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A collage of images showing students in a laboratory setting, wearing masks and gloves, working with various equipment and materials. The text "MUSTASA TEA" is overlaid on the right side of the collage.

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Measuring the levels of knowledge and attitudes regarding advance directives of families of patients admitted in UERMMMCI: A descriptive cross-sectional study

Karlos Pio H. Alampay Airah Gizelle A. Abacan, , Jearwin C. Angeles, Jimuel D. Añonuevo, Ralph Lorenz R. Apilado, Bett Shannen M. Carpio, Monica Castro, Ma. Felilia Noela M. Cataquis, Kathleen Jessica S. Cheng, Christian Leo T. Chua, Jennifer M. Naites, MD, MSPH^a

Abstract

Introduction Advance directives are documents by which a person makes provisions for health care decisions in the event that, in the future, that person becomes unable to make those decisions. There is a lack of studies on the knowledge and understanding towards advance directives among patients and their families. The purpose of this study is to address this lack of research regarding advance directives by measuring the level of knowledge and attitudes of families of hospitalized patients.

Methods A descriptive, cross-sectional study design was used to describe the attitudes and the level of knowledge on advance directives of the families of patients. Data were collected directly by the researchers via assisted questionnaires. Descriptive statistics and frequencies were reported.

Results A total of 79 participants consisting of immediate family members of patients from UERMMMCI were enrolled. Only 24% reported having discussed advance directives with the patient's physician. Those respondents whose families had no discussion with their physician about advance directives had the same score as those who had. Overall, 61% of participants have only medium to low knowledge of advance directives, while 70% have positive attitudes regarding advance directives.

Conclusion The study showed that the family members of patients had a reasonable understanding of advance directives in terms of basic knowledge, and positive attitudes on advance directives. Those who denied having discussed advance directives were comparable in the knowledge of advance directives with those who did.

Keywords: Advance directives, knowledge, attitude

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An advance directive is an intrinsic component of end-of-life care programs in many developed countries. It is a document that specifies a person's preferences for treatment, should that person lose the capacity to make treatment decisions in the future.¹ Advance directives have been most commonly used in end-of-life care settings to direct medical care treatment decisions.² Advance directives consist of a living will and a durable power of attorney.³ A living

will is a written document that expresses a preference for or against specific types of treatment; while a durable power of attorney is a document that empowers an individual surrogate to assume decision-making authority as soon as the patient loses decisional capacity because of injury, illness, or diminished capacity. These are two distinct means designed to safeguard autonomous choice in patients who are incapable of participating in the decision to use life-sustaining interventions when necessary. Participation of patients in decision making is an essential element which helps in empowering patients. If proper and adequate participation of patients in hospital care is achieved, this would lead to more appropriate and cost-effective services, and ultimately enhanced health outcomes, quality of life, and satisfaction of patients.⁴

Although advance directives and protocols do exist in the country, there is a lack of knowledge and awareness of their availability and implementation. A law is yet to be passed regarding advance directives.⁵ There is also a dearth of research and dissemination of information regarding advance care directives in the Philippines.

This lack of knowledge regarding advance directives is a problem not only for chronically ill and dying patients but also for the general population. Discussing end-of-life issues is something one should not avoid nor be afraid of. As family is almost always involved, they should be informed with regards to decisions such as advance directives. Being knowledgeable about the benefits of having advance directives would help improve the treatment planning and the participation of the patient and his/her family in these plans. The purpose of this study is to measure the level of knowledge and attitudes of families of hospitalized patients.

Methods

A descriptive, cross-sectional study design was used to describe the attitudes and the level of knowledge on advance directives of the families of patients confined in the Medical, Neurology and Surgical Wards of UERMMMCI using an assisted questionnaire patterned from similar studies which was pretested prior to data collection.^{4,6-8}

The participants of the study were immediate adult family members or legal guardians of admitted

patients who were involved in the care of the patient. The study was limited to only the service wards of the Departments of Medicine, Neurology, and Surgery, respectively. The study involved a purposive sampling design. A sample size of 71 participants was calculated based on a previous study.⁹

The questionnaire consisted of eight items on knowledge and six items on attitude. The questions on knowledge covered definition of terms, patient rights about advance directives, and the logistics and legal implications of advance directives. The questions on attitude were on the participants' trust in physicians and the ability of the family to respect and understand patient decisions. Higher scores were interpreted as high level of knowledge and satisfactory attitude, respectively.

Results were analyzed using SPSS version 23. Standard deviations of the items related to attitudes regarding advance directives were taken. Results were coded, and descriptive statistics and frequencies were tabulated. The researchers transmuted those negatively worded statements. A score of more than 3 and less than 5 would indicate a neutral attitude. A score of 5 or more would indicate a positive attitude, and more trust in families and in the health care system to fulfill patient autonomy, while a score of 3 or less would indicate a negative attitude, with the respondent seeing little need for advance directives.

Results

Out of the 104 recruited participants, 79 met the inclusion criteria. The participants' characteristics are presented in Table 1. Most of the participants were female and married. Majority were at least 31 years old, Catholic, with at least high school education and were classified as poor. The participants that comprised the largest group among family members were the spouses of the patients (40%). Most of the participants had not had discussions on advance directives with their physician (76%).

The levels of knowledge regarding advance directive are presented in Table 2. Males and females are equally knowledgeable. Patients' parents are the most knowledgeable on advance directive. Those respondents whose families had no discussion with their physician about advance directives had the same score as those who had. Respondents also scored the

same regardless of the wards their patients were admitted. Although the mean score of the 79 participants showed adequate knowledge (5.21 ± 1.23), 31 (39%) had a high knowledge score of 5 or more, while more than half (53%) had medium knowledge (score more than 3 but less than 5) and six (8%) had a low knowledge score of 3 or less.

Table 1. Sociodemographic profile of 79 respondents.

Demographic Characteristic	Frequency (%)
Sex	
Male	31 (39.2)
Female	48 (60.8)
Age (yr)	
18-30	15 (20.0)
31-40	25 (33.3)
41-50	20 (26.7)
51-60	15 (20.0)
≥ 61	4 (5.1)
Marital Status	
Single	25 (31.6)
Married	46 (58.2)
Widowed	4 (5.1)
Divorced/separated	4 (5.1)
Education	
No formal education	1 (1.3)
Grade school	3 (3.8)
High school	34 (43.0)
College	35 (44.3)
Postgraduate	6 (7.6)
Religion	
Roman Catholic	69 (87.3)
Others	10 (12.7)
Financial status (estimated gross monthly income)	
Less than 7,890 (E)	50 (63.3)
7,890 - 31,560 (D)	28 (35.4)
31,560 - 78,900 (C)	1 (1.3)
Relationship with patient	
Spouse	32 (40.5)
Parent	18 (22.8)
Child	16 (20.3)
Sibling	13 (16.4)
Discussion with physician	
Yes	19 (24.1)
No	60 (75.9)
Ward admitted	
Medicine	28 (35.4)
Neurology	25 (31.6)
Surgery	26 (33.0)

The attitudes regarding advance directive are presented in Table 3. Females had a higher median attitude score than males, and 41-50-year-old

Table 2. Levels of knowledge regarding advance directives of 79 respondents.

Demographic Characteristic	Frequency (%)	Mean Average Score \pm SD
Sex		
Male	31 (39.2)	4.84 \pm 1.42
Female	48 (60.8)	5.46 \pm 1.03
Age (yr)		
18-30	15 (20.0)	4.73 \pm 1.39
31-40	25 (33.3)	5.64 \pm 1.25
41-50	20 (26.7)	5.05 \pm 2.81
51-60	15 (20.0)	5.13 \pm 2.56
≥ 61	4 (5.1)	
Marital status		
Single	25 (35.2)	5.16 \pm 1.40
Married	46 (64.8)	5.22 \pm 1.11
Education		
High school	34 (43.0)	5.53 \pm 1.31
College	35 (44.3)	5.03 \pm 1.10
Others	10 (12.7)	
Religion		
Roman Catholic	69 (87.3)	5.19 \pm 1.22
Others	10 (12.7)	
Financial Status (estimated gross monthly in-come in PHP)		
Less than 7,890 (E)	50 (63.3)	5.20 \pm 1.12
7,890 - 31,560 (D)	28 (35.4)	5.21 \pm 1.42
31,560 - 78,900 (C)	1 (1.3)	6.00
Relationship with patient		
Spouse	32 (40.5)	5.03 \pm 1.26
Parent	18 (22.8)	5.61 \pm 0.78
Child	16 (20.3)	5.56 \pm 1.26
Sibling	13 (16.4)	4.69 \pm 1.44
Discussion with physician		
Yes	19 (24.1)	5.16 \pm 1.17
No	60 (75.9)	5.23 \pm 1.42
Ward admitted		
Medicine	28 (35.4)	5.46 \pm 1.03
Neurology	25 (31.6)	5.16 \pm 1.25
Surgery	26 (33.0)	5.00 \pm 1.38
Total		5.21 \pm 1.23
High knowledge (5)	31 (39.2)	
Medium knowledge (>3 and <5)	42 (53.2)	
Low knowledge (<3)	6 (7.6)	

participants had the highest median score among the age groups. Married individuals, mostly the spouses of the patients (70%) had higher positive attitude score than the single members. Although there was no difference in the median attitude scores between

Table 3. Attitudes regarding advance directives of 79 participants.

Demographic Characteristic	Frequency (%)	Median score
Sex		
Male	31 (39.2)	5.08
Female	48 (60.8)	5.50
Age (yr)		
18-30	15 (20.0)	5.00
31-40	25 (33.3)	5.42
41-50	20 (26.7)	5.67
51-60	15 (20.0)	4.83
Marital status		
Single	25 (35.2)	5.33
Married	46 (64.8)	6.00
Education		
High school	34 (43.0)	5.33
College	35 (57.0)	5.17
Religion		
Roman Catholic	69 (87.3)	5.33
Others	10 (12.7)	
Financial status (estimated gross monthly in-come in PHP)		
Less than 7,890 (E)	50 (63.3)	5.33
7,890 - 31,560 (D)	28 (35.4)	5.50
31,560 - 78,900 (C)	1 (1.3)	
Relationship with patient		
Spouse	32 (40.5)	5.50
Parent	18 (22.8)	5.00
Child	16 (20.3)	5.25
Sibling	13 (16.4)	5.00
Discussion with physician		
Yes	19 (24.1)	5.33
No	60 (75.9)	5.50
Ward admitted		
Medicine	28 (35.4)	5.50
Neurology	25 (31.6)	5.50
Surgery	26 (33.0)	5.25
Total		5.33
Positive attitude	55 (69.6)	
Neutral attitude	18 (22.8)	
Negative attitude	6 (7.6)	

respondents whose families had discussion with their physician about advance directives, results showed that 55 (70%) had positive attitudes about advance directives, while six (8%) had negative overall attitudes about them and eighteen (22%) were about neutral.

The participants' knowledge on advance directives is presented in Table 4. Among the four terms defined, only a few participants have correct knowledge on the definition of advance directives (15%), while majority have correct living will (87%), durable power of attorney (75%) and "do not resuscitate" order (76%). Among the four statements regarding knowledge of logistics, most of the

Table 4. Proportion of participants with correct answers of knowledge items.

Knowledge of Terms	Frequency (%)
An advance directive expresses the preferences of an individual via verbal communications only, for future health and personal care, and helps prepare people for health-care decision making in times of medical crisis.	12 (15)
A living will is a written document that expresses the preference for or against specific types of treatment.	69 (87)
A durable power of attorney in healthcare is a document that empowers a group of people to assume decision making authority as soon as patient loses decisional capacity.	59 (75)
A Do Not Resuscitate order (DNR) states a patient's decision regarding the desire to avoid cardiopulmonary resuscitation.	60 (76)
Knowledge of Logistics	
Patients have a right to accept or refuse medical or surgical treatment	74 (84)
Creating a document that provides directions for end-of-life or life-sustaining care requires the involvement of a lawyer	39 (49)
Once someone has documented their wishes for end-of-life or life-sustaining care, he/she cannot change his/her mind	37 (47)
More than 1 person may be named to speak on behalf of an individual regarding end-of-life care wishes in the event he/she is unable to speak for himself/herself.	62 (78)

participants (94%) know that patients have a right to accept or refuse medical or surgical treatment while almost half of them (49%) do not know that "creating a document that provides directions for end-of-life or life-sustaining care does not require the involvement of a lawyer" and thought that "once someone has documented their wishes for end-of-life or life-sustaining care, they cannot change their mind (47%).

Participants' attitude on advance directive are presented in Table 5. Majority of the participants have positive attitudes such as understanding of the patient's wishes for life-sustaining treatment, the patient being able to communicate his/her wishes even if family did not totally agree and relying on the ability of the physicians to carry out wishes for life-sustaining treatment." However, 39% of the respondents felt that "patients should NOT trust the medical system to make decisions about their medical care if they should become unable to communicate their wishes," while 19% were neutral about it. Forty three percent of the respondents felt that discussing wishes for life-sustaining treatment with family would only lead to disagreement and conflict, while an equal percentage felt otherwise. Only 14% were undecided.

Discussion

Out of the 79 participants enrolled in the study, only 24% reported having discussed advance directives with the patient's physician. This low report of discussion of advance directives is consistent with studies that there is a clear lack of knowledge or awareness regarding advance directives.⁹⁻¹¹ Surprisingly, the proportion of those who reported having discussed advance directives with their physician is similar to those reported in studies in countries where advance directives are practiced.⁴ This may be due to the fact that Filipinos tend to avoid disagreeing with more authoritative groups, leading to bias, with the participants simply answering what they thought the researcher may have wanted to hear.⁹

The participants demonstrated a generally positive attitude towards advance directives. Despite only low to intermediate scores (61% with scores less than 5 out of 8), majority (70%) had positive attitudes with regards to advance directives, and only a few (8%) had negative views. This current study rated similarly or even higher than those by a previous study.⁷

All these suggest that though only a small proportion of participants have had discussion of advance directives, it showed display of good trust

Table 5. Attitudes of participants toward advance directives.

	Answered negatively to the item (≥3)	Had neutral attitudes about the item (4)	Answered positively to the item (≥5)
Knowledge of Terms			
Patients should NOT trust the medical system to make decisions about their medical care if they would become unable to communicate their wishes.	33 (42%)	15 (19%)	31 (39%)
Discussing wishes for life-sustaining treatment with family would only lead to disagreement and conflict.	34 (43%)	11 (14%)	34 (43%)
My family understands the patient's wishes for life-sustaining treatment.	8 (10%)	5 (6%)	66 (84%)
The patient can rely on his/her family to communicate his/her wishes even if his/her family does not all agree with his/her wishes.	11 (14%)	9 (11%)	59 (75%)
Physicians clearly understand the patient's wishes for life-sustaining treatment.	8 (10%)	6 (8%)	65 (82%)
The patient can rely on physicians to carry out wishes for life-sustaining treatment even if he or she disagrees with the patient's wishes.	10 (13%)	11 (14%)	58 (73%)

in their families and health care providers to respect health care decisions and patient autonomy. This is similar to a study in that, despite the low number of patients with knowledge about advance directives, many more were interested in advance directives.¹¹ Similarly, in another study, even if most of the participants had never heard of advance directives but after being informed of what advance directives were, they showed positive attitudes toward advance directives.⁸

In this current study, most had the misconception that advance directives could be communicated verbally only. This is similar to a study where patients wanted to verbally express their directives rather than use written documentation and that the involvement of family or a physician was desired.⁶ In the statement "creating a document that provides directions for end-of-life or life-sustaining care requires the involvement of a lawyer," a little more than half believed creating a document requires the participation of a lawyer, when in truth it does not. This misconception that legal consultation is necessary may be a barrier to availing of advance directives because of socioeconomic reasons. It is also consistent with a study wherein about one-third reported that they believe that initiation of an advance directive requires a lawyer.⁷ More than half believe that advance directives are final and binding. This may also be a possible barrier because they may be hesitant to commit to an end-of-life decision. This is also reported in another study where respondents believe that advance directives may influence the course of treatment.¹²

In this study, the researchers concluded that only relatively few participants were informed about advance directives, but the proportion still exceeded the expected outcomes. This is consistent with another study which suggested that nurses and physicians had little interest in educating patients about advance directives.⁹ Time limitations, difficulty bringing up and dealing with the subject matter, and possible legal ramifications were identified as major reasons why physicians do not usually discuss advance directives. However, the study also shows that majority of the family members have a reasonable understanding about some terms regarding advance directives but are not fully knowledgeable about the process of availing of them.

The scope of the study was limited to the immediate families of patients who were not critically

ill admitted specifically in the Medical, Neurology, and Surgery Wards. The study focused on families and not the patients themselves due to ethical concerns of involving at-risk patients. Second, advance directives are decided on by the patient, but the family should be informed about it since major decisions in Filipino culture are usually discussed as a family. Finally, the family members are also the ones called on to be health proxies, so they should be able to understand what is being asked of them and what the patient wants.

The topic of advance directives in the Philippines shows promise as a largely unexplored field both legally and in the academe. The researchers recommend studies to be conducted, involving a larger number of hospitals to get a representation of advance directives in the Philippine healthcare system. Future studies should also focus on more vulnerable patients who are more likely to avail of advance directives such as cancer patients, heart failure patients, and the elderly, because they are most likely to be informed by their doctors about advance directives, would reduce confounding variables and have better homogeneity of the sample.

Inconvenient as it may sound, death is a reality for patients with diseases with high mortality rates, which is why planning out advance directives, while difficult to talk about, is important when discussing the patient's future care. For a patient's condition wherein a terminal phase is probable, a discussion with the patient's doctor about future care and advance directives, ideally should come up at some point. The researchers recommend that patients be better informed and educated about their rights and privileges, not only to improve their health but also to improve the service of physicians and health care providers.

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Effectiveness of neem seed oil methanolic extract shampoo versus permethrin shampoo in the reduction of head lice infestation in children*

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Abstract

Introduction This study compared the effectiveness and safety of neem (*Azadirachta indica*) seed oil methanolic extract shampoo against permethrin shampoo in reducing head lice infestation among children.

Methods A single-blind, non-inferiority, randomized clinical trial was conducted on children aged 6-14 years with pediculosis. Using block randomization, the participants were assigned to receive either 10% neem seed oil methanolic shampoo, 1% permethrin shampoo, or pure shampoo for three treatment applications at 10-day intervals. The presence of head lice after each application was determined by standard quadrant counting and compared with the baseline count within and among treatment groups.

Results There was a statistically significant difference in lice count after treatment for both neem and permethrin, with mean reductions of 17.8 ± 23.97 ($p = 0.043$) and 22.5 ± 23.47 ($p = 0.014$), respectively. Repeated Measures ANOVA showed a summary p-value of 0.041 for neem, 0.013 for permethrin, and 0.193 for the shampoo alone with a linear trend indicating a significant decrease in the lice counts from the baseline to the third application of neem and permethrin shampoo, but not in the shampoo group. There was no significant difference in the mean decrease in lice count from baseline to the third application between the neem and permethrin shampoo groups.

Conclusion Neem seed oil methanolic extract shampoo is non-inferior and comparable to permethrin in the reduction of head lice count. There were no reported dermatologic adverse effects such as burning sensations, redness, skin irritation, and allergic reactions.

Keywords: Pediculosis, neem seed shampoo, *Azadirachta indica* methanolic seed extract shampoo

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Head lice infestation remains to be a worldwide health concern that affects all age groups, especially preschool and elementary-age children. Females are infested more often than males, probably due to more frequent head to head contact, length of hair and autonomy in their bath and body care, and common behaviors such as sharing of hair ornaments, combs and brushes.¹ In tropical countries like the Philippines, where head lice infestation is a common health problem, environmental factors such as air temperature and humidity influence the longevity and fecundity of *P. humanus capitis*,

particularly the propagation of lice populations and their dispersion.²

Pediculosis has been treated by methods that include the physical removal of lice, various domestic treatments, and conventional insecticides.³ Commercially available medicated shampoos are often used as treatment of pediculosis for infestations not manageable by manual nit and lice removal because of their affordability and accessibility. Kwell Shampoo™ a pediculocidal shampoo considered as standard of treatment, contains the active ingredient permethrin. Permethrin is a synthetic pyrethroid proven to kill lice and nits quickly and exhibits extremely low mammalian toxicity. The potent insecticidal action of permethrin arises from repeated neuronal depolarization, leading to a reduction in neuronal excitability causing sensory hyperexcitability, incoordination, prostration, paralysis and eventual death.⁴ However, evidence shows that the extensive use of commercial pediculicides has resulted in resistance and cross-resistance.⁵ Non-toxic alternatives are hence needed for head lice treatment and prevention, and plant-derived natural products may offer safer alternatives with good pediculocidal activity.

Among these natural products is neem (*Azadirachta indica*) which contains azadirachtin, a complex tetranortriterpenoid limonoid found to cause toxicity to a wide spectrum of agricultural and household insects such as lice. Neem seeds with azadirachtin are comparable to synthetic chemicals in terms of efficacy and are deemed safe for human use according to previous studies. In addition, neem has added components such as azadirachtin B, nimbin, and salannin, which prevent the development of lice resistance against these products.⁶ These natural products are useful sources of bioactive components in the development of new drugs and pharmaceutical targets due to their low toxicity to mammals and easy biodegradability.

The aim of this study was to determine the effectiveness of neem (*Azadirachta indica*) seed oil methanolic extract shampoo compared with permethrin in the reduction of head lice, and to determine the occurrence of adverse effects with its use.

Methods

The study utilized a single-blind, non-inferiority, randomized dual controlled clinical trial on a study

population of males and females aged 6-14 years old from Barangay San Perfecto, San Juan City that was selected by purposive sampling. Mass screening was conducted through a house-to-house survey among school children belonging to the target age group in the identified barangay. The screening process included subjects who had eggs, nits, nymphs and adult lice. Excluded from this study were those who had 1) been previously treated with any pediculocidal solution within the last three months prior to the study, 2) erythema, scalp wounds and alopecia, and 3) hypersensitivity to the 1% permethrin treatment.

The presence of head lice was confirmed with a nit comb after thorough combing and detangling of dry hair with a wide-toothed comb. Having at least one head louse was considered positive for pediculosis infestation. A baseline lice count using a standard quadrant counting was performed prior to the administration of the treatment. Each subject's hair was partitioned into four major quadrants (Q): Q1 at the top of the head, Q2 and Q3 behind the ears and Q4 at the back of the head/above the nape. The hair was combed using a wide-toothed comb to detangle, followed by the use of the nit comb over the Q4.

The minimum required sample size of ten participants per treatment group was computed using the formula for the estimation of population mean using a 95% alpha error and 80% beta error and a mean and standard deviation of 1.96 with a margin of error of 10%, based on a previous study regarding cashew nut and its effect in reducing pediculosis.⁷ A total of 30 participants was divided equally into three treatment groups.

The neem seeds utilized in this study were collected from the Bureau of Plant Industry in Manila. The leaves and seeds were collected, air dried, securely stored in resealable plastic bags and transported to the Division of Botany and Zoology of the National Museum of the Philippines for identification and authentication of plant species, as part of the requirements for herbal research. The seeds were then submitted to the Department of Science and Technology (DOST) Central Office in Taguig City for plant extraction and shampoo formulation. Under the protocols of the Chemicals and Energy Division-Pharmaceuticals Section of the DOST Central Office, the seeds were dried in an incubator at 60°C for 3 hours. The shells were removed to obtain the kernels inside which were then ground

and utilized for the extraction of the active component of neem seeds, azadirachtin. A suspension of 250g of ground neem seed kernel in one liter of hexane was stirred at 40°C for 2 hours and filtered. The hexane extract was concentrated in vacuo to give 106g of neem oil. The defatted marc was then extracted with one liter of methanol in the same manner as n-hexane extraction. The methanolic was dissolved in 100mL of 90% aqueous methanol and partitioned twice with 50mL hexane to remove remaining oil and other non-polar compounds. Mixtures were then filtered using Whatman No.2 filter paper. The solvent was removed from the oil using a rotary evaporator. The extracted oil was stored in aliquots of 10 mL vials and stored at 4 °C until use. A concentration of neem seed oil:shampoo at a dilution of 1:10 mL/mL was made as this was the concentration needed to exert its pediculocidal effect. This was assigned as Treatment A for this study.

The negative control, Treatment C, was the pure (pearlized) shampoo provided by the DOST, identical to the one used as the carrier shampoo for the neem seed methanolic extract. It contained a pearlizing agent known as glycol stearate which acts as an 1) opacifying agent, making it less transparent and giving the shampoo a pearly appearance, 2) skin conditioner to smooth and soften the tissue, 3) emulsion stabilizer which helps to hold oil particles in the water solution to prevent production separation, and 4) thickener. None of the said ingredients has any pediculocidal effect. The pediculocide shampoo Kwell™, with 1% permethrin (10 mg/g) as its main active ingredient, was used as Treatment B, the positive control.

Using block randomization technique, the participants were randomly assigned to their treatment groups through a computer software randomizer. The 10% neem seed oil methanolic shampoo treatment was given to Group A, permethrin shampoo was given to Group B as positive control, and pearlized shampoo was given to group C as negative control.

A regular comb, a fine-toothed nit comb, and a shower cap were provided for each participant in this study. Hair washing, and administration of treatments were done by the investigators to ensure proper administration of the treatments. The treatment was kept on the hair for about 10-15

minutes during each application before it was thoroughly rinsed with water. The counting of head lice using the standard quadrant counting was done using a fine nit comb after every treatment application. Results in each quadrant were based on lice yield after stroking thrice using a fine-toothed nit comb. The number of live lice was then counted and recorded. Observation and documentation of any dermatologic side effects were also done by the investigators, participants, and their parents or guardians. Treatments were given at 10-day intervals for three applications. A 10mL solution of the respective shampoos were applied per treatment day, for a total of 30mL of shampoo applied throughout the study, in all treatment groups. At the end of the study, the Kwell™ permethrin shampoo was distributed and applied to groups A and C as standard treatment for pediculosis. Health education on pediculosis was also conducted to increase awareness and improve hygiene practices.

The study was approved by the Ethics Review Committee of the Medical Center. Written informed consent from the parents or guardian of the children, and assent from the children, as applicable, were obtained. Measures were taken to ensure confidentiality and to protect the privacy of the participants.

Results

Among 36 potential participants screened, 30 children were included in the study and randomly assigned to one of three treatment groups. The mean age of the participants was 10.2 years; more than 80% were girls and 80% were in Grades 1 to 6. All participants except two belonged to socioeconomic classes D and E. There was no statistically significant difference among the three treatment groups in terms of age, sex, grade level and socioeconomic status as seen in Table 1.

Compared with baseline, all groups showed a reduction in lice count following the first week of application, with the greatest reduction seen in the permethrin group. On Weeks 2 and 3, the permethrin and neem group continued to demonstrate a reduction in lice count. The group that received pearlized shampoo had an increase in lice yield on the third week. Repeated measures ANOVA showed that there was a significant decrease in the number of lice from baseline to Week 3 in the neem and

permethrin groups, with summary p-values of 0.041 and 0.013, respectively. There was no significant change in the pearlized shampoo group (Figure 1).

There was a decrease in the lice count from baseline to the third application in both the neem shampoo and the permethrin shampoo groups. The differences between the mean lice count in the two

groups at Applications 1, 2 and 3 were not statistically significantly (Table 2). Upon completion of treatment, paired t-test showed a statistically significant difference of lice count from baseline to Application 3, in both the neem shampoo and permethrin shampoo groups (Table 3). Independent t-test showed no statistically significant difference between the

Table 1. Comparison of demographic characteristics of 30 participants in neem seed and permethrin shampoo groups.

Characteristics	Neem seed shampoo	Permethrin shampoo	Pearlized shampoo	p-value
Age (yr)				
Mean	9.4	10.4	10.8	0.309 ^a
6-8	3	2	2	
9-11	6	6	4	
12-14	1	2	4	
Sex				0.383 ^b
Male	1	1	3	
Female	9	9	7	
Grade level				0.506 ^a
Preschool	2	0	0	
Grades 1-3	4	3	4	
Grades 4-6	3	5	5	
Grades 7-10	1	2	1	
Socioeconomic status				0.733 ^a
AB	0	0	0	
C	1	0	1	
D	5	5	3	
E	4	5	6	

^aOne-way ANOVA

^bChi-square test

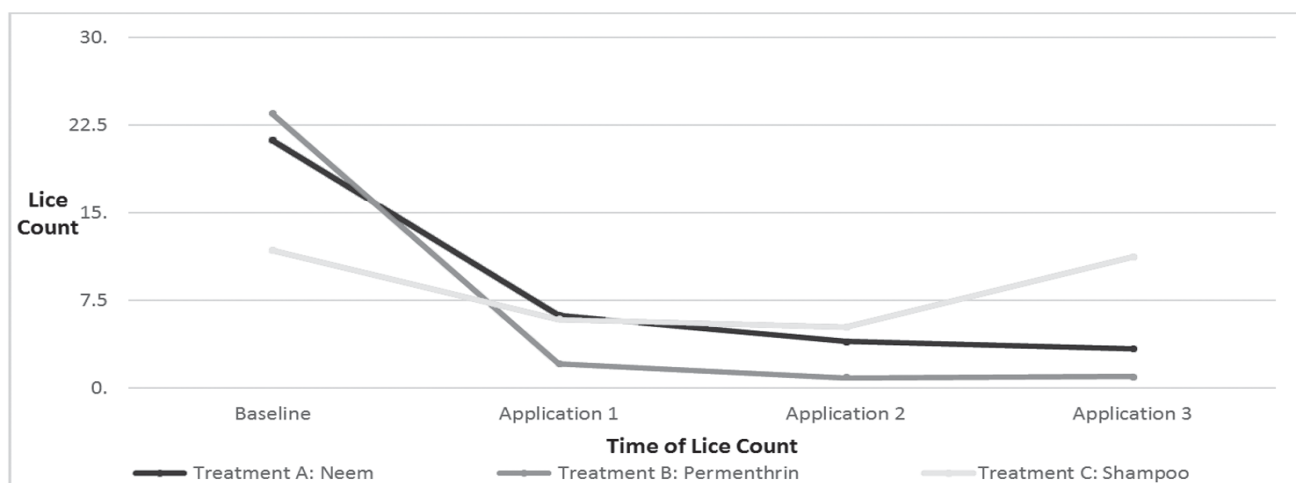


Figure 1. Mean count of head lice per treatment group at the baseline and after each treatment application.

reduction of head lice count after completion of treatment in the neem shampoo and permethrin shampoo groups as seen in Table 4.

No dermatologic adverse effects such as burning sensation, redness, skin irritation, allergic reactions or other adverse reactions were reported by the participants or observed by the investigators in any of the treatment groups.

Discussion

The larger percentage (83.3%) of female participants reflects the findings of Canyon and Meinking that pediculosis frequently affects girls more than boys.^{8,9} Most of the subjects were familiar with each other as playmates and neighbors and were therefore prone to increased head-to-head contact. Having longer hair in females and sharing hair accessories (especially among the siblings in the current study participants), also increases the odds of transmission of head lice from one host to the other.¹⁰ However, whether hair length indeed affects pediculosis capitis infestation is still debated upon in the literature. The participants had also recently resumed their classes

during the study, which is consistent with the findings of Canyon that the highest rates of pediculosis occur from April to August where transmission is higher due to clustering of the children at a common site.⁸ Abd El Raheem identified hygienic practices and overcrowding to be specific determinants influencing pediculosis prevalence.¹¹ However, the researchers were not able to obtain an average number of household members for the barangay, households of the study participants nor a baseline measure of the participants' hygienic practices, which could have supported this finding. Majority of the study participants belonged to the D and E socioeconomic strata, which reflects the findings of Willems that socioeconomic status is a significant factor in the increased incidence of pediculosis in children.¹²

The difference in effect of permethrin and neem shampoo on head lice reduction was not statistically significant, which may reflect that neem shampoo produces a comparable reduction of head lice to the standard treatment. These are consistent with the findings of Schmall that neem seed extract shampoo is pediculocidal and ovicidal.¹³

Table 2. Comparison of mean lice count in the neem seed and permethrin shampoo groups.

	Neem seed shampoo	Permethrin shampoo	p-value
Baseline	21.2 ± 8.41	23.5 ± 7.37	0.839
Application 1	6.2 ± 2.84	2.1 ± 0.78	0.497
Application 2	4.0 ± 1.5	0.9 ± 0.5	0.611
Application 3	3.4 ± 1.6	1.0 ± 0.7	0.663

Table 3. Comparison of reduction in lice count with from baseline to third application in the neem and permethrin shampoo groups.

	Baseline	Trial 3	Difference	p-value
Neem seed shampoo	21.2 ± 26.61	3.4 ± 5.28	17.8 ± 23.97	0.043
Permethrin shampoo	23.5 ± 23.30	1.0 ± 2.31	22.5 ± 23.47	0.014

Table 4. Comparison of mean differences from baseline to Application 3 between neem and permethrin shampoo.

	Mean	Standard deviation	Std. Error Mean	p-value
Neem seed shampoo	12.30	20.78	4.64	0.994
Permethrin shampoo	12.25	19.82	4.43	

The neem tree (*Azadirachta indica*), from the Meliaceae family, originally grown in East India and Burma, is considered as one of the most versatile plants for its therapeutic, ethnomedicinal, and cosmetic values, rendering it as the "wonder tree" of India.¹⁴ Due to its wide range of uses, it has been included in the top 10 list of plants studied and used for development by the international scientific community. The WHO has also identified this plant as an environmentally powerful natural pesticide for its potential use in pest management, environment protection, and medicine.¹⁴

According to Asher, the neem tree has a wide spectrum of activity which includes anti-helminthic, anti-feedant, parasiticide, insecticide and pediculocidal properties.¹⁴ The plant's seed is enclosed by a sweet pulp containing 1-3 kernels, in which azadirachtin, a biologically active compound known to be toxic to insects, is contained. Azadirachtin is a complex tetranortriterpenoid limonoid, and this compound affects both physiologic and behavioral aspects in a wide variety of insects: anti-feedancy, severe growth reduction, increased mortality, and abnormally delayed molts. Neem seeds also contain other triterpenoid components such as 3-tigloylazadirachtin (azadirachtin B), nimbin, and salannin. These components have an additive effect when combined with azadirachtin and are useful in preventing resistance.⁶

According to Schmall, another important effect of neem is stunting in the development of immature insect stages.¹⁵ Due to the reduced food intake by adults, postembryonic development of species is delayed. Neem also causes disruption of the endocrine system leading to disturbances of molting, pupation, and adult emergence. The reproduction of insects is also greatly affected, due to egg sterility and shortening of the longevity of male and female insects.

Deshmukh showed neem extract flowing into the opening of the aeropyles of the eggs, and slowly surrounding its surface.¹⁶ It also penetrated the lateral tracheal openings in the motile stages of the lice. In the adult and larval stages, the tracheoles or the insect's respiratory tubes were also clogged. By covering the tracheoles and aeropyles, oxygen transfer between the water layer and the cell was essentially blocked resulting in the disruption of oxygen uptake by the insect's body muscles, heart, and other organs. Within just a short period, oxygen became depleted while

the amount of lethal carbon dioxide increased. Sinha showed that 5 to 10 minutes of incubation with neem extract produced significant larvicidal and pediculocidal effects.¹⁷ This protocol was implemented in the current study, and similar results were obtained among the 10 children utilizing neem shampoo.

The study of Schmall on the efficacy of neem extract showed that it was highly effective against all stages of head lice, with less side effects compared to the chemical based standard shampoo. The study tested a neem-based anti-lice shampoo as a single treatment vivo and in vitro. After a short incubation period of just 10 minutes, results show that none of the lice survived after a 22-hour observation and after 7 days of treatment. A second group of children was treated for 20 minutes with identical results. In vitro results of the said study show that only three minutes of submersion was needed to effectively kill both larval and adult stages of head louse. Both in vivo and in vitro results did not reveal freshly hatched larval stages of lice, suggesting the product's ovicidal activity.¹⁵ Asher likewise demonstrated that a 1:10 dilution of the neem extract shampoo was sufficient to kill head lice in vivo within three minutes, or within 10-15 minutes within the wet hair of children.¹⁴

No side effects such as burning sensations, redness, skin irritation, allergic reactions or alopecia were observed and reported in any of the 10 children using neem seed extract shampoo, replicating the findings of Asher and Trob that neem seed extract shampoo has no associated toxicological risks and is suitable for human use.¹⁴

The overall results of the trials indicate that neem seed oil methanolic 1:10 mL/mL shampoo given at 10-day intervals for three applications is non-inferior and comparable in lice reduction to the standard treatment, permethrin 1% shampoo. There were no reported dermatologic adverse effects such as burning sensation, redness, skin irritation, allergic reactions based on the observation and feedback from subjects. The study offers support in the potential application of natural compounds for lice infestation reduction.

Factors such as physical removal of lice, difference in the hair length, reinfestation from other family members within the household and hygiene practices of each subject could have affected the results but were beyond the control of the researchers,

thus it is recommended that the future studies should consider and control such factors when feasible.

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Association between parental authority prototype and perceived self-esteem of adolescent nursing students at UERMMMCI

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Abstract

Introduction Parental authority prototype may greatly influence how adolescent nursing students can demonstrate skills on how they provide quality nursing care to patients in the future. This study aimed to determine the association between parental authority prototype and perceived self-esteem among adolescent nursing students.

Methods The researchers administered the Parenting Authority Questionnaire and the Rosenberg Self-Esteem Scale to nursing students 16 to 19 years old to determine the maternal and paternal parenting types of the respondents and their level of self-esteem, respectively. Chi-square was used to determine the association between parenting style and self-esteem.

Results Normal levels of self-esteem were seen in 70% of respondents and almost one-third were considered to have low self-esteem. The most common parenting style among both the respondents' mothers and fathers was authoritative. There were more authoritative mothers than fathers and twice as many authoritarian fathers than mothers. Paternal parental authority prototype was associated with the respondents' self-esteem ($\chi^2 = 19.19$, $p < 0.05$) but the maternal authority prototype was not.

Conclusion Paternal, but not maternal, parental prototype is associated with the perceived self-esteem of adolescent nursing students. The most common parenting style was authoritative for both mothers and fathers.

Keywords: Parental authority prototype, perceived self-esteem

Parental authority prototype or parenting style refers to the actions and reactions of parents

towards their children and includes their beliefs, support and expectations, and how they discipline their children. The term "parenting style" was coined by Baumrind in the 1970s.¹ She interviewed and observed parents and children and concluded that there are three types of parenting based on levels of demandingness (control, supervision, maturity demands) and responsiveness (warmth, acceptance, involvement). Based on this study, there are three distinct prototypes of parental authority: permissive, authoritarian, and authoritative.¹ These parental authority prototypes

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play a pivotal role in the development of children and adolescents.

Authoritarian parenting involves having strict rules that must be followed, and adolescents are punished if those rules are not followed. The punishment is usually harsh and abusive, and may be physical and/or emotional. This style is low in parental responsiveness yet highly demanding.¹ Authoritarian parents are not very emotional or affectionate; they exhibit low levels of trust and interaction with their children, discourage open communication, and engage in strict control.² Adolescents from most authoritarian families have poor social skills, low levels of self-esteem, and high levels of depression.³

Authoritative parents are high in responsiveness and highly demanding, and exhibit more supportive than harsh behaviors.⁴ These parents provide consistent boundaries, clear and appropriate expectations, communicate well, listen to their children, and adapt to different situations. This parenting style is most often associated with positive adolescent outcomes and has been found to be the most effective and beneficial style of parenting among most families. It is well-established that authoritative parenting fosters adolescents' positive well-being.⁵ This style also encourages independence, teaching adolescents that they are capable of accomplishing things on their own.

Permissive parents look at their offspring as equals rather than as children of a parent. This parenting style involves being nurturing and warm, and is reluctant to impose limits. It also rejects the notion of keeping children under control. Permissive parenting is characterized by high levels of responsiveness and low levels of demandingness. It does not set rules, avoids engaging in behavioral control, and sets few behavioral expectations for adolescents.¹ Adolescents from permissive families report a higher frequency of substance use, school misconduct, and are less engaged and less positively oriented to school compared to individuals from authoritative or authoritarian families.⁶ Permissive parenting is also associated with low self-esteem.

Self-esteem is an overall evaluation of oneself, including feelings of general happiness and satisfaction.⁷ Harter stated that the foundation of self-esteem is laid early in life.⁷ Parental involvement, acceptance, support, and exposure have a big influence on self-esteem. Deshpande found that parents who

were unable to understand their children because of a generation gap gave less than optimal acceptance or support.⁸ Parental acceptance or support has been positively related to adolescents' self-esteem as those who experience an accepting attitude from their parents have a higher self-esteem because they view their parents as their caretakers and protectors.

This study aimed to determine the association between parental authority prototype and perceived self-esteem among adolescent nursing students from the UERMMMCI College of Nursing. Specifically, this study aimed to determine the association between the paternal and maternal authority prototypes with self-esteem. The results of this study may provide insights to faculty members, guidance counselors and mentors to understand adolescents better especially those who deal with self-esteem issues. This study did not determine which parenting style was associated with a high self-esteem.

Methods

The study employed a cross-sectional research design to determine the association between parental authority prototypes and the perceived self-esteem among adolescent students enrolled in the UERMMMCI College of Nursing. The researchers administered the Parenting Authority Questionnaire and the Rosenberg Self-Esteem Scale to determine the maternal and paternal parenting types of the respondents and their level of self-esteem, respectively. Chi-square was used to determine the association between parenting style and self-esteem.

Students enrolled in the College of Nursing aged 16 to 19 years were invited to join the study. Those who agreed and gave their informed consent were included. Potential respondents were selected by simple random sampling to attain a sample size of 400 based on the following parameters: proportion with positive self-esteem 85%, level of significance 0.1, power 80%.⁹

The researchers used the Rosenberg Self-Esteem Scale (RSE), a 10-item tool that measures feelings of global self-worth, to determine the respondents' level of self-esteem. The scale ranges from 0-30; scores above 25 mean high self-esteem; scores between 15 and 25 imply a normal self-esteem and scores below 15 suggest low self-esteem. The RSE has high internal reliability (0.92) and strong construct validity. This instrument is unique because it is designed to measure

global self-esteem, so items do not specify exacting areas of activity or qualities that individuals must take into consideration when judging themselves. Individuals who score high in the RSE scale reflect the feelings that they are "good enough" in self-worth and self-respect.¹⁰

The Parenting Authority Questionnaire (PAQ), consisting of 30 Likert-type items each for maternal and paternal styles, was used to determine one of three parenting styles: authoritarian, authoritative and permissive. There were 10 questions each for authoritarian, authoritative and permissive styles, respectively. The tool provided different situations wherein the respondent selected which response --- strongly disagree, disagree, neither agree nor disagree, agree, and strongly agree --- best fit his/her father and mother, respectively.¹¹

The study was explained to potential respondents and the questionnaires were administered to those who gave their informed consent. The respondents were given sufficient time to answer the survey tools. The identities of the respondents were not revealed to protect their privacy and confidentiality. The study was approved by the Ethics Review Committee of the Medical Center.

The responses gathered from the survey were analyzed using SPSS version 19.0. The researchers utilized chi-square statistics in analyzing the significance of any association between parenting style and self-esteem. Those whose answers were incomplete were excluded from the analysis.

Results

A total of 74 nursing students qualified and agreed to join the study. Fifty-two (70%) gave valid responses; the invalid responses were incomplete, with major portions of the survey left blank, thus, these were excluded from the study. The mean age of the respondents was 18.4 years; more than half were 19 years old and almost two-thirds were female, as seen in Table 1.

Table 2 shows that 7 out of 10 respondents had normal self-esteem and almost one-third were considered to have low self-esteem. The most common parenting style among both the respondents' mothers and fathers was authoritative (82.7% and 69.2%) as seen in Table 3. There were more authoritative mothers than fathers (43 vs 36) and twice as many authoritarian fathers than mothers (10 vs

5). The permissive style was least common in both parents. As seen in Table 4, paternal parental authority prototype was associated with the respondents' self-esteem ($\chi^2 = 19.19$, $p < 0.05$) but the maternal authority prototype was not.

Table 1. Demographic characteristic of respondents (N = 52).

Characteristics	Frequency (%)
Age (yr)	
17	5 (9.6)
18	20 (38.5)
19	27 (51.9)
Sex	
Female	34 (65.4)
Male	18 (34.6)

Table 2. Perceived self-esteem of 52 respondents.

Level of Self-esteem	Frequency (%)
High	1 (1.9)
Normal	37 (71.2)
Low	14 (26.9)

Table 3. Parental authority prototypes of both parents of 52 respondents.

Parental Authority Prototype	Maternal	Paternal
Authoritative	43 (82.7%)	36 (69.2%)
Authoritarian	5 (9.6%)	10 (19.2%)
Permissive	4 (7.7%)	6 (11.6%)

Discussion

Previous studies have linked self-esteem and parenting style, and many of them show that there is relationship between certain parenting styles and global self-esteem. The hypothesis of this study is that both maternal and paternal parental authority prototypes are significantly associated with perceived self-esteem, but the results show that it is the paternal parenting style which is significantly associated with the adolescent nursing student's perceived self-esteem. The results also showed that the most common paternal and maternal parenting style is authoritative.

The results of this study provide further evidence that parental authority prototype is associated with perceived self-esteem in adolescent students enrolled in the College of Nursing. This study however, did not investigate which particular parenting style is associated with a high self-esteem. Future studies that examine this possible relationship may provide a basis for the College of Nursing to encourage parents to adopt a parental authority prototype which will boost rather than lower one's self-esteem. Self-esteem, especially among adolescent nursing students, greatly influences how an individual can demonstrate skills and how one can provide quality nursing care to patients. Understanding what boosts self-esteem, as early as the formation years for student nurses, can help enable them to foster proactive coping behaviors, which would influence the way they respond to stressful situations in the clinical areas, and thus affect the way they render care to patients.¹² Nurses with high self-esteem come across less difficulties in communication with colleagues and patients. They have more empathy and efficacy, and have better collaboration with colleagues and patients, and consequently, better performance at work.¹³

The investigators showed that paternal, but not maternal, parental prototype is associated with perceived self-esteem of adolescent nursing students. The most common parenting style is authoritative for the mothers and the fathers.

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Effects of 70% isopropyl alcohol wipes and bleach-based wipes in disinfecting nurses' mobile phone: a quasi-experimental approach

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Abstract

Introduction Concerns have been increased about the use of mobile phones in hospitals as they may be vehicles for the transmission of hospital-acquired infections. This study aimed to compare the effectiveness of 70% isopropyl alcohol wipes with bleach-based wipes in decreasing bacterial colony counts of mobile phones of staff nurses.

Methods Mobile phones of staff nurses in the UERM Hospital were assigned to be disinfected with 70% isopropyl alcohol wipes or bleach-based wipes. Mobile phones were swabbed using standard techniques before and after disinfection with 70% isopropyl alcohol wipes or bleach-based wipes. Post-disinfection colony counts were compared with baseline counts in each group and compared between the two test groups.

Results There was a significant decrease in the post-disinfection mean colony count compared with the mean baseline colony count in both the 70% isopropyl alcohol wipes ($p < 0.001$) and bleach-based wipes ($p = 0.002$) groups. The decrease in the 70% isopropyl alcohol wipes group was bigger (121,635 vs 85,769 CFU/mL). The mean post-disinfection colony count of the 70% isopropyl alcohol wipes was significantly lower ($p = 0.007$) than the other group.

Conclusion Both 70% isopropyl alcohol wipes and bleach-based wipes are effective in decreasing bacterial colony counts of mobile phones of staff nurses. The alcohol wipes resulted in a greater decrease in colony count compared with the bleach wipes.

Keywords: Contamination, colony count, disinfection, mobile phones

In the world of modern technology, majority of the population use different kinds of gadgets, the

most common of which is the mobile phone. A study by Naeem in 2014 showed the mobile phone to one of the most widely-used objects in the world today.¹ It is highly indispensable in improving the quality of health care in hospital settings. However, Pal found that mobile phones are perfect habitats for microbes to be transmitted and cause health care-related infections.²

In recent times, the health care providers have increasingly used mobile phones before and after patient care and they have been placed anywhere without hand-washing, proper dusting or cleansing, which may increase risk of acquiring microorganisms. The pathogens may be transferred from one place to another. Furthermore, mobile phones of health care workers are exposed inside and outside the hospital.

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Zakai showed that mobile phones were commonly used in health care settings for rapid communication.³

Concerns have been increased about the use of these devices in hospitals, as they are used everywhere, even in toilets. Shakthivel revealed that out of 50 mobile phones screened for microbial colonization, 90% had Gram positive pathogenic bacterial and commensal bacteria and 10% had Gram negative bacteria.⁴ Brady showed that 78% of physicians were aware that mobile phones could carry pathogenic bacteria, but only 8% of them cleaned their mobile phones regularly.⁵

Two agents available as wipes may be used to disinfect mobile phones and other items - alcohol and sodium hypochlorite. Ethyl alcohol is an intermediate-level germicide which is the most available and commonly used in health care settings because it is least expensive compared with the other products. Alcohol is safe for the skin although it may cause dermatitis in certain prone individuals. Long term use of alcohol as a disinfectant may cause discoloration, swelling, hardening and cracking of rubber and certain plastics. Alcohol is flammable; precautions against accidents that may cause fires are necessary.

Sodium hypochlorite is effective in killing bacteria, fungi, and viruses such as the influenza virus. In addition, it is also used for disinfecting hard surfaces.^{6,7} According to Rutala, sodium hypochlorite in a solution shows a broad spectrum antimicrobial activity and is used in a variety of settings including healthcare.⁷ It is normally diluted in water and a 0.5% solution is used for disinfecting areas that are contaminated with body fluids and high-volume blood loss. Dilution of household bleach with water at 1:9 inactivates *Clostridium difficile* and the human papilloma virus. Bleach can irritate the skin, eyes, nose, throat, and in some cases cause dermatitis if there is direct skin contact. Prolonged exposure to the fumes of bleach may lead to nausea and vomiting. Bleach can also corrode metals and damage painted objects. The use of bleach in conjunction with other household products such as those containing ammonia should be avoided as the mixture creates a gas that may cause injury or death. Exposure to sunlight creates a toxic gas.

Matthew has accepted the conundrum inherent in cleaning mobile technology devices and stated that wipes moistened with alcohol or bleach are

effective in reducing levels of pathogenic bacterial load on mobile devices.⁸ The researchers aimed to compare the effectiveness of 70% isopropyl alcohol wipes with bleach-based wipes in decreasing bacterial colony counts in mobile phones of staff nurses.

Methods

This was a quasi-experimental study where mobile phones of nurses in the UERM Hospital were assigned to be disinfected with 70% isopropyl alcohol wipes or bleach-based wipes. Post-disinfection colony counts were compared with baseline counts in each group and compared between the two test groups. The participants were randomly selected from a list of nurses was obtained from the Nursing Service. The mobile phones of those who agreed to join and give their informed consent were assigned by drawing of lots to either 70% isopropyl alcohol wipes or bleach-based wipes groups. The computed sample size was 20 mobile phones per group.

The mobile phones were swabbed using a sterile cotton swab and inoculated directly into a sterile test tube containing a 6 mL of nutrient broth. Mobile phones were swabbed using standard techniques before and after disinfection with 70% isopropyl alcohol wipes or bleach-based wipes. All specimens collected were placed in a clean ice box during transport to avoid further contamination and were brought immediately to the University Laboratory to be incubated for 24 hours. After 24 hours of incubation, the incubated specimen was diluted into three dilutions (1:10, 1:100, 1:1000) of melted nutrient broth. Each 1 mL of dilution was plated in a nutrient agar to determine the number of colonies found using a colony counter.

The mean pre- and post-disinfection colony counts in each group were compared using a paired t-test. The difference in colony counts between the 70% isopropyl alcohol wipes and bleach-based wipes groups was compared using an independent t-test. The level of significance was set at 0.05.

The study was approved by the Ethics Review Committee of the Medical Center. Informed consent was obtained from the nurses prior to including their mobile phones in the study. Measures were instituted to maintain the confidentiality of the data and the privacy of the nurse participants.

Results

Forty-one staff nurses consented to have their mobile phones included in the study. As seen in Table 1, more than half of phones in each group had baseline colony counts of at least 150,001 CFU/mL. There were thrice as many phones in the 70% isopropyl alcohol wipes group with 1,000 to 50,000 CFU/mL.

Table 2 shows that there was no significant difference in the mean baseline counts of the 70% isopropyl alcohol wipes and bleach-based wipes groups ($p = 0.064$). There was a significant decrease in the post-disinfection mean colony count compared with the mean baseline colony count in each group: $p < 0.001$ and 0.002 for the 70% isopropyl alcohol wipes and bleach-based wipes groups, respectively. The decrease in the 70% isopropyl alcohol wipes group was bigger (121,635 vs 85,769 CFU/mL). The difference in the mean post-disinfection colony counts between the two groups was significant ($p = 0.007$) and favored the 70% isopropyl alcohol wipes over the bleach-based wipes.

Discussion

The results show that all except 11 mobile phones tested were heavily contaminated. Factors such as

commuting from home to work, placing the phone on furniture, bringing the phone when going to the comfort room or when with animals, and the phone not being disinfected daily may have contributed to the phone's exposure to microorganisms before going on duty.

However, after disinfecting 21 mobile phones with 70% isopropyl alcohol wipes, 18 units were observed to have colony counts less than 250,000 CFU/mL. This is consistent with the findings of Durano.⁹ In addition, it is stated that there was a significant difference in the APC of mobile phones after disinfection with 70% isopropyl alcohol. Eleven of 20 mobile phones disinfected with bleach-based wipes had colony counts less than 250,000 CFU/mL, however, nine were still heavily contaminated despite disinfection. The colony count on a plate of 1 mL of agar is countable at ranges of 25-250 CFU/mL, and anything above that range on a standard-sized petri dish is considered as highly contaminated.¹⁰ Since the sample from mobile phones was swabbed with a dilution from nutrient broth (1:10, 1:100, and 1:1,000), the countable range was then reported as 2,500-250,000 CFU/mL.

Both 70% isopropyl alcohol wipes and bleach-based wipes are effective in decreasing bacterial colony

Table 1. Comparison of colony counts of mobile phones before disinfection with 70% isopropyl alcohol wipes ($n = 21$) and bleach-based wipes ($n = 20$).

Colony count (CFU/mL) ^a	70% isopropyl alcohol n (%)	Bleach n (%)
1,000 to 50,000	7 (30.0)	2 (10.0)
50,001 to 100,000	1 (5.0)	0
100,001 to 150,000	0	0
150,001 to 200,000	0	2 (10.0)
200,001 to 250,000	13 (65.0)	16 (80.0)

^aColony Forming Unit

Table 2. Comparison of pre- and post-intervention colony counts of 70% isopropyl alcohol wipes ($n = 21$) and bleach-based wipes ($n = 20$)

Colony count (CFU/mL)	70% isopropyl alcohol Mean \pm SD	Bleach Mean \pm SD	p-value
Pre-intervention	164,270 \pm 113,558.6	220,200 \pm 69,469.1	0.064 ^b
Post-intervention	42,635 \pm 88,039.3	134,431 \pm 113,824.3	0.007 ^b
Mean difference	-121,635	-85,769	
p-value	< 0.001 ^a	0.002 ^a	

^aPaired t-test

^bIndependent t-test

counts of mobile phones of staff nurses. The alcohol wipes resulted in a greater decrease in colony count compared with the bleach wipes.

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Effectiveness of immature *Mangifera indica* Linn (mango) fruit in reducing the *Ascaris lumbricoides* infection among children: a non-inferiority randomized controlled trial

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Abstract

Introduction This study aimed to compare the effectiveness of immature *Mangifera indica* L. (mango) fruit with albendazole in reducing *Ascaris lumbricoides* infection among children.

Methods Children aged 2 to 14 years were enrolled in a randomized, controlled, non-inferiority trial. Participants were randomly allocated to receive 250 mL immature mango fruit puree daily for 3 days or one dose of albendazole 400 mg tablet. Egg reduction rates and cure rates were computed and compared. Adverse effects were monitored during and after administration of treatment.

Results There was a statistically significant decrease between the pre- and post-treatment EPG of those who took immature mango fruit ($p < 0.001$) and those who took albendazole ($p < 0.001$). There was a higher ERR and CR for the albendazole group, but the difference was not significant ($p = 0.472$, $p = 0.785$, respectively). Risk analysis of reduction in intensity showed mango is non-inferior to albendazole (RR = 0.80, 95% CI 0.67, 0.97; $p = 0.026$). Risk analysis of cure showed mango is non-inferior to albendazole in both PP (RR = 0.92, 95% CI 0.68, 1.25; $p = 0.607$) and ITT (RR = 0.79, 95% CI 0.58, 1.08; $p = 0.139$).

Conclusion Immature *Mangifera indica* Linn is non-inferior to albendazole in terms of effectiveness in the reduction of ascariasis infection.

Keywords: Ascariasis, soil transmitted helminthiasis, helminth, mango

Soil transmitted helminthiasis (STH) is a group of parasitic infections which can be caused by

Ascaris lumbricoides (intestinal roundworm), *Trichuris trichiura* (whipworm), and *Necator americanus* or *Ancylostoma duodenale* (hookworm).¹ According to the World Health Organization (WHO), STH is classified as a neglected tropical disease. STH infections worldwide are confirmed to be over 1 billion, with another 4.5 billion individuals at risk of infection, and school-age children considered as the highest risk group.²⁻⁴ Ascariasis is the most common STH infection.⁵ These STH infections occur largely in areas with inadequate sanitary disposal facilities and poor water supply.¹ In the Philippines, a baseline survey revealed that provincial regions across the country have a

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prevalence of STH infections above 50%.⁶ The Philippine government's response to address this issue has been the Department of Health's (DOH) Soil Transmitted Helminth Control (STC) Program. The STC Program attempts to deworm millions of children across the nation on a biannual basis, through the administration of albendazole. The WHO also handles its deworming programs with similar and traditional drugs.¹

Research regarding natural alternative treatments for STH has gained attention due to the possibility of drug resistance to current therapy for STH.⁷ Studies show that synthetic antihelmintics generally used are not very safe because of their side effects and toxicity. The increase in helminth infection and their growing resistance to most broad-spectrum chemotherapeutics is a major problem facing human health.^{8,9} Reports also show antihelmintic resistance due to frequent use of drugs. Factors that have led to the development of antihelmintic resistance were incorrect dosing, widespread use and increased frequency of treatment. Hence, there is a need for development and discovery of new substances that are derived from plants having antihelmintic properties.⁷

This study evaluated the capacity of *Mangifera indica* Linn (mango) as a natural, alternative treatment for ascariasis. Relatively inexpensive and readily available in the Philippines, mangos are an ideal candidate for study and a potential alternative to current treatment. Potential antihelmintic attributes of plants such as *Mangifera indica* L., and their extracts are credited to secondary metabolites, such as alkaloids, terpinoids or polyphenols.¹⁰ A polyphenol found in aqueous extracts of *Mangifera indica* L. known as tannin is largely responsible for antihelmintic action.¹¹ Additionally, flavonoids found in mango can potentially be applied as larval growth inhibitors as well.¹¹

The general objective of this study is to determine the effectiveness of immature *Mangifera indica* L. fruit in the reducing *Ascaris lumbricoides* infection among children aged 2-14 years compared to albendazole. Specifically, this study aimed to 1) determine the egg reduction rate (ERR) in children taking immature mango fruit compared to those taking albendazole; 2) determine the cure rate (CR) in children taking immature mango fruit compared to those taking albendazole; and 3) determine the hazard ratio of any adverse effects experienced by children taking

immature mango fruit compared to those taking albendazole.

Methods

This was a randomized, controlled, non-inferiority trial. The sampling population consisted of residents of Barangay San Isidro Labrador, Rodriguez, Rizal, aged 2 to 14 years. Enrolled in the study were all those who submitted an assent form and whose guardians submitted a consent form, had not undergone treatment with antihelmintic drugs for the past month, and positive for ascariasis during baseline analysis. Those with co-existing infections (cough, colds, fever) during the study, had known allergy to either treatment modality, gastroesophageal disease, and known autoimmune diseases were excluded. Participants were randomly allocated to receive immature mango puree or albendazole. Each participant was assigned an identification code that was written on a piece of paper, placed in a bowl, and randomly drawn and alternately assigned to treatment groups. Sample size was based on 91% cure rate of immature mango fruit, with confidence level set at 95%, alpha error at 1.96, and level of precision at 0.05.¹²

Demographic data consisting of age, sex, average family income, height, and weight were collected from each participant using a survey form patterned according to the World Health Organization (WHO) recommended survey, STH and Schistosomiasis School Survey Child Form. Stool samples were collected for pre-treatment analysis. Kato-Katz technique was used to analyze one fecal smear per participant. Pre-treatment egg counts were obtained and used to classify the intensity of ascariasis infections into light (1-4,999 epg), moderate (5,000-49,999 epg), or heavy ($\geq 50,000$ epg) infections based on the WHO guidelines.

Participants were then randomly allocated to treatment groups. The control group received a single dose of albendazole 400 mg tablet. The experimental group received 250 mL immature mango fruit puree, prepared fresh each morning, each day for three consecutive days. Guardians of participants helped in assuring proper compliance with the treatments. Stool samples were collected from each participant after one month and analyzed using the Kato-Katz technique. All participants from either treatment group who were still found positive during post-

treatment analysis were administered a single dose of albendazole 400 mg tablet.

Participants who were not able to provide a stool sample for post-treatment analysis were considered dropouts. Egg reduction rates (ERR) and Cure Rate (CR) were computed to assess the antihelminthic effectiveness of immature mango fruit puree. percentage drop of post-treatment mean EPG from pre-treatment mean EPG, measured after the administration of treatment. The egg reduction rate was the percentage drop of post-treatment mean EPG from pre-treatment mean EPG, measured after the administration of treatment. Cure was indicated by the absence of *A. lumbricoides* eggs in one post-treatment fecal smear processed by the Kato Katz technique. Adverse effects were monitored during and after administration of treatment. Guardians were advised to contact the investigators for any untoward symptoms during the one-month interval between pre-treatment and post-treatment analysis.

Analysis was performed using Stata 14 statistical software. T-test or Wilcoxon-Shapiro Test, and chi-square were used to compare demographic data (age, sex, average family income, height, and weight), pre- and post-treatment egg count, ERR and CR. The risk ratios for both ERR and CR, respectively, were computed in per protocol (PP) and intention-to-treat (ITT) worst-case scenario analysis to account for the effect of dropouts. All dropouts in the immature fruit puree group were assigned as not having

reduced EPG or not cured status while those in albendazole group as having reduced EPG or cured status for the worst-case scenario analysis. For ERR analysis, the change in intensity of infection of each participant of both treatment groups was considered. Only those who experienced a decrease in intensity of infection were noted as having reduction. Decrease corresponded to at least one-degree reduction on the intensity of infection. Those without at least one degree decrease in intensity or had an increase in intensity were noted as having no reduction even in the presence of actual reduction in mean EPG.

The research was approved for implementation by the UERMMMCI Research Institute for Health Sciences Ethics Review Committee. The researchers obtained informed consent, and assent as applicable, from all participants. Measures were taken to ensure the privacy and confidentiality of the children-participants.

Results

A total of 96 children were enrolled in study of which 6 and 3 children were dropped from the immature mango fruit and albendazole groups, respectively. The baseline socio-economic demographics and clinical characteristics, as summarized in Table 1, indicate that both treatment groups were comparable at the start of the trial.

Table 1. Comparison of baseline characteristics of immature mango (n=46) and albendazole (n=41) groups.

Demographic Characteristic	Immature Mango Fruit Group	Albendazole Group	p-value
Sex (n (%))			0.240
Male	26 (56.5)	18 (43.9)	
Female	20 (43.5)	23 (56.1)	
Age (yr)			
Mean \pm SD	6.5 \pm 3.22 (95% CI 5.54, 7.46)	6.5 \pm 3.61 (95% CI 5.37, 7.65)	0.942
Weight (kg)			0.446
Mean \pm SD	19.2 \pm 6.71 (95% CI 17.16, 21.14)	17.8 \pm 5.20 (95% CI 16.11, 19.40)	
Height (cm)			0.882
Mean \pm SD	110.3 \pm 19.84 (95% CI 104.42, 116.21)	110.4 \pm 20.56 (95% CI 103.89, 116.87)	
Average family income (PHP)			0.258
Mean \pm SD	8693.48 \pm 5897.81 (95% CI 6942.04, 10444.91)	9146.34 \pm 4323.257 (95% CI 7781.75, 10510.93)	
Initial EPG			0.249
	7857.7 \pm 16232.86 (95% CI 3037.15, 12678.28)	8592.9 \pm 11833.93 (95% CI 4857.68, 12328.18)	

There was a statistically significant decrease between the pre- and post-treatment EPG of those who took immature mango fruit ($p < 0.001$) and those who took albendazole ($p < 0.001$), as seen in Table 2. Table 3 shows a higher ERR and CR for the albendazole group, but the difference was not significant ($p = 0.472$, $p = 0.785$, respectively).

Risk analysis of reduction in intensity showed mango is non-inferior to albendazole in intention-to-treat (ITT) analysis but a statistically significant inferiority in per protocol (PP) analysis, implying that the results were altered by the dropouts. Tables 4 and 5.

Summarize the total subjects with and without reduction in both treatment groups. Meanwhile, risk analysis of cure showed mango is non-inferior to albendazole in both PP and ITT analysis.

Tables 6 and 7 summarize the total cured subjects in both treatment groups. Thus, immature mango fruit puree is found non-inferior to albendazole in terms of effectiveness in reduction of ascariasis infection; but is not non-inferior in terms of CR.

No adverse effects were recorded for both treatment groups.

Table 2. Comparison of mean pre- and post-treatment EPG in immature mango and albendazole groups.

Treatment Group	Pre-treatment (Mean \pm SD)	Post-treatment (Mean \pm SD)	p-value
Immature mango fruit	7857.7 \pm 16232.86 (95% CI 3037.15, 12678.28)	2319.1 \pm 6916.53 (95% CI 265.20, 4373.06)	$p < 0.001$
Albendazole	8592.9 \pm 11833.93 (95% CI 4857.68, 12328.18)	732.3 \pm 1723.96 (95% CI 188.14, 1276.44)	$p < 0.001$

Table 3. Comparison of ERR and CR in immature mango and albendazole groups.

	Immature Mango Fruit	Albendazole	p-value
ERR	86.7% \pm 0.24	93.9% \pm 0.12	0.472
CR	63.04%	68.29%	0.785

Table 4. Comparison of reduction in intensity of infection in both treatment groups (per protocol analysis).

Treatment Group	Participants with Reduction in EPG	Participants without Reduction in EPG	RR (95% CI)	p-value
Immature mango fruit	38	8	0.92 (0.78, 1.08)	0.303
Albendazole	37	4		

Table 5. Comparison of reduction in intensity of infection in both treatment groups (worst case scenario analysis).

Treatment Group	Participants with Reduction in EPG	Participants without Reduction in EPG	RR (95% CI)	p-value
Immature mango fruit	38	14	0.80 (0.67, 0.97)	0.026
Albendazole	40	4		

Table 6. Comparison of CR in both treatment groups (per protocol analysis).

Treatment Group	Participants Cured	Participants not Cured	RR (95% CI)	p-value
Immature mango fruit	29	17	0.92 (0.68, 1.25)	0.607
Albendazole	28	13		

Table 7. Comparison of CR in both treatment groups (worst case scenario analysis).

Treatment Group	No. of Participants Cured	No. of Participants not Cured	RR (95% CI)	p-value
Immature mango fruit	29	23	0.79 (0.58, 1.08)	0.139
Albendazole	31	13		

Discussion

The anthelmintic ability of the immature mango is attributed to the phytochemicals, specifically the polyphenols present in the pulp. Polyphenols are present in mango puree concentrate and have been characterized through high precision liquid chromatography (HPLC) with diode array and mass spectrometric detection.¹³⁻¹⁵ Among the polyphenol subclasses, phenolic acids (gallic acid and aepiginin) and flavonoids (tannin, mangiferin, gallotannins, quercetin, isoquercetin, ellagic acid, and β -glucogallin) exhibit anthelmintic properties.^{16,17} One kilogram of immature mango fruit contains a total phenolic content of 27.8 ± 2.21 mg GAE/g which contains 20mcg/mL tannin, 4.4 mg/kg mangiferin, and 6.9 mg/kg gallic acid.

Among the polyphenols, tannin has the most potent anthelmintic property.¹⁷ Tannin and its other form, condensed tannin (CT) or proanthocyanidin, has the capacity to bind to the cuticle of infective larva that can lead to decreased nutrient availability, causing larval starvation and death.^{17,18} The tannins bind to the site of the cuticle crucial for transport of non-nutrient organic solutes, and other small organic molecules.¹⁸ The cuticle serves as a highly impervious barrier between the host and its environment. It maintains the body morphology and integrity, and has a critical role in locomotion and uptake of nutrients of helminths.¹⁹ As the tannin binds to the cuticle of the larva, it interferes with energy generation by inhibiting or dissociating the 'coupling' or 'joining' of the electron transport and the

phosphorylation (uncoupling oxidative phosphorylation) reactions resulting in inhibition of ATP synthesis, immobilization and starvation, and eventually the death of the larva.

A study by Williams showed that extracts which had the greatest number of CT were the most potent against *Ascaris suum*, as evidenced by a reduced migratory ability of newly hatched third-stage larvae, and reduced motility and survival of fourth-stage larvae recovered from pigs. *A. suum* is similar to *A. lumbricoides* and is even suggested to be of the same species.^{20,21} Another study by Niezen reported that feeding a diet with CT in lambs inhibited the effects on the egg hatching of *Trichostrongylus colubriformis*. These findings indicate that CT can be detrimental to both egg hatching and larval development by reducing migration and motility, suggesting that CT can have a marked effect on the subsequent development of nematode larvae.²² A study by Terrill showed that CT is not absorbed in the digestive tract since none was detected in the blood of sheep and all were found in the digestive tract. Therefore, these CT are all concentrated in the feces, which have more effects on the hatchability of the eggs.¹⁴ Condensed tannins are currently the most studied natural class of compounds for reducing egg counts in ruminants infected with gastrointestinal nematodes such as *Ascaris lumbricoides*.²³

In vivo and in vitro studies by Nery have indicated anthelmintic effects, specifically the inhibition of larval development and fecal egg count reduction, against mixed ovine gastrointestinal nematodes.¹³ Tannins

and flavonoids were the primary metabolites identified in the phytochemical analysis of the extracts of unripe *M. indica* L. var. Uba. Another in vitro test of aqueous extracts of immature mango showed effective inhibition of larval development at a concentration of 50 mg/mL and the metabolite extracts were mostly proanthocyanidin (CT), hydrolysable tannin, triterpenes and saponins. Another study used chestnut tannin solutions in geometrical scale concentrations from 0.32 to 20.48 g/L, which significantly reduced nematode egg hatching.²⁴

The induction of mangiferin also leads to a significant decline in the number of helminths during the larval stages. Moreover, mangiferin is observed to inhibit the production of reactive oxygen species including nitrous oxide that mediates the intestinal pathology of the helminths. The inhibition of the production of reactive oxygen species may have affected the adult female embryogenesis.²⁵

Gallic acid, which is found in significant amounts in mango pulp, and apigenin has similar mechanism of action as tannin.²⁵ Gallic acid causes a dose dependent paralysis and death of *Pheretima phostuma* (earthworm), and at least 10 mg of gallic acid was needed to produce this effect. *Pheretima phostuma* and *Ascaris lumbricoides* share the same collagenous cuticle structure. The apigenin, on the other hand, can also inhibit larval growth. Kawasaki showed inhibition of larval growth in *Caenorhabditis elegans* (roundworm) was associated with DAF-16 activation. Apigenin acts as a stressor to either stimulate DAF-16 activity directly or inhibit DAF-2/insulin signaling, which reduces the inhibitory effect of DAF-2 on DAF-16. In either case DAF-16 is activated, and eventually leading to larval arrest.²⁶

In contrast to the polyphenols, the prime effect of albendazole is flaccid paralysis of ascaris, which allows easier expulsion of the worms by gastrointestinal peristalsis. Albendazole increases the chloride ion conductance of worm muscle membrane, which produces hyperpolarization and excitability reduction, which leads to flaccid paralysis of worms.²⁶

Both experimental and standard treatments for ascariasis showed significant decrease in infection. After administering for 3 days, the experimental treatment of immature mango fruit puree had 86.68%

ERR, while the standard treatment, albendazole, showed 93.93% ERR. In addition, the CR of immature mango fruit puree is 63.04% while albendazole is 68.29%. These values are consistent with that obtained by Belizario wherein the CR of standard drug, albendazole, against ascariasis was 69.7%.⁶ The ERR and the CR of immature mango fruit puree are high and comparable to that of albendazole.

The RR for reduction of intensity is only significant upon ITT analysis. The dropouts in the study, however low, affected the results. Meanwhile, there is no difference in RR for cure rate in both PP and ITT analysis. The cure rate of immature mango fruit puree was comparable to albendazole. This can be due to rapid reinfection of *A. lumbricoides*. Numerous studies showed that rapid reinfection of *A. lumbricoides* was observed post-treatment even with standardized chemotherapy against *A. lumbricoides* infection.²⁷⁻²⁹ Upatham compared the predisposition to reinfection of different intestinal helminths after chemotherapy.³⁰ The results showed that *A. lumbricoides*, as compared to hookworms and *T. trichiura*, has the strongest predisposition to reinfection; it occurs very rapidly in children that live in tropical countries and are younger than 15 years of age.

In conclusion, immature mango puree was found to be non-inferior to albendazole in terms of effectiveness in reduction of ascariasis infection.

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Effectiveness of *Brassica juncea* (mustard green) leaf decoction as an adjunct in the treatment of type 2 diabetes mellitus among Filipinos: a randomized clinical trial

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Abstract

Introduction This study aimed to determine the effectiveness of mustard green leaf decoction as an adjunct to drug treatment in controlling blood glucose among Filipinos with type 2 diabetes mellitus.

Methods Participants were randomly assigned to receive mustard green decoction or a placebo solution for eight weeks on top of their oral anti-hyperglycemic medication. Fasting blood sugar and complete blood counts were determined at baseline, Week 4 and Week 8, and compared within and across the two groups.

Results There was a decreasing trend in the blood sugar level in the mustard green group while the opposite was noted in the placebo group. The mean FBS levels of the mustard green group were significantly lower than that of the placebo group at the Week 8 determination (6.10 vs 8.69 mmol/L, $p = 0.004$). The decrease in blood sugar level on the eighth week in the mustard green group was significant compared with the baseline level ($p = 0.008$).

Conclusion This study has demonstrated that the intake of *Brassica juncea* decoction can significantly decrease blood sugar levels among type 2 diabetics compared to metformin alone.

Keywords: Mustard green, *Brassica juncea*

Diabetes is one of the leading causes of morbidity among older adults. In the Philippines, it is

estimated that 1 in 5 Filipinos is diabetic.¹ In a survey published by the Food and Nutrition Research Institute of the Philippines, the prevalence of high fasting blood sugar (FBS) based on the World Health Organization criteria of fasting blood glucose >125mg/dL for individuals older than 20 years old was 5.4%, which showed a 0.6% increase compared with a similar study in 2008.¹ Those living in the urban areas, and who were in the 60 to 69 years age bracket in both sexes showed the highest prevalence and was found among the richest in the wealth index. This tells us that there is an alarming upward trend in diabetes not only in the Philippines but also worldwide.²

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The Organization of Diabetes Care in the Philippines has conducted lay fora in several settings wherein screening, education, and management are delivered. An example would be at the level of the barangay health center, the health worker must deliver diabetes self-management education, blood pressure monitoring, weight/BMI monitoring. At the level of the RHU/City or Provincial Health Office the diabetes clubs are established to encourage patients to learn from other patients. At all levels of the health care system, education and training are being given so health care workers will have the skills and materials needed for screening, management, and education.³

Currently, management of diabetic patients consists of blood sugar control, home blood sugar testing, HbA1C testing, cardiovascular risk control which includes lifestyle modifications, and intake of oral anti-hyperglycemic agent such as metformin, and insulin for advanced diabetes management.⁴ In the data obtained from IMS Health in 2007 gathered from the retail sector indicate that the total consumption of oral hypoglycemic agents in the Philippines in 2007 was 114 million defined daily doses (DDD) which doubled from the 2000 figure of 50 million DDD. Four major oral anti-hyperglycemic agents are being taken by Filipinos (metformin, gliclazide, glimeprimide, and glibenclamide) which comprises 84% of DDD, while the consumption of insulin increased from 5 million DDD in 2000 to 12 million DDD in 2007.⁵

Since diabetes mellitus affects Filipinos across socio-demographic backgrounds, many have tried, and some are still constantly trying to find cheaper and more effective medications. *Brassica juncea* or mustard green is a widely known plant used as an oil source, and a green vegetable that has a lot of medicinal potential. The leaf extracts of *Brassica juncea* have been reported to exhibit antioxidant, anti-nociceptive, and anti-hyperglycemic properties both in vitro and in vivo.⁴ The anti-hyperglycemic effect may be due to isothiocyanate, glycoside sinigrin, protein and fixed oil. These substances have been shown to increase activity of glycogen synthase and decrease glycogenolysis and gluconeogenesis, resulting in a decrease of glycogen phosphorylase and gluconeogenic enzymes.¹³

Different hematologic effects have been reported with the intake of *Brassica juncea*. One study showed that the administration of the seed extract may cause anemia, leukocytosis, and thrombocytopenia.¹² The

main component of the seed extract that causes anemia, and leukocytosis is isothiocyanate, which is both present in the seed and leaf extracts.^{12,15} Despite these side effects, *Brassica juncea* is considered to be generally safe by the Department of Health and has many beneficial properties such as antibacterial and antioxidant, and helps improve blood circulation.

According to the World Health Organization, the Philippines ranks 15th for diabetes prevalence. Considering the proportion at risk for diabetes mellitus, the researchers focused on the hypoglycemic properties of mustard green in lowering blood sugar levels for those with FBS ≥ 7.0 mmol/L.⁷ The main objective of the study was to determine the effectiveness of mustard green leaf decoction as an adjunct to drug treatment in controlling blood glucose among Filipinos with type 2 diabetes mellitus. The specific objectives of the study were to determine the changes on the fasting blood sugar levels (mmol/L) of those taking mustard green leaf decoction as treatment adjunct compared to placebo and to measure the hematologic changes with regards to WBC, RBC, and platelets and its possible hematologic side effects. This study can benefit not only patients but also other practicing doctors as they could use this study in the future to further research on mustard green and its effects on diabetes, and they can also include this in managing their patients as this has the potential to be an adjunct in managing patients with type 2 diabetes mellitus.

Methods

The study was a randomized, double-blinded, clinical trial. A measured set of *Brassica juncea* decoction or a placebo solution consisting of FDA-approved food coloring were given to the participants to be taken for 8 weeks. Fasting blood sugar and CBC with platelet count were checked every 4 weeks and compared.

The study participants were diabetic residents of Taytay, Rizal identified from a list of patients obtained from the barangay health centers. The potential participants were identified, contacted, and those who replied favorably were screened. They were selected by purposive sampling. The participants were 40 to 59 years, had type 2 diabetes mellitus with FBS range of 7.0-12.0 mmol/L living in Taytay, Rizal, and diagnosed at least one year prior to the study.^{7,10} Recent FBS results not

exceeding three months were used for the initial screening. In addition, potential participants must only be taking metformin 500 mg once a day as maintenance medicine. Those who were pregnant and/or lactating, had other chronic diseases, and/or had a history of stroke or myocardial infarction were excluded. Those who were anemic, and/or thrombocytopenic were also excluded from the study. Additional participants referred by the barangay health workers were also screened to meet the required sample size. The computed sample size for each group was 10 with level of significance set at 95% with 80% power.⁹ Two additional participants per group were included to account for possible dropouts.

Those who were qualified were randomly allocated to either *Brassica juncea* decoction (experimental) or placebo (control) group using an online randomizer. Each participant was assigned a numerical code known only by the logistician. After obtaining informed consent, participants were asked to complete a profile sheet. Baseline CBC with platelet and FBS were taken before they were started on the decoction or placebo. The researchers were blinded accordingly: the logistician who was the only person who had access to everything and knew of the participants' assignment. The participants did not know which group they were in.

Mustard green (*Brassica juncea*) was procured from Balintawak Organic Market and from Good Foods Community. It was then verified by the Botany Division of the National Museum of the Philippines. The authenticated fresh leaves were carefully washed under running tap water and any foreign matter was completely removed. They were kept in a dry, well-ventilated place, protected from light and dried at room temperature for 7 days.⁸ Once dried, they were carefully weighed and placed in a closed container for storage. The decoction was prepared weekly for eight weeks with a measurement of 2g/250 mL. A total of 348 grams dry weight was used to decoct 48 liters of mustard green decoction. Eight grams of dried leaves were placed in a stock pot containing 1 liter of water and heated until it boiled. The solution was cooled at room temperature. After cooling, it was strained and then transferred to clean 1L bottles, coded and kept refrigerated overnight at 4 °C.

The placebo decoction was prepared weekly as well. One liter of placebo decoction was prepared

using 1 liter of distilled water mixed with 0.5 mL of FD & C Yellow No. 6, 0.1 mL of FD & C Yellow No. 5 and Blue No. 1 and 0.1 mL of FD & C Red No. 40 and Red No. 3. Each clear bottle was then coded and refrigerated overnight at 4°C.

The experimental group was given mustard green decoction while the control group was given placebo for eight weeks. They were given 4000 mL of mustard green decoction or placebo placed in four pieces of one-liter bottles that were refilled at each weekly visit. The participants were given a measuring cup for uniform intake. Participants were instructed to take approximately one cup (250 mL) of the assigned liquid after breakfast and dinner daily for eight weeks.¹¹

FBS levels and CBC with platelet of both groups were measured after every 4 weeks. Common symptoms of possible side-effects were explained to the participants. They were instructed to immediately inform the researchers if any side effects were noted such as anemia, hypoglycemia, allergic reactions. Blood specimens such as CBC with platelets were analyzed using a Samsung LABGEOHC10 Hematology Analyzer at Evergreen Medical and Diagnostic Center, Inc.¹²

The data were recorded in MS Excel 2013 and constantly checked for any inconsistencies then analyzed using SPSS v. 23. The researchers used a paired t-test for comparing means within groups. An independent t-test was used in comparing means between treatment and control groups. Chi square was utilized to analyze proportions and distributions. ANOVA was also utilized to compare mean FBS levels of both groups within different points in the study (baseline, 4 weeks and 8 weeks).

This study was approved by the Ethics Review Committee of the UERMMMCI before it was implemented. Informed consent was obtained for every participant before the start of the assigned treatment. All participants were told of all the risks and benefits that they may encounter in the study. They were informed that there is a 50% chance of not receiving the mustard green decoction. Whatever information they would hand over to the researchers will be kept confidential. No personal information was published. Their identities were concealed, and each participant was given a corresponding unique code. The information was only accessible by the researchers and only used for the study.

Results

From the list of diabetic patients provided, 50 individuals were invited to join the study and 24 were enlisted. There were two dropouts each in the mustard green and placebo groups. The mustard green group was significantly older ($p = 0.038$), had more females (75.0% vs 58.3%) and more married participants (83.3% vs 58.3%), though the differences were not significant, as seen in Table 1. All the participants in the control group were non-smokers.

As seen in Table 2, the baseline FBS levels in the mustard green and placebo groups were comparable. There was a decreasing trend in the mustard green group while the opposite was noted in the placebo group. The mean FBS levels of the mustard green group were significantly lower than that of the placebo group at the eighth week determination (6.10 vs 8.69 mmol/L, $p = 0.004$). The decrease in blood

sugar level on the eighth week in the mustard green group was significant compared with the baseline level ($p = 0.008$).

A decrease in the RBC count and increases in the WBC and platelet counts were seen in the mustard green group but the changes were not significant, as shown in Table 3. All values were still within normal limits. Increases in all three parameters were noted in the control group (Table 4), with all values within normal limits.

Discussion

The results of this study showed that mustard green, taken as an adjunct to metformin, was able to provide better blood glucose control than metformin alone. The significant decrease in the blood sugar levels of diabetic patients in this study, according to

Table 1. Sociodemographic profile of 24 participants.

Sociodemographic Information	Mustard Green Frequency (%)	n (%)	Control Frequency (%)	p-value
Age (yr) [mean \pm SD]	56.1 \pm 4.44		50.00 \pm 8.22	0.038
Sex				0.386
Female	9 (75.0)		7 (58.3)	
Male	3 (25.0)		5 (41.7)	
Civil Status				0.178
Single	2 (16.7)		5 (41.7)	
Married	10 (83.3)		7 (58.3)	
Occupation				0.638
Self-employed	5 (41.7)		3 (25.0)	
Employed	4 (33.3)		6 (50.0)	
Housewife/husband	3 (25.0)		3 (25.0)	
Highest educational attainment				0.549
Attended elementary	1 (8.3)		0	
Elementary graduate	1 (8.3)		1 (8.3)	
Attended high school	2 (16.7)		0	
High school graduate	4 (33.3)		4 (33.3)	
Attended college	1 (8.3)		1 (8.3)	
College graduate	3 (25.0)		6 (50.0)	
Smoking				0.028
Yes	8 (83.3)		0	
No	4 (33.3)		12 (100.0)	
Coffee drinking (cups/day)				0.537
1	6 (50.0)		3 (25.0)	
2	4 (33.3)		2 (16.7)	
3	1 (8.3)		1 (8.3)	
None	1 (8.3)		2 (16.7)	

Table 2. Mean fasting blood sugar (mmol/L) per testing period for mustard green and placebo groups.

	Mustard green (Mean \pm SD)	Control (Mean \pm SD)	p-value (between groups)
Baseline (n=12)	8.32 \pm 1.87	8.24 \pm 1.90	0.918
Four weeks (n=10)	7.77 \pm 2.03	8.54 \pm 0.05	0.388
Eight weeks (n=10)	6.10 \pm 0.65	8.69 \pm 2.14	0.004
P-value (within groups)	0.008	0.638	

Table 3. Comparison of eighth week vs baseline complete blood count of mustard green group.

	Baseline (Mean \pm SD)	Eight weeks (Mean \pm SD)	p-value
RBC (x 10 ⁹ /L)	5.03 \pm 0.28	4.96 \pm 0.42	0.501
WBC (x 10 ⁹ /L)	8.12 \pm 1.33	8.86 \pm 1.37	0.050
Platelets (x 10 ⁹ /L)	256.80 \pm 74.67	298.20 \pm 60.95	0.077

Table 4. Comparison of eighth week vs baseline complete blood count of control group.

	Baseline (Mean \pm SD)	Eight weeks (Mean \pm SD)	p-value
RBC (x 10 ⁹ /L)	5.06 \pm 0.38	5.37 \pm 0.32	0.007
WBC (x 10 ⁹ /L)	6.61 \pm 1.44	7.20 \pm 0.96	0.234
Platelets (x 10 ⁹ /L)	306 \pm 52.09	319 \pm 54.88	0.259

previous studies, may be due to isothiocyanate, glycoside sinigrin, protein and fixed oil.¹³ Another reason may be the increased secretion of insulin from the pancreas that leads to increased uptake of glucose or may be due to the inhibition of glucose from the gut to the bloodstream.⁹ In terms of hematologic effects, it is stated that the causes of leukocytosis are isothiocyanate and nitric oxide which affect the bone marrow.¹⁴ Other side effects such as anemia and thrombocytopenia were not seen in any of the participants, most probably because of the limited intake of the decoction. One particular study stated that intake of mustard green for 60 days will not have any adverse effects.

Type 2 diabetes mellitus has one of the highest prevalences among the non-communicable diseases in the Philippines. This study has demonstrated that the intake of *Brassica juncea* decoction can significantly decrease blood sugar levels type 2 diabetics compared to metformin alone. This study focused on determining the effect of mustard green

as an adjunct therapy on lowering the blood sugar of the participants diagnosed with type 2 diabetes mellitus and maintained on metformin alone. The results of this study may not apply to diabetic patients with other comorbidities or those taking other medications. Measurement of changes in blood sugar levels was limited to the use of FBS. The results of the study add to the evidence of the potential of mustard green as an effective adjunct therapy for type 2 diabetes mellitus. Further investigation into its beneficial effects under different circumstances can help in establishing mustard green as a viable alternative in supplementing diabetes therapy. It is of benefit if the specific active component of mustard green can be identified as it can result in the development of new diabetes drugs in the future. For the patients, the positive results can be encouraging as mustard green is readily available in the local market and can provide affordable dietary supplements for those trying to control their blood sugar levels.

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A quasi-experimental study on the effect of a nursery rhyme on the comfort of infants after vaccination in selected barangay health centers in Quezon City

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Abstract

Introduction Administration of parenteral medications may cause pain in infants. This study aimed to assess the efficacy of a nursery rhyme in increasing the comfort of infants after vaccination.

Methods Infants who were brought to six barangay health centers for vaccination were recruited. Infants from three barangay health centers were randomly assigned to the experimental group, while infants from the other three were assigned to the control group. A Filipino nursery rhyme *Tatlong Bibe* was played to the experimental group. The comfort of each infant was then assessed by a pediatrician prior to, immediately after, and two minutes post-vaccination using the COMFORT-B scale. Results were analyzed using independent t-tests.

Results Prior to vaccination, the control and experimental groups had mean COMFORT-B scores of 12.46 and 12.74 ($p = 0.634$), respectively. The immediate post-vaccination mean COMFORT-B scores were 22.14 and 21.63 ($p = 0.420$), while the 2 minutes post-vaccination mean COMFORT-B scores were 16.40 and 16.49 ($p = 0.927$), respectively. There were no significant differences between groups for the three determinations.

Conclusion Based on the study results, the nursery rhyme had no significant effect on the comfort of infants after vaccination.

Keywords: Music therapy, vaccination, Filipino nursery rhyme

Pain is ubiquitous in the clinical setting. Aside from the disease process, methods of treatment,

especially those which are invasive, may also cause pain. Administration of parenteral medications come at the cost of inflicting trauma to the injection site, thus causing pain. Although some vaccines may be administered orally, such as the oral polio and rotavirus vaccines, others are administered parenterally through the intramuscular, intravenous, and subcutaneous routes. Thus, conferring protective active immunity on an individual would entail some form of pain or discomfort in pediatric patients.

One of the most cost-effective, readily available, and easy to use modalities for decreasing pain

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perception in children is music. According to Kemper and Danhauer, music is known to effectively reduce anxiety and improve the mood of adult and pediatric medical and surgical patients, since music can distract them from pain and other unpleasant symptoms, thoughts, or feelings.¹ An observational study by Ozdemir and Tufekci found that a musical mobile with both visual and auditory elements effectively distracted infants from vaccination pain.²

Distraction is one of the interventions of pain management and though it does not eliminate the distress or pain, distracting patients helps them focus less on pain.³ A study by Moon elicited a differential response in infants who listened to a voice in their native language and in a foreign language, interpreted as a preference by newborns for their native language.⁴ Soley and Hannon found that familiarity of music had a powerful influence in the allocation of attention early in life, and familiarity preferences may facilitate the acquisition of culture-specific perceptual and cognitive abilities.⁵ Hence, music in the form of nursery rhymes is proposed as an adjunct therapeutic modality and way of distracting infants during immunization due to its affordability and availability with no known side effects.

Aside from adding to the body of knowledge in the field of medicine and possibly decreasing the usual pain experienced during vaccination, the results of this study may also eventually contribute to diminishing learned crying behavior among children during health check-ups and vaccination as infants may vicariously learn from other infants crying in a healthcare setting. Likewise, when distraction and decrease in pain perception through nursery rhymes is found to be effective and is implemented, infants may also learn to be more comfortable through observation of other infants who are comfortable in these settings. They may learn that clinics or hospitals are not anxiety-provoking and may consequently have a more positive perception of them.

This study aimed to determine if a nursery rhyme in Filipino significantly increased the comfort of infants after vaccination. Specifically, this study compared the mean COMFORT-B scores of infants exposed and those not exposed to the nursery rhyme *Tatlong Bibe* prior to and after vaccination via intramuscular and subcutaneous routes.

Methods

This was a quasi-experimental study performed in the immunization rooms of six barangay health centers in the Second District of Quezon City: Barangays Holy Spirit, Batasan Hills, Commonwealth, Lupang Pangako, NGC, and Payatas B. These were assigned by the City Health Officer in pairs based on location. Hence, a matched-pair clustered randomization was employed by performing a toss coin for each pair to divide the six centers into experimental and control groups. Participants belonging to centers in the experimental group were exposed to the nursery rhyme with Filipino lyrics *Tatlong Bibe* played repeatedly and continuously in the immunization room throughout the duration of the experiment, while no nursery rhyme was played in the immunization rooms of the control centers. *Tatlong Bibe* has a simple tune and is rhythmically repetitive. It was popular at the time of the study and was constantly played on radio and television. The comfort of infants was assessed by a pediatrician using the COMFORT Behavior Scale pre-vaccination, immediately post-vaccination, and 2-minutes post-vaccination. The scores of the two groups were compared using an independent t-test.

The target population consisted of infants aged 1 year old and below who were brought to the barangay health centers for measles, MMR (measles-mumps-rubella), or pentavalent vaccination. Participants were then conveniently sampled from those present at the assigned health centers on the days of the experiment. Infants whose families use Filipino as their main mode of communication at home were included, since use of native language was considered a form of familiarity with nursery rhymes. Those who were premature, had hearing impairment, nervous system disorders, congenital anomalies and/or disorders, as determined by a pediatrician, as well as those given pain relievers within 24 hours prior to the study, were excluded, since these may affect their expression and perception of pain. Based on a similar study which explored the effects of a music therapy intervention in the PICU as measured by the Comfort Behavior Scale, the computed sample size was 32 infants each for the experimental and the control group, resulting to a total sample size of 64.⁶

The comfort of the infants was measured using the COMFORT Behavior Scale, a scale based on

observable behavior, mainly used to assess pain and distress in pediatric intensive care patients 0 to 3 years old. It is a modified version of the COMFORT Scale developed by Ambuel, wherein two physiologic factors originally included are omitted without compromising the quality of data.⁷ Also considered as a "discomfort scale", higher final scores in the COMFORT Scale correspond to less comfort and lower scores imply a greater degree of comfort. Although this scale is primarily used in assessing postoperative infants or those in PICU, it was used in healthy infants subjected to heel prick, a procedure similar to vaccination which also induces acute pain.⁹ In addition, this scale was used in a study also involving music and comfort.⁶ Hence, the COMFORT Behavior Scale was selected based on its use in previous researches similar to this study, the way it relates the constructs of comfort and pain, and the availability of a free online training module upon request from its authors. To ensure the quality of the scores, a pediatrician who completed the online training module provided by the author of the scale assessed the participants prior to, immediately after, and two minutes after vaccination.

Written informed consent was secured from parents at least 18 years, who accompanied the infant to the health center. Anonymity and confidentiality were ensured by using patient codes. The study was approved by the University of the East Ramon Magsaysay Memorial Medical Center, Inc. Research Institute for Health Sciences Ethics Review Committee.

Results

Out of 82 infants recruited, a total of 70 participants, 35 in each group, were included for analysis. Majority were female, three-fourths were Catholic and half of them came from Holy Spirit-Catalina and NGC Sentrong Sigla Health Centers. Their demographic profile is shown in Table 1.

The mean scores of the experimental and control groups in the respective times are shown in Table 2. No significant differences in the mean COMFORT-B scores at baseline were noted. A uniform increase in discomfort was then observed immediately post-vaccination, with mean scores of 21.63 and 22.14 for the experimental and control groups, respectively. At two minutes post-vaccination, both groups had lower mean scores compared to the immediate post-

Table 1. Demographic profile of 70 participants.

Demographic characteristic	n (%)
Sex	
Female	38 (54.3)
Male	32 (45.7)
Age	
0 to 6 months	36 (51.4)
≥ 6 months	34 (48.6)
Religion	
Roman Catholic	54 (77.1)
Iglesia ni Cristo	6 (8.6)
Others	10 (14.3)
Participants per Barangay Health Center	
Barangay Holy Spirit - Sta. Catalina	19 (27.1)
Barangay NGC Sentrong Sigla	16 (22.9)
Barangay Batasan Hills Super	11 (15.7)
Payatas B	11 (15.7)
Barangay Commonwealth	10 (14.3)
Lupang Pangako	3 (4.3)
Vaccine administered	
Pentavalent	37 (52.9)
MMR	20 (28.6)
Measles	13 (18.5)

vaccination scores, though these scores remained higher than baseline. The group exposed to the nursery rhyme had lower increases in the immediate post-vaccination ($p = 0.420$) and 2 minutes post-vaccination scores ($p = 0.927$) but the differences were not significant.

A significant increase in the COMFORT-B scores, shown in Table 3, from baseline to immediately post-vaccination in both experimental ($+8.89$, $p < 0.001$) and control ($+9.68$, $p < 0.001$) groups. A significant but smaller increase from baseline was also noted 2 minutes post-vaccination in both experimental ($+3.75$, $p < 0.001$) and control ($+3.94$, $p < 0.001$) groups, as seen in Table 4. As shown in Table 5, the route of administration of the vaccines was a significant factor in the comfort of the infant participants immediately post-vaccination ($p = 0.007$) and at 2 minutes post-vaccination ($p = 0.003$).

Discussion

The pre-vaccination and post-vaccination mean scores for the group with nursery rhyme were expected

Table 2. Comparison of mean COMFORT-B scores.

	With Nursery Rhyme (n=35)	Without Nursery Rhyme (n=35)	p-value
Pre-vaccination	12.74	12.46	0.634
Immediately post-vaccination	21.63	22.14	0.420
2 min post-vaccination	16.49	16.40	0.927

Table 3. Comparison of pre-vaccination and immediately post-vaccination mean COMFORT-B scores within groups.

		Mean	p-value
Without nursery rhyme	Pre-vaccination	12.46	< 0.001
	Immediately post-vaccination	22.14	
With nursery rhyme	Pre-vaccination	12.74	< 0.001
	Immediately post-vaccination	21.63	

Table 4. Comparison of pre-vaccination and 2 minutes post-vaccination mean COMFORT-B scores within groups.

		Mean	p-value
Without nursery rhyme	Pre-vaccination	12.46	< 0.001
	2 minutes post-vaccination	16.40	
With nursery rhyme	Pre-vaccination	12.74	< 0.001
	2 minutes post-vaccination	16.49	

Table 5. Comparison of Pentavalent versus Measles/MMR mean COMFORT-B scores.

	Vaccine Type	Mean	p-value
Immediately post-vaccination	Pentavalent (n=37)	22.68	0.007
	MMR and measles (n=33)	21.00	
2 minutes post-vaccination	Pentavalent (n=37)	17.70	0.003
	MMR and measles (n=33)	15.03	

to have no significant difference. Likewise, the scores of the group without nursery rhyme were hypothesized to have a significant difference, which could have implied that infants became less comfortable after vaccination. However, contrary to the expected results, not only did the pre-vaccination and post-vaccination scores of the control group turn out to be significantly higher, so did those of the group with nursery rhyme. Hence, the baseline level of

comfort was not maintained despite the nursery rhyme.

This study aimed to test the hypothesis that nursery rhymes in Filipino significantly increase the comfort of infants after vaccination. However, it was found that there was no significant difference between the COMFORT-B scores of those exposed to the nursery rhyme *Tatlong Bibe* and those who were not. This may be attributed to several factors. Most

studies on music therapy and pain in infants made use of music without lyrics and were performed in a controlled environment. Ozdemir and Tufekci, used both visual and auditory elements as part of the intervention in the form of a musical mobile and this was found to decrease pain scores and crying duration among infants.² In this study, a purely auditory intervention with lyrics was used in a field experiment, where there was less control of the environment. Another possible contributory factor may be the use of the COMFORT Behavior Scale, which was more strongly validated for use in NICU and PICU settings and procedures rather than in healthy infants in acute pain.

It was found that the route and type of immunization and the age of the participants, which are closely related in this study given that immunizations are given at particular ages, were significantly different immediately post-vaccination and 2-minutes post-vaccination between infants who received the pentavalent vaccine and those who received measles/MMR.

Unlike other similar researches which used audiovisual materials as independent variable, the intervention in this study was purely auditory. In addition, only one nursery rhyme was used, *Tatlong Bibe*. The acoustics, geographic location, and other logistical characteristics of the health center were not under the control of the researchers. Although having more than one rater may have been more ideal, a single expert was invited due to budget constraints, the time and days she was required to participate, and the small area for observation in the immunization room of barangay health centers. Furthermore, the type and route of the vaccines received by the participants were not limited to a single type or route, which may have confounded study results.

The results showed that the nursery rhyme used did not significantly increase comfort in infants after vaccination. The researchers suggest the use of audiovisual materials such as music videos or animated videos of nursery rhymes, use of other nursery rhymes, a pain measurement scale more suitable for healthy infants in acute pain, and strict control of the environment where the infant would be vaccinated, in future studies.

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Instructions to Authors

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This should be presented in logical sequence in the text, tables, and figures, avoiding repetitive presentation of the same data. Measurements should be in International System (SI) units. This section should not include material appropriately belonging to the discussion. Results must be statistically analyzed when appropriate.

Discussion

Data mentioned in the results should be explained in relation to any hypothesis advanced in the introduction. This may also include an evaluation of the methodology and the relationship of new information to previously gathered data. Conclusions should be incorporated in the final paragraph and should be commensurate with and completely supported by data gathered in the study.

Acknowledgments

Only persons who have made genuine contributions and who endorse the data and conclusions should be acknowledged. Authors are responsible for obtaining written permission to utilize any copyrighted text and/or illustrations.

References

References cited in the text shall be written as Arabic numerals in superscript in the order in which they appear in the text. Use the format in the *Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals 2017* which is available at www.icmje.org. Titles of journals may be abbreviated in the reference list according to the style used in Index Medicus. Unpublished observations and personal communications may not be used as references. Examples of the correct manner of listing references are illustrated below:

Standard journal article

(List all authors when six or less; when seven or more, list only the first three then add "et al".)

Francis D, Hadler SC, Thompson S, et al. The prevention of hepatitis B with vaccine: Report of the Centers for Disease Control multicenter efficacy trial among homosexual men. *Ann Intern Med* 1982; 97: 362-6.

Krugman S, Overby LR, Mushahwar IK, et al. Viral hepatitis type B: studies on the natural history and prevention reexamined. *N Engl J Med* 1979; 300: 101-6.

Nyland LJ, Grimmer KA. Is undergraduate physiotherapy study a risk factor for low back pain? A prevalence study of LBP in physiotherapy students. Retrieved from: <http://www.Biomed-central.com/1471-2474/4/22>. 2003. [Accessed August 27, 2011].

Rankin J, Tennant PW, Stothard KJ, et al. Maternal body mass index and congenital anomaly risk: A cohort study. *Int J Obes* 2010; 34(9): 1371-80. Available from: <http://ncbi.nlm.nih.gov/pubmed/20368710>. [Accessed August 27, 2011].

Books and other monographs

Personal authors

Adams RD, Victor M. Principles of Neurology. New York: McGraw-Hill; 1981.

Chapter in a book

Corbett S. Systemic Response to Injury and Metabolic Support. In: Brunnicardi FC (editor). Schwartz's Principles of Surgery. 10th ed. New York: McGraw-Hill; 2015: 13-50.

Tables

These should be typed on a separate sheet (NOT EMBEDDED IN THE TEXT), numbered with Arabic numerals and accompanied by a title and an explanatory caption at the top. Each table must be referred to in the text and an indication of the preferred position in the text should be given. Other explanatory materials should be placed in footnotes below the tables. All non-standard abbreviations should be explained in the footnotes. Vertical and horizontal rules between entries should be omitted.

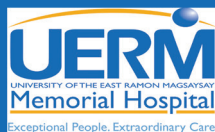
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