of patients with a diagnosis of Lupus Nephritis were generated. Group sample sizes of at least 24 and 40 achieve 80% power to detect a difference of 20 in GFR between the 9 mos and 6 months group with the estimated group standard deviation of 26.3_2 at the 5% level of significance using a one sided two sample t test.

Data present in PhililippineChildrens Medical Center medical records of patients with diagnosis of lupus nephritis from 2000-2008was reviewed. Data gathered were divided into two groups, those patients who underwent treatment of cyclophosphamide for 6 month alone and patients who continued the treatment for 9 months. Baseline characteristic of included patient such as age, gender, age of onset of lupus nephritis and glomerular filtration rate prior to induction therapy were included in data analysis.

The only difference for both protocol was only the duration of treatment. Patients at first were closely follow up every two weeks with CBC and urinalysis. All patients were advised to record in their notebook symptoms experienced after every therapy and followed up every month. After 1 month of treatment a repeat creatinine, CBC and urinalysis was requested. In case during treatment patient will experienced persistent adverse effects of cyclophosphamide such as nausea and vomiting, bone marrow suppression, stomach ache, diarrhea, darkening of the skin/nails, alopecia (hair loss) or thinning of hair, changes in color and texture of the hair, and lethargy. Patient will not be included in the study. After completion of 6 months or 9 months regimen, patient will have a series of laboratory workup such as estimated glomerular filtration rate using the schwartz formula, quantitated 24 hour proteinuria, C3, serum bun and creatinine, serum albumin and urinalysis.

For those with complete remission IV cyclophosphamide was discontinued and prednisone was taper. For those with partial remission, continue IV cyclophosphamide for 3 months and include prednisone (2mg/kg/day) for 4 weeks.

Records of subjects in each group were reviewed up to 2 years after induction.
At the beginning of induction therapy,following parameters were determined and compared at the end of induction therapy thus identify response rate of pediatric patients with lupus nephritis.The same parameter at 24 month follow up after induction therapy was reviewed to determine remission or relapse rate of patients. Death of patients within the observation period of the study was included as an outcome.

Data were described using means, standard deviations and frequency counts. T-test for both paired and independent samples were used to analyse the data. For comparison of correlated continuous data of more than two groups, ANOVA one way for correlated samples was used. McNemar's test for frequency data before and after treatment was also employed. For comparison of categorical variables, chi-square and Fischer's exact test, whichever was appropriate was used. For continuous variables of paired samples of less than or = 10, we used Wilcoxon Rank Signed test .

For all tests, a 95% confidence level was considered significant.

Table 1. Profile of Subjects

	Protocol for Cyclophosphamide Induction Chemotherapy		
	6 months	9 months	P value
Age in yrs, mean + sd	13.2 + 3.3	12.8 + 3.3	0.750
Sex			
Male	2	2	0.952
Female	21	14	
Age at onset of Lupus, in yrs	13.4 + 3.3	12.9 + 3.2	0.952
Age at onset of lupus nephritis, in yrs	13.4 + 3.3	13.4 + 3.4	0.968
Interval between onset of lupus and lupus nephritis, in yrs	0.39 years (4.7 months)	0.5 years (6 months)	0.968

RESULTS

The mean age of patients diagnosed lupus nephritis on the average was 13yrs old with gender distribution predominantly female. Age at onset of lupus and lupus nephritis was about 13 yrs old also, and this is true for both groups.

Table 2. GFR Levels After Treatment With Cyclophosphamide Induction Chemotherapy Protocols

	Protocol		
	6 months	9 months	P value
GFR			
Baseline	108.9 + 43.9	129.8 + 38.5	0.389
At 6 months post treatment	111.0 + 40.5	121.3 + 42.1	0.446
At 24 months post treatment	114.9 + 44.2	126.9 + 38.1	0.379
P value	0.592	0.446	
% increase at 6/9 months	15%	0.2%	<0.05
% increase at 24 months	23%	8%	>0.05
No. and proportion of subjects who showed > 25% increase from baseline levels			
Post treatment(6 and 9 months)	4/23	2/14	1.00
At 24 months	6/21	3/14	0.711

Table 2 shows that although the baselineGFR was lower in the 6 months Protocol group compared to the 9 months protocol group, the difference was not statistically significant. Hence, baseline level was not considered as confounding, and therefore comparison of GFR levels at 6 and 24 months was done without adjusting for baseline levels.

GFR levels of both groups did not significantly differ from each other at 6 and 24 months. Within the 6 months protocol group, GFR levels increased steadily over time, but the increases at 6 and 24 months were small and were not statistically different from baseline. For the 9 months protocol group, there was noted a decrease after 9 months, and an increase at 24 months, but this increase did not exceed baseline levels. Changes in GFR level within this group over time was not statistically significant.

With regards to percentage increase from baseline, we found significant difference between the two groups, with the 6 months protocol group showing a higher percentage increase than the 9 months protocol group (15% vs 0.2%) post treatment. At 24 months , however, no significant difference was found.

In terms of proportion of subjects who showed > 25% increase in GFR from baseline, we found no significant difference between the two groups at 6, 9 months and at 24 months.

Table 3. Serum Creatinine and Albumin Levels

	Protocol		
	6 months	9 months	P value
Creatinine			
Pre treatment	63.5 + 30.3	68.2 + 33.4	0. 648
Post treatment (at 6 and 9 months)	59 + 22.9	63.2 + 31.2	0.627
P value	0.119	0.226	
Percent decrease	6%	7%	>0.05
Albumin	N=10	N=9	
Pretreatment	17.1 + 6.6	21.8 + 9.1	0.253
Posttreatment	27.2 + 6	29.2 + 3.0	0.386
P value (Wilcoxon rank sign test)	<0.005	0.01 (one tailed)	
Percent increase	58%	33%	>0.05

- P value vertical= from t-test for matched pair
- P value horizontal = from t-test for independent samples

Table 3 shows that serum creatinine levels did not significantly differ between the two groups post treatment. Within each group, the absolute average amount of decrease was also not significant. In terms of percent decrease from baseline, again we did not find significant difference between the two groups also.

For serum albumin, we found that both protocols resulted in significant increase post treatment. The percent increase from baseline, was nearly similar and statistically, there was no significant difference between the two groups. Likewise, comparing the average serum levels of albumin posttreatment to baseline levels, no significant difference was found.

Table 4. Results of Urinalysis Pre and Post treatment

	Protocol			
	6 months		9 months	
CHON	Pre	Post	Pre	Post
0-trace	0	16	1	13
+1-2	10	1	9	1
+3-4	7	0	4	0
P value = <0.0001			P value= <0.0001	
RBC				
0-1	1	10	2	13
2-6	3	6	3	1
7-11	7	1	5	0
12-16	2	0	1	0
16+	4	0	3	0
P value=<0.0001			P value=<0.0001	

*McNemar's Test

There was marked improvement in the urinalysis results before and after treatment for both groups. Proteinuria and hematuria were markedly improved posttreatment in both groups.

Table 5. Flare-ups and Mortality

	Protocol		
	6 months N=23	9 months N=16	P value
+ Flare	5 (21%)	2 (13%)	0.677
Mortality	1	1	1.00

*Fischer's exact test

The proportion of flare-ups during the study period was higher in the 6 months protocol, but the difference was not significant. Two mortality cases were recorded, one in each group. The proportion of mortality and was not significantly different between the two groups.

DISCUSSION

This is a preliminary study done in tertiary government hospital in comparison between 9 month versus 6 month Cyclophosphamide induction chemotherapy in the management of Lupus Nephritis. The comparison of two protocols in the administration of intravenous cyclophosphamide (IVCY) did not show significant difference between the two in terms of changes in GFR levels, but we did find some evidence of a greater percent increase from baseline with the 6 months protocol post treatment. In this study a very low rate of chronic renal failure was observed. In one patient that progressed to end stage renal disease was lost to follow up for 5 months the had poor compliance to treatment due to lack of funds. According to study by Gunnarssonet. Al, prospective studies have shown that delaying the start of induction therapy more than three months after diagnosis is associated with a progression towards end stage renal disease. On the other hand, the initial response to treatment also influences the long-term evolution of the disease: complete and partial remission are accompanied by greater renal survival than in those cases with no response to treatment. Poverty may account for some of this explanation. In a population based ecological study, Ward reported that lower socio-economic areas had higher incidence of endstage renal disease due to SLE suggesting that limited access to care results in poorer SLE renal outcomes.

Creatinine, albumin and urinalysis profile among 39 subjects did not also differ between the two groups. Creatinine levels within each group changed insignificantly over time up to 24 months, but albumin levels improved significantly post treatment, for both groups.

Proteinuria and hematuria were also markedly improved posttreatment in both groups. The proportion of subjects with renal flare ups and who expired during the study period were also essentially similar between the two groups. Limitation of this study was only few numbers had quantitated 24 hour urine protein and C3 at the end of the induction therapy, some of the patients had a missed follow up schedules of protocol. In study of S. K. Annavarajula et.al, they demonstrated that a number of previously neglected or rarely studied predictors were important prognostic markers. It confirms the predictive importance of serum creatinine, 24-h urinary excretion of protein, C3, and of the activity and chronicity indices on biopsy(16).

Nausea and vomiting were nearly universal with infusion of cyclophosphamide. Hemorrhagic cystitis was not seen. No occurrence of malignancy was reported.

The proportion of flare-ups during the study period was essentially similar in the two groups. 5 of the patients had a doubling of serum creatinine and one of them was dialysis dependent. The improved outcome may have been definitely influenced by the use of cyclophosphamide. The persistence of nephritic syndrome for more than 6 months is a strong risk factor for ESRD.(17). Limitation of this study is long term follow up with patients is not done. On the other hand 2 of the patients had renal flare after 1 month post 9months induction therapy which described as proteinuria. No

literature published regarding nine months protocol.

IVCY seems efficacious if given at the very beginning of the flare and at the start after patient was diagnosed with lupus nephritis. One can gain much information by performing a protocol biopsy prior to induction and after induction therapy with mean interval of at least 2 years. Diligent follow up is needed for further studies. Its usefulness is obvious at third to six months among clinical and biological data in patients with severe lupus nephritis or renal flare. It seems that long term outcome on the renal function is not modified.

CONCLUSION

The comparison between 9 month versus 6 month Cyclophosphamide induction chemotherapy in the management of Lupus Nephritisdid not show significant difference between the two in terms of changes in GFR levels. In this study, 39 subjects were included in the study predominantly female and age of onset of lupus nephritis was 13 years old. 4 dropouts was observed, 2 for each group. Patients were lost to follow up during treatment. Other parameters such as creatinine, albumin and urinalysis profile among 39 subjects did not also differ between the two groups. Creatinine levels within each group changed insignificantly over time up to 24 months, but albumin levels improved significantly post treatment, for both groups.

Proteinuria and hematuria were also markedly improved posttreatment in both groups. The proportion of subjects with renal flare ups such as increasing in creatinine by 50 % and proteinuria in urinalysis were observed in between months of protocol weresimilar between the two groups. Nausea, vomiting and headache are among side effects observed of cyclophosphamide in both 6th and 9th month protocol. Usually observed during first month of induction and eventually outgrow as treatment proceed. Anti emetics and pain relievers were given to lessen the symptoms. No significant difference on side effects observed in both protocols.

IVCY seems efficacious if given at the very beginning of the flare and at the start after patient was diagnosed with lupus nephritis. Diligent follow up is needed for further studies.

This study may have been limited by the lack of power due to insufficient sample size. In the estimation of sample size, we assumed a difference of 20 in GFR. However, results showed a much smaller difference, so the computed sample size may have lacked power to show significant difference. We also had 4 dropouts, 2 for each group. The data on their status post treatment could have improved the analysis of this study. The retrospective nature of the study poses a limitation to follow-up. A prospective type of study is recommended wherein serum creatinine, 24-h urinary excretion of protein, C3, and of the activity and chronicity indices on biopsy will be included pre and post treatment. A diligent followup of patients will be needed for the specificity of the results. A larger sample size is also needed to show significant difference.

BIBLIOGRAPHY:

1. De Bandt M, Goycochea MV, Meyer O, Delahousse M, Palazzo E, M'Bappé P, Kahn MF. Treatment of acute systemic lupus erythematosus with intravenous infusions of Cyclophosphamide.Value and limitations. Ann Med Interne (Paris). 1994;145(2):75-87.

2. David Askenazi& Barry Myones&AnkurKamdar& Robert Warren & Maria Perez & Marietta De Guzman & Anna Minta& M. John Hicks & Arundhati Kale Outcomes of children with proliferative lupus nephritis:the role of protocol renal biopsy. PediatrNephrol 10 (2007):

3. Austin HA 3rd, Klippel JH, Balow JE, le Richie NG, Steinberg AD, Poltz PH, Decker JL. Therapy of lupus nephritis. Controlled trial of prednisone and cytotoxic drugs. N Engl J Med (1986) 314:614–619

4. Donadio JV Jr, Holley KE, Ferguson RH, Ilstrup DM (1978) Treatment of diffuse proliferative lupus nephritis with prednisone and combined prednisone and cyclophosphamide. N Engl J Med 299:1151–115

5. Bogdanovic R, Nikolic V, Pasic S, Dimitrijevic J, Lipkovska-Markovic J, Eric-Marinkovic J, Ognjanovic M, Minic A, Stajic N. Lupus nephritis in childhood: a review of 53 patientsfollowed at a single center. PediatrNephrol(2004) 19:36–44

6. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB,Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, NagataM. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am SocNephrol(2004) 15:241–25

7. Steinberg AD, Steinberg SC (1991) Long-term preservation of renal function in patients with lupus nephritis receiving treatmentthat includes cyclophosphamide versus those treated with prednisone only. Arthritis Rheum 34:945–950

8. Tangnararatchakit K, Tapaneya-Olarn C, Tapaneya-Olarn W.The efficacy of intravenous pulse cyclophosphamide in the treatment of severe lupus nephritis in children.J Med Assoc Thai. 1999 Nov;82Suppl 1:S104-10.

9. Hagelberg S, Lee Y, Bargman J, Mah G, Schneider R, LaskinC,Eddy A, Gladman

10. D, Urowitz M, Hebert D, Silverman E Longtermfollowup of childhood lupus nephritis. J Rheumato (2002) l29:2635–2642

11. Niaudet P. Treatment of lupus nephritis in children. PediatrNephrol (2000) 14:158–166

12. McCurdy DK, Lehman TJ, Bernstein B, Hanson V, King KK,Nadorra R, Landing BH Lupus nephritis:prognosticfactors in children. Pediatrics (1992) 89:240–246

13. Yang LY, Chen WP, Lin CY Lupus nephritis in children—a review of 167 patients. Pediatrics 94:335–340

14. Barbano G, Gusmano R, Damasio B, AlpigianiMG, BuoncompagniA, Gattorno M, Perfumo F. Childhood-onset lupus nephritis: asingle-center experience of pulse intravenous cyclophosphamidetherapy. J Nephrol(2002) 15:123–129

15. Keisha L. Gibson et.al Predictors of Relapse and End Stage Kidney Disease in Proliferative Lupus Nephritis: Focus on Children, Adolescents, and Young Adults.October 2009, doi: 10.2215/CJN.00490109

16. S. K. Annavarajula. The outcome of proliferative lupus nephritis with pulse cyclophosphamide therapy. Arthritis Rheum. 2002 Apr;46(4):995-1002.

17. Esdaile JM, Levinton C, Federgreen W, Hayslett JP, Kashgarian M. The clinical and renal biopsy predictors of long-term outcome in lupus nephritis: a study of 87 patients and review of the literature. 1989 Sep;72(269):779-833.