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Contents

Transovarial transmission of dengue virus in *Aedes aegypti* in Quezon City*

Ralph Julius Bawalan, MSTM[a] and Nelia P. Salazar, PhD[b]

Abstract

Introduction Due to the changing nature of dengue epidemiology and control, this study was conducted to determine and describe evidence for transovarial transmission of the dengue virus.

Methods This was a quantitative experimental study on the transovarial transmission of the dengue virus from field-collected *Aedes aegypti* in an animal model. Viremia was detected by reverse transcriptase-polymerase chain reaction. Mosquito homogenate was used for intracranial inoculation of the virus into suckling mice. The brains of the suckling mice were extracted and inoculated intraperitoneally into 3 to 4 week old mice for recording of disease manifestations.

Results The mice infected intraperitoneally with dengue virus from field Aedes mosquitoes showed evidence of dengue disease manifested through physical signs, thrombocytopenia and histopathologic changes in affected organs.

Conclusion These observations indicate that transovarial transmission of dengue virus can occur in a highly urbanized locale like Quezon City where dengue cases are high, and virulence may translate into dengue disease when inoculated in an animal model.

Key words: dengue, animal model, vector control, histopathology, molecular diagnostics, virus inoculation

Transovarial transmission of the dengue virus is an etiologic phenomenon responsible for the persistence of the dengue virus during inter-epidemic periods.[1] Certain in-vivo and in-vitro studies confirmed that mosquitoes demonstrate viral transmission through vertical transmission via the transovarial route[2,3,4,5,6] and there are even other studies that provided evidence of transmission up to the seventh generation.[6,7]

The changing nature of dengue epidemiology and how dengue virus is maintained even during these inter-epidemic periods form questions that led to different researches to evaluate the importance of transovarial transmission in dengue virus.[5] However, the Philippines, one of the countries which suffered substantially from the dengue epidemic, still has limited studies related to the transmission of dengue virus and on useful animal models to understand dengue pathogenesis. Given that Quezon City has been declared by the Department of Health as an urban dengue hotspot, the transovarial transmission of the dengue virus by *Aedes aegypti* must still be considered to contribute in the maintenance of the virus in the area, and as a potential source for introduction of the dengue virus during a possible epidemic period. This study aimed to determine and describe evidences of transovarial transmission of dengue virus from field collected *Aedes aegypti* and describe virulence in an animal model based on disease manifestation.

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Methods

This was a quantitative experimental laboratory study. Field larval *Aedes aegypti* samples from Quezon City (endemic Philippine strain) were used for viral analysis. Suckling mice and 3 to 4 week old mice were obtained from the Research Institute for Tropical Medicine (RITM) Veterinary Medicine and housed in specific pathogen-free conditions at the UP Manila Department of Biochemistry and Molecular Biology Laboratory. All procedures were approved by the Animal Ethics Committee of the RITM Veterinary Medicine.

Mosquito samples

Larval samples were collected in containers with tight lids to prevent escape and reared in the laboratory until they developed into adults. Five male and five female mosquitoes from each location (Basila, Camiling, RITM) were then randomly selected and allowed to lay their eggs until they hatched. These samples were then reared for the next generation. The F1 progeny were reared to adulthood and were retained for 5 to 7 days after emergence in order to guarantee mating. Pupae were collected routinely in order to prevent the adult mosquito from emerging in the larval rearing container. Adults were kept in sturdy cages. A similar procedure was performed until the F2 generation.

Molecular diagnosis and viremia detection

Detection of dengue virus in adult mosquitoes was performed with serotype-specific primers by using reverse transcriptase-polymerase chain reaction (RT-PCR). Mosquito pools were homogenized with a sample disruptor and then centrifuged. Total RNA was extracted from the mosquito suspension. Using the RNA extracts as template, the dengue viral genes were amplified by real-time PCR. Nested RT-PCR of dengue virus RNA was carried out with dengue virus consensus and serotype specific primers. Five microliters of RNA in 50uL reaction volume were used with QIAGEN OneStep RT PCR kit (QIAGEN™). RT-PCR was carried out according to the manufacturer's instructions with a 55°C annealing temperature. The resultant PCR product was diluted in 1:100 in water. Nested PCR was carried out with 5uL of diluted RT-PCR product in a 50uL reaction volume with the TaqPCR Master Mix Kit (QUIAGEN™). Initial denaturation of 3 minutes at 94°C was followed by 25 cycles, each consisting of 94°C for 30 seconds, 50°C for 1 minute, and 72°C for 1 minute, followed by a final extension step of 72°C for 10 minutes.

Dengue serotypes were identified and detected for the 15 male and 15 female mosquitoes of the parent generation using RT- PCR. Only those that turned out positive for viral presence were continued for monitoring and were bred again for the succeeding generation. Those that turned negative were discarded; one pair was maintained as the control. This procedure was repeated until the second generation (F2). PCR products were analyzed by gel electrophoresis using a 2% agarose gel (Vivantis™) containing Gel Red (0.5 ug/mL). A 100 bp ladder was used as a size standard. A band on the agarose gel of the correct size (200 to 500 bp depending on the viral type) was interpreted as a positive result. A faint band of the correct size was considered an equivocal result while the absence of bands was interpreted as a negative result. Shaved ICR mice, which were RT-PCR verified to be dengue negative were used as blood source for the female *Aedes* mosquitoes.

Animal injection

1) Intracranial injection of dengue virus in mice.

Living *Aedes aegypti* female mosquitoes were held for 24 hours, identified and frozen. Pools of approximately 10 to 15 *Aedes* mosquitoes were ground and homogenized and suspended in phosphate buffered saline (PBS). The supernatant obtained by centrifugation at 10,000 rev/min for 30 minutes was inoculated intracranially into the 1 to 2 day old suckling albino ICR mice. The amount of viral-positive extract injected was calculated accordingly at 2 mg/uL per gram body weight.

Two suckling mice per site and per generation per treatment were injected with the extract, and the treatments included: 1) parent mosquito extracts, 2) F1 adult mosquito progeny extracts, 3) F2 adult mosquito progeny extracts and 4) PBS-injected mice that served as the control. Quantitative determination of virus activity was based upon the intracranial inoculation. In the intracranial inoculation of the suckling mice, the site (mid frontal area) and depth of inoculation (2 mm) were carefully controlled in

order to ensure greatest uniformity and sensitivity of virus detection.

Disease manifestations in the mice - fatal or otherwise - were recorded. On the 7th day, the mice were terminated using standard procedures, and blood was recovered by heart puncture for dengue RT-PCR and complete blood count, and the brains of infected and uninfected control were removed with sterile syringes and prepared as a 20% PBS solution. The mice brains were macerated and the suspensions were centrifuged, while the supernatant were aliquoted and stored. This was then inoculated intraperitoneally to another set of 3 to 4 week old female mice.

2) Intraperitoneal infection in mice

Juvenile mice 3 to 4 weeks old were inoculated intraperitoneally with 150 uL infected viral suspension (from brains of suckling mice) per treatment (parent, F1, F2, and control per site) and with 150 uL PBS for the control. Mice were kept for 21 days after inoculation and during this time, occurrence of disease and signs of specific infection, fatal or otherwise were noted. One mouse from each generation (parent, F1, and F2) of each site (Basila, Camiling, RITM) and one control mouse were sacrificed for brain and other organ analysis on the 21st day post-inoculation.

Temperature

The 3 to 4 week old mice were housed in a temperature- and humidity-controlled environment with a 12:12 hour light-dark cycle and with food and water available for 21 days. Mouse anal temperature was measured with a thermocouple thermometer (model BAT-10R) using a RET-3 probe on day 21. The mean temperature reading was recorded once the value settled for at least 5 seconds.

Platelet count

Platelet count was performed using standard methods. Whole blood extracted through a capillary tube was immersed in 40 uL RBC lysis buffer and 20 uL 0.1% formaldehyde for preservation prior to platelet count. After the baseline blood extraction, more blood from the tail vein region was obtained on the 1st, 3rd, 6th, 9th, 12th, and 15th day. Platelets were counted under a light microscope using a hemocytometer. For the purpose of calculating the total platelet count, the following variables were considered: area of the counting chamber, dilution factor and constant were also processed. ANOVA was used to compare mean platelet count of the groups (Basila, Camiling and RITM) with the control.

Analysis of the brain, kidney, liver and spleen

All mice were sacrificed on the 21st day; the kidney, liver, brain and spleen were removed for histological analysis. Euthanasia using cervical dislocation was done according to standard guidelines. Infection in the brain was determined performing histological analysis of cerebrocortical areas. Macroscopic examination was performed for physical manifestations of infection. The kidneys, livers and spleens of the mice were removed and macerated to obtain a cell suspension. Microscopic analysis for evidence of virus infection in these organs was documented.

Results

Diagnosis of dengue virus in mosquito samples

Results showed a detectable and strong dengue viral presence, predominantly DEN-2, DEN-3, and DEN-4, in the *Aedes aegypti* mosquito bodies of the research samples (Basila, Camiling, RITM) for each generation, and there was no detectable dengue viral presence in the negative controls. The profile of dengue virus serotypes for each group (Basila, Camiling, RITM) and for each generation is summarized in Table 1.

Survival Rate and Virus Proliferation in Suckling Mice

There was a decrease in survival of the suckling mice among the aggregate groups of parent (67% by day 7) and F2 generation (67% by day 7) coming from Basila, Camiling and RITM as shown in Figure 1. All mice from the control group survived. Infection was clearly observed between day 1 and day 3 in the parent suckling mice group (Basila, Camiling, RITM). Two out of the six mice did not exhibit definite paralysis but showed weakened motor movement, three of the six had marked paralysis of one or two legs, and one was classified as sick. Two

Table 1. Profile of dengue virus serotypes (DEN-1, DEN-2, DEN-3, DEN-4) present in field collected *Aedes aegypti* mosquitoes from Quezon City and RITM using RT-PCR.

Sample Code*	Ordinary PCR for specific DEN serotype**				Remarks***
	DEN TS1	DEN TS2	DEN TS3	DEN TS4	
B Parent	-	+	+	-	Strongly positive for DEN virus
B F1	+	+	-	+	Strongly positive for DEN virus
B F2	-	+	+	+	Strongly positive for DEN virus
C Parent	-	+	+	+	Strongly positive for DEN virus
C F1	+	+	-	+	Strongly positive for DEN virus
C F2	+	+	+	+	Strongly positive for DEN virus
R Parent	+	+	+	-	Strongly positive for DEN virus
R F1	-	-	+	+	Strongly positive for DEN virus
R F2	+	+	+	+	Strongly positive for DEN virus
NC Parent	-	-	-	-	Negative
NC F1	-	-	-	-	Negative
NC F2	-	-	-	-	Negative

* B - Basila; C - Camiling; R - RITM; NC - Negative Control
** "+" means presence of PCR product, "-" means absence of target PCR product
*** Strongly positive means more than two positive scores or with presence of DEN 2. Weakly positive means < 2 positive scores and signal is weak or PCR product size is not the one expected (ie possible alternative genotype)

mice (33%) with marked paralysis (one Basila, one RITM), died on day 4 with signs of hemorrhage. Four of the six mice (67%) from the F1 suckling mice group (Basila, Camiling and RITM) were categorized as weak clinical condition but had no definite paralysis. Two out of the six mice (33%) were categorized as slight paralysis of one or both hind legs. There was mortality after 7 days of observation. Three of the six mice (50%) from the F2 suckling mice group (Basila, Camiling, RITM) were categorized as weak condition with no definite paralysis. One of the three improved its condition on day 5, with faster movement and reflexes. Two mice with slight paralysis (33%) and one mouse (17%) with marked paralysis died on day 5 (RITM and Basila) with signs of hemorrhage. There was no apparent change in the negative control group within the 7 day observation period.

Survival Rate and Disease Manifestation in Mice

There was a decreased survival of the 3 to 4 week old mice among the aggregate groups of parent (89% by day 15), F1 generation (78% by day 15) and F2 generation (78% by day 15) coming from Basila, Camiling and RITM as shown in Figure 2. All mice from the control group survived. For the parent group of Basila, Camiling and RITM, five out of the nine mice (56%), were categorized as weak; they exhibited no definite paralysis but had weakened physical activity with ruffling of their fur; three of the nine (33%) had slight paralysis of one or both legs and continuous ruffling of fur; and one (11%) was classified as marked paralysis. Conditions usually improved before reaching day 15. One mouse from Basila (P) categorized with marked paralysis died on day 1.

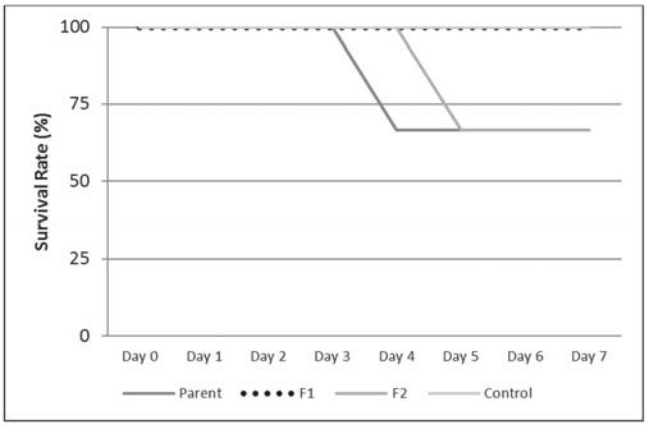

Figure 1. Survival rate among the control and suckling mice inoculated intracranially with dengue virus (Parent, F1, F2) at post infection, n=20.

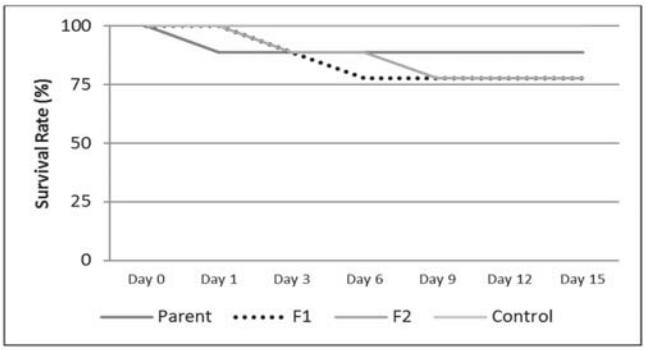

Figure 2. Survival rate among the control and mice inoculated intraperitoneally with dengue virus using suckling mice brain suspension (Parent, F1, F2) at post infection, n=30.

Four of the nine mice (44%) from the F1 group (Basila, Camiling, RITM) were categorized as weak but had no definite paralysis, weakened physical activity and ruffling of fur. Four of the nine mice (44%) were categorized as slight paralysis of one or both hind legs, and ruffling of fur; one mouse (11%) was categorized as sick. While most of the mice showed improved reflexes and mobility before reaching day 15, these mice were becoming heavier and fatter, meaning their livers may have been affected. There were two mortalities, Basila and RITM F1, after day 3 and day 6, respectively.

Four of the nine mice (44%) from the F2 group (Basila, Camiling, RITM) were categorized as weak condition with no definite paralysis and continuous ruffling of fur. All of the mice's condition improved on day 12. One mouse was categorized as sick but also improved its condition by day 12. Two mice (22%) categorized as slight paralysis and as marked paralysis died (Camiling and RITM) on days 3 and 9, respectively.

In general, after inoculation of the viral brain suspension into the 3 to 4 week old mice, weanling mice appeared well until day 3, when a number of these mice became lethargic, developed ruffled fur, and even stopped feeding. Five of the 27 mice (19% mortality) died by the end of the first week; death was preceded by tonic seizures. There was no apparent change in the negative control group within the 15-day observation period.

Temperature

The normal body temperature of the mice was 35.8°C to 37.6°C. By day 21 post-infection, 13 of the 30 mice (43%) had higher than normal temperatures, indicative of mild to severe fever. Five of the thirty mice died at various days of the observation period.

Platelet count

The infected mice for Basila, Camiling and RITM showed significant reduction of the platelet count between day 3 and day 10 after infection as seen in Figures 3 to 5; the platelet count subsequently returned to normal. Mice group of Basila (parent, F1 and F2) had the lowest platelet count, and were also the slowest to recover and increase their platelet count by day 12 and day 15. The Camiling and RITM groups had higher platelet counts compared to the control a day after intraperitoneal injection of suckling mice brain suspension. The Camiling group had fluctuating mean platelet levels starting day 3 and returned to baseline levels by day 15.

Table 2 shows significant differences among control, parent, F1 and F2 in the Basila, Camiling and RITM groups. The subset analysis for the Basila group shows that control and F2 are similar but different from parent and F1 (P = 0.004). The Camiling group shows that F2 is significantly different from control, parent and F1 combined (P = 0.029). The RITM group shows no significant differences among control, parent, F1 and F2 platelet counts (P = 0.287).

Table 2. Comparison of control with mean platelet count of treatment groups (B, C and R), 21-days post infection in mice.

	Mean (cells/mL)	Subset	F-value	P-value
Basila				
Control	9718.479	1		
Parent	6169.512	2	5.797	0.004
F1	6573.824	2		
F2	9389.039	1		
Camiling				
Control	9718.479	1		
Parent	9389.039	1	3.559	0.029
F1	10916.44	1		
F2	7457.323	2		
RITM				
Control	9718.479	1		
Parent	10676.85	1	1.332	0.287
F1	8805.031	1		
F2	9029.65	1		

Histopathological Analysis

Signs of hemorrhage and inflammatory perivascular cuffing were observed in the brains of the infected mice. Although there was no definite astrocytosis (an indication of viral infection), there was evidence of lymphocytic infiltration and nucleolar structure alteration for both sub-cortex and cerebral cortex of the infected mice brains. These alterations in neurons showed degenerative changes wherein nuclei appeared pushed to the corner and were condensed into chromatin masses.

There were no significant hemorrhagic lesions in the spleens of the infected mice. Their spleens did not appear enlarged compared to the controls. The livers of infected mice showed inflammatory cell infiltration. Other areas of the slides showed liver damage demonstrated by the presence of perivascular cuffing and vacuolation of hepatic cells in a major vessel. There was also moderate liver damage characterized by centrilobular hepatocellular degeneration showing nuclear destruction. There were no significant lesions in the livers of the control group. Focal areas of perivascular cuffing in the cortico-medullary junction of kidneys of infected mice.

Discussion

This study presented data and observations which confirmed transovarial transmission of dengue virus in Quezon City and that transovarial transmission may play an important role in initiating and maintaining the vector-borne disease in the locality. This study tested the presence of infectivity through virus detection in adult stages of the mosquito, until the F2 generation, and the inoculation of infection in mice to determine disease manifestations.

Since the experimental mice were inoculated with dengue virus from field mosquitoes, there was strong presumptive evidence of the etiology of the disease observed. Previous studies reported that mice inoculated intracranially with active dengue virus may develop infection that is not only fatal but may also be asymptomatic.[8] This study used suckling mice to aid in the proliferation of the limited virus present in mosquito samples. Many successful arbovirus isolations have been made through intracranial inoculation into suckling mice, since these suckling mice are considered a universal host system. The brains of the suckling mice were extracted and the suspensions were prepared and injected intraperitoneally to the 3 to 4 week old mice after 7 days. The clinical behavior and various disease manifestations of the mice were found to be typical for dengue virus infection. These included the onset of high fever, rash, joint, muscle pain with apparent decrease in movement, partial or severe paralysis, some degree of hemorrhage, low platelet counts, and liver damage. This study used parameters such as mice anal temperature, behavioral changes or clinical condition and progression of disease until day 15, platelet count, and analysis of the brains and other organs for morphological and histopathological abnormalities indicative of dengue infection.

Platelet count served as a useful marker in any animal model during dengue virus infection.[9] In this study, thrombocytopenia was observed as early as day 3 until day 12. By day 21, animals became viremic, indicating that the virus had already spread systemically and infected other organs as evidenced by the mononuclear infiltrates and perivascular cuffing of lymphocytes in the brain and other organs. The absence of neutrophilic infiltrates strengthens the presumption that the infection is viral in origin. Several studies have demonstrated that splenic cells, hepatocytes and lymphocytes were targets of infection of the dengue virus in mice.[9] Involvement of the central nervous system was confirmed through the observed changes in physical activity, motor strength and presence of seizures.

The ecology and mechanisms of transovarial transmission of dengue virus with respect to disease outbreak may not yet be fully explored especially in a country where dengue is dominant.[10] Yet, these findings suggest occurrence of transovarial transmission of dengue virus by *Aedes aegypti* in the selected sites of Quezon City. Viral infection pattern of the progeny was observed until the F2 generation. The study presented the complexities of the relationship between the transovarial transmission in mosquitoes with the mice survival, viral replication, and clinical condition. Early mice mortality correlated directly with the amount of virus inoculated. The use of a comprehensive animal model, whereby a suckling mice model was used for dengue virus proliferation in the mice brain and a 3 to 4 week old mice model was used to determine disease manifestation provided evidences, may aid

in understanding dengue pathogenesis specific to transovarial transmission of the virus. Collectively, these observations suggest that transovarial transmission of dengue virus can occur in nature, and that its virulence can translate into dengue disease when inoculated in an animal model.

This study provided evidences and confirmed occurrence of transovarial transmission of dengue virus in field adult *Aedes aegypti* mosquitoes in Quezon City. The examination of dengue virus for field adult *Aedes* mosquitoes yielded positive results for all four dengue serotypes until the F2 generation. An animal model was also developed to determine if disease manifestation typical of dengue infection was observed upon inoculation of the dengue positive mosquito homogenate. This animal model showed that when inoculated intraperitoneally, dengue manifested with both clinical and physical signs. This study presents that based on the model used, ICR mice can be a reservoir of the virus and may be implicated in the maintenance of the virus in nature and in the locality.

While vertical transmission may be responsible for the persistence of dengue virus in nature and eventually may play an important role in virus maintenance, its current implications to the disease epidemiology, and up to some extent, on vector control (especially larval control), must be further studied. It is also important to consider genetic diversity among viruses within a serotype. Factors such as the biology and biting habits of the vector mosquitoes, retention of virulence on succeeding generations, environmental factors and immune responses of the population and host susceptibility must also be put into consideration.

In general, despite the various intensive vector surveillance and control programs advocated by the health sector to address challenges of dengue control, transovarial transmission may have the potential to increase the probability of dengue outbreaks, and may contribute to persistence of dengue hemorrhagic cases in the locality.

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Conflict of Interest Declaration

The author and co-authors declare that there is no conflict of interest.

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A non-concurrent cohort study on the use of *Euphorbia hirta Linn* (tawa-tawa) in dengue: patients' platelet response

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Abstract

Introduction This study aimed to document the manner by which *Euphorbia hirta* is used among hospitalized dengue patients and to determine differences in platelet levels between dengue patients who had taken *Euphorbia hirta* and the control group.

Methods A non-concurrent cohort design was used. The cohort group comprised 46 verified dengue patients who had taken *Euphorbia hirta* during the course of the illness and 47 subjects in the control group who did not. Subjects in the cohort group were interviewed regarding the use of *Euphorbia hirta*. Platelet counts were compared within and between the cohort and control groups.

Results The use of *Euphorbia hirta* in the cohort group varied in terms of its preparation, dosage, frequency and duration of intake. Controlling for day of illness, the mean platelet counts did not show significant differences between the groups. However percentage change of platelet counts at each day of illness was favorable among the *Euphorbia hirta* subjects compared to the control group. The initial drop in platelet during the first 4 days of illness was greater in the control group. The rise beginning day 5 to 6 was twice greater in the *Euphorbia hirta* group compared to the control group.

Conclusion The beneficial effect of *Euphorbia hirta* may be on the degree of change in platelet levels experienced by the cohort group. The expected decline during the initial days of illness was less precipitous and the rise in platelet levels was twice greater compared to the control group.

Key words: *Euphorbia hirta*, dengue, platelet count, tawa-tawa

Dengue is one of the major disease problems in the Philippines and in about 100 countries worldwide[1] where the mosquito vectors thrive. According to WHO reports, since the identification of dengue hemorrhagic fever in the 1950s, the average annual trend of dengue incidence and case fatality rates have increased tremendously. In the Asia Pacific region, 70% of the population is now at risk of this disease.[2] The past 5 years showed a rising trend in dengue morbidity and mortality rates in the Philippines. Furthermore, the 2010 Department of Health (DOH) report from sentinel hospitals in the country showed a 142.89% rise in the number of cases recorded over the same period in the previous year.[3] Undoubtedly, dengue has exceeded the epidemic threshold set in 2010. The dengue problem prevailed in spite of all the educational campaigns,[4] treatment guidelines[2,5] and other interventions introduced by the government and private sectors.

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The varied medicinal value of *Euphorbia hirta* Linn has been thoroughly explored in several countries.[6-11] In the Philippines, the seemingly unabated dengue problem has prompted the consideration of *Euphorbia hirta* Linn as an alternative intervention that may complement western medicine. It is believed to have platelet increasing properties. Because of this, basic investigations on its properties have been conducted. Most of these local studies, however, were animal experiments.[12-16] With conflicting findings and the dearth of scientific analytic and clinical studies on human beings, *Euphorbia hirta's* value in the treatment of dengue still needs thorough scientific exploration so that development of policies and guidelines on its use in dengue among humans can really be evidence based.

The main objective of this non-concurrent cohort study was to determine if mean platelet count responses significantly differed between hospitalized dengue patients who took *Euphorbia hirta* preparations from those who did not. It may provide evidence on its use as a medicinal preparation for subsequent investigations. Lastly, it may shed light for the thousands of Filipinos who are in a quandary as to *Euphorbia hirta*'s value in dengue.

Methods

This is an analytic study of the historical cohort design. The study population included 93 dengue cases admitted between 2011 and 2012 identified through clinical records from a hospital in Metro Manila. The subjects in the cohort group consisted of 46 verified dengue cases that had oral intake of any preparation of *Euphorbia hirta* at any time during the course of their illness. The control group included 47 dengue cases who did not take the medicinal plant preparation at any time during the course of the illness. Both study groups received concomitant dengue management as prescribed by their respective attending physicians.

Identification of subjects was based on the attending physician's final diagnosis of dengue as it appeared in the patients' charts. The revised dengue case classification was used to reconcile the old case definition and classification with the updated version.[17] This was further verified through the presence of manifestations referable to the disease as documented in the history and physical examination sections of the patients' hospital records. Subjects included only those whose data information was complete. Enrollment of subjects was finalized after obtaining consent for participation in this study. Moreover, a sub-sample of 30% (26 patients) of these subjects consented to have blood examinations for Dengue NS1, ELISA test for IgG or IgM or both. This was done to serologically verify the diagnosis of dengue fever. All subjects were interviewed regarding the use of *Euphorbia hirta*, its preparation, dosage and administration.

Interview, clinical records review and for a proportion of the subjects, blood examinations (NS1, ELISA IgG, IgM), and focused group discussions were conducted to verify the accuracy of data collected through records. Data gathered from the subjects' clinical records included basic demographic information, chart records of fever, nausea, vomiting, rash, hemorrhagic manifestations, retro-orbital pain, arthralgia; laboratory data on NS1, immunoglobulin determination during confinement and serial platelet counts.

Informed consent from the parent/guardian and assent of the subjects (when appropriate) for participation in the study, interview, blood extraction were obtained by the research team.

Results

The characteristics of the 93 subjects are presented in the succeeding tables. In general, there was homogeneity in the age and gender distribution of the subjects. The mean ages were 13.5 ± 5.9 and 13.9 ± 7.2 years for the cohort and control groups, respectively.

Table 2 shows distribution of the study groups according to the classification of dengue, based on the attending physician's final discharge diagnosis. Table 3 shows that more subjects in the cohort group were admitted at later days of illness. More than 50% of these subjects were admitted at day 3 or later and

Table 1. Age distribution of the cohort and control groups.

Age groups	Cohort	Control	Total
<5 years old	5	6	11
5 - 10	7	13	20
11 - 14	15	8	23
15 - 20	13	15	28
21 - 24	4	2	6
25 and older	2	3	5
Total	46	47	93

even up to the 7th day of illness while those in the control group were admitted at earlier days up to the 5th day of illness. Thus, subsequent analyses are grouped according to day of illness.

Table 2. Classification of subjects according to discharge diagnosis.

Dengue classification	Cohort Group	Control Group	Total
Dengue without warning signs: (DF & DF1)	18	21	39
Dengue w/ warning signs (G2, G3, G4)	28	26	54
Total	46	47	93

Table 3. Distribution of subjects according to day of illness on admission.

Day of Illness	Cohort Group	Control Group	Total
Day 1 to 2	3	10	13
Day 3	12	13	25
Day 4	13	15	28
Day 5	7	5	12
Day 6 & 7	12	0	12
Total	42	43	95

Table 4 shows important clinical manifestations recorded for each of the subjects. Hemorrhagic manifestations were recorded in 28% of the subjects. Only about 60% of the subjects had tourniquet tests done on them, of which only 40% turned positive. Table 4 shows the distribution of subjects according to presence of clinical manifestations.

Information on *Euphorbia hirta* obtained through interview presented in Tables 5 and 6 shows that there was neither a standardized way, nor amount of intake of this herbal preparation. Fifty percent of the group used decoctions prepared at home by means of simple boiling the leaves, stem or the whole plant. The frequency, duration and dosage of intake likewise varied from patient to patient. Only about 60% of subjects took the preparation for 3 days to one week. The amount per intake ranged from one-half glass decoction or tea to as low as only one glassful per day.

Table 4. Distribution of subjects according to presence of clinical manifestations.

Manifestations	Cohort Group		Control Group	
	#	%	#	%
Fever	46	100	47	100
Headache	32	69	29	55
Vomiting	34	74	35	67
Abdominal/Epigastric pain	31	67	30	58
Malaise	27	59	21	40
anorexia	25	54	27	52
Rash	23	50	26	50
Bleeding	11	24	12	23
Petechiae	13	28	14	27
Retro-orbital pain	2	4	3	6
Arthralgia	6	13	9	17

Table 5. Types of *Euphorbia hirta* preparation used by cohort group.

Preparation Type	Number	Percentage
Decoction using leaves	7	15.2%
Decoction using stem	1	2.2
Decoction using whole plant	15	32.6
Commercial tea	14	30.4
Commercial capsule and liquid forms	9	19.6
Total	46	100%

Table 6. Duration of intake of *Euphorbia hirta* preparations in the cohort group.

No. days on intake	Number	Percentage
2 days or less	10	26.3
3 -4 days	14	36.8
5 days to 7 days	14	36.8
Total	38	100.00%

Missing data= 8(17.4%)

Figure 1 shows a comparison of the mean platelet trends between the study groups according to days of illness. For both groups, mean platelet counts progressively started to decline from the 2nd day of illness until the 6th day, thereafter, the platelet counts began to increase (Table 7, Figure 1). The mean platelet values were generally higher in the control group but did not significantly differ between the study groups ($P > 0.05$, Levene's test) even if these

were controlled according to the subjects' discharge diagnosis and day of illness. The absolute difference in the mean platelet values between the study groups are likewise shown in Table 7. When analyzed within groups, these platelet changes from day to day were significant ($P < 0.05$, T-test for equality).

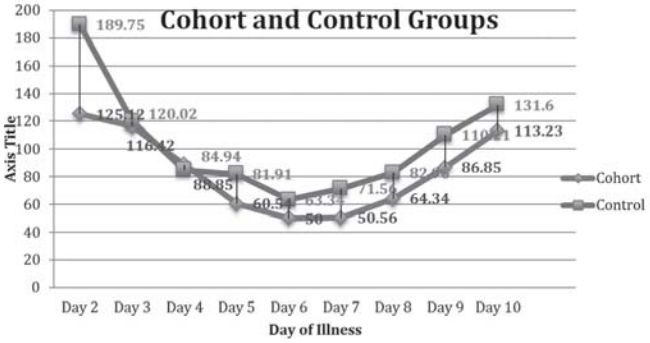

Figure 1. Mean platelet counts and absolute difference between cohort and control groups.

In terms of percentage change in mean platelet count in between days of illness (Table 8, Figure 2), the percentage drop observed in the cohort group was generally less drastic than the changes in the control group. The rise or improvement in platelet count trend was observed to start from day 6 to 7. When these changes were analyzed using percentage change in mean platelet counts between days of illness within each study group, the cohort group exhibited an increase of about 30% to 50% more compared to the control group (Figure 2). This was observed from day 7 and onwards. There was also a precipitous drop observed in platelet count during the initial days of illness in the control group compared to the cohort group.

Discussion

The use of *Euphorbia hirta* is diverse worldwide[17] but in the Philippines, it has been popular for generations

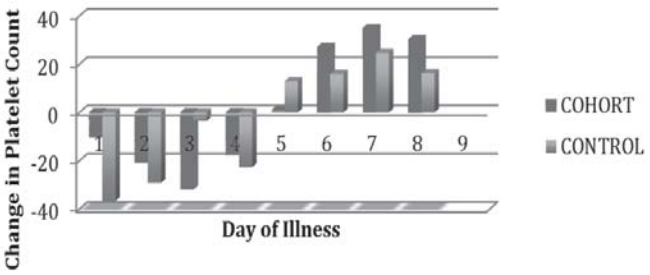

Figure 2. Percentage change mean platelet count by day of illness cohort vs. control.

Table 8. Percentage change in mean platelet count in between days of illness within groups and between groups.

Day of Illness	Cohort	Control
Day 2 and 3	10.30% drop	36.73% drop
Day3 and 4	20.8 4% drop	29.25% drop
Day 4 and 5	31.85% drop	3.50% drop
Day 5 and 6	17.41% drop	22.67% drop
Day 6 and 7	01.12% rise	12.94% rise
Day 7 and 8	27.25% rise	15.93% rise
Day 8 and 9	34.99% rise	24.70% rise
Day 9 and 10	30.37% rise	16.20 % rise

Table 7. Comparison of mean platelet counts (SD) and absolute difference between cohort and control groups by day of illness.

Day of Illness	Cohort Mean (Standard Deviation)	Control Mean (Standard Deviation)	Absolute Difference in Mean Platelet Count	Independent t Test p-value*
Day 2	125.12 (66.3)	189.75 (30.0)	64.63	0.277
Day 3	116.42 (35.2)	120.05 (44.7)	3.63	0.546
Day 4	88.84 (42.0)	84.94 (38.4)	3.91	0.768
Day 5	60.54 (37.7)	81.91 (49.9)	21.37	0.070
Day 6	50.00 (32.8)	63.34 (43.3)	13.34	0.296
Day 7	50.56 (34.0)	71.56 (51.3)	19.00	0.056
Day 8	64.34 (45.1)	82.96 (49.7)	18.62	0.147
Day 9	86.85 (41.7)	110.21 (62.8)	23.36	0.125
Day 10	113.23 (45.1)	131.60 (73.2)	18.37	0.440

*t-test for equality of means (all not significant)

as a folk remedy for dengue. Yet, there is a dearth of scientific investigations on the use of this medicinal plant in dengue among humans. Inconsistent platelet patterns or responses after *Euphorbia hirta* exposure have been observed in the few experiments done in rats.

The result of this study is centered on *Euphorbia hirta's* effect on the mean platelet levels of patients. The drop in mean platelet count trend of subjects who took *Euphorbia hirta* preparations were not as drastic as those observed in the control group. The expected rise in platelet levels from the 6th or 7th day were twice as much in the cohort compared to the control group. The absence of an existing standard recommendation on the use of *Euphorbia hirta* among human subjects in dengue may have contributed to the inconsequential difference in mean platelet counts between the study groups. In spite of this, there is an interesting observation involving the degree of platelet changes between the study groups. A lesser degree of decline and a steeper rise in mean platelet counts, measured as the percentage change, is seen favoring the *Euphorbia hirta* group. The drop in platelet counts during the first 5 days was lower by more than twice in the cohort group compared to the controls. Moreover, the rises in platelet counts from day 7 onwards were almost twice as much among those who took *Euphorbia hirta* compared to the percentage change observed in the control group. No studies so far have measured changes in terms of percentage change as observed daily during the entire course of illness. This observation needs to be analyzed more closely as this requires bigger sample size. In this study however, the estimated percentage change values still fall within the estimated confidence intervals.

If reduction in platelet counts can be retarded promptly and its rapid, steeper rise to normal values enhanced through intake of *Euphorbia hirta* preparation, this would be beneficial to dengue patients. This is the unexplored value of *Euphorbia hirta* as an adjuvant in dengue management. Further studies using a specific preparation, dose and duration of *Euphorbia hirta* intake, with endpoint measures centered on quantity and degree of change in platelet counts can be explored.

Experimental animal studies on *Euphorbia hirta*[12,13,14] recorded significant differences in the observed increase in platelet counts. Standardized doses of *Euphorbia hirta* decoction and ethanolic extract of *Euphorbia hirta* were used in the two studies respectively. On the other hand, some recent studies both in animals and normal human volunteers have reported contrasting results. Reductions in platelet counts of experimental animals given *Euphorbia hirta* were observed in one study.[16] Likewise, among normal human volunteers who were randomized to receive *Euphorbia hirta* tea preparations and those in the control group, no significant difference in platelet counts at days 5 and 10 of its administration were observed.[18]

In the studies cited above, varied formulations, dosages, frequencies and durations of *Euphorbia hirta* were used. The method of induction of thrombocytopenia likewise differed, and so did the outcomes. This study showed wide variation in the use of *Euphorbia hirta* preparations in dengue patients, as well as in procedures in its preparation, part of the plant used, frequency, duration and dose. A comparison of the platelet and WBC counts between the group who took *Euphorbia hirta* and those who showed no significant difference. However, the percentage change in platelet count from day to day was found to differ between the groups, favoring the cohort group. In spite of the variations in the use of this medicinal plant among our subjects, a steeper rise and a rather lower degree of initial decline in platelet count were observed among the *Euphorbia hirta* group compared to the control group. The need for further analysis in this regard and the use of experimental study designs in humans are now in order so that more conclusive evidence on the use of *Euphorbia hirta* in dengue can be scientifically documented.

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Focused group discussion on the use of *Euphorbia hirta* Linn (tawa-tawa) in the treatment of dengue fever

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Abstract

Introduction *Euphorbia hirta* Linn has been used as a folk remedy for dengue. Despite numerous anecdotal reports, there is a lack of precise documented information regarding its actual use, perceptions of effectiveness and safety. This study aimed to elucidate the use of *Euphorbia hirta* Linn in the treatment of dengue.

Methods Previously confined pediatric patients and their parents were invited to attend focused group discussions on their use of *Euphorbia hirta*. The discussions centered on sources of information, sourcing, preparation and dosing, the effects of treatment and willingness to endorse its use.

Results Eight volunteers participated. The common source of information was word-of-mouth endorsement. They used commercial preparations and administered it as an infusion. *Euphorbia hirta* was given at various doses and durations. Respondents claimed that it made their patients feel better and that they were willing to recommend its use.

Conclusion: The study showed the potential value of *Euphorbia hirta* for the treatment of dengue, as perceived by the care givers.

Key words: *Euphorbia hirta*, tawa-tawa; dengue

Euphorbia hirta Linn. is a slender-stemmed annual pantropical plant that is ubiquitous in the Philippines and is considered to be a common weed. Referred to as tawa-tawa, gatas-gatas, bolobotonis and golondrina, this plant has been in the news recently as a folk remedy for dengue fever. *Euphorbia hirta* has been a topic of interest worldwide as a medicinal plant for varied medical conditions such as dysentery, diabetes, intestinal parasitism, seizure disorder and even for wound care. Although used for such diverse conditions, its use as treatment for dengue is apparently unique to the Philippines.

The alarmingly high numbers of dengue-infected individuals at times counting in the tens of thousands[1] has overwhelmed local medical facilities. In the face of this situation, there is a persistent and regular interest regarding the use of *Euphorbia hirta* as a potential treatment for the disease. However, there are no definitive studies confirming or denying its safety or efficacy in humans. Medical authorities, on the other hand, have been slow to investigate this phenomenon and have been prudent in advising the general public to refrain from its use[2] even though

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there already seems to be significant awareness on the part of the public for its potential as gleaned from the availability of commercial preparations of this herb in the market.

The authors believe that in the face of an increasing threat from the disease vis-à-vis the logistical constraints of the healthcare delivery system there should be a more active search for alternatives to existing treatment strategies. Despite numerous anecdotal reports regarding the use of *Euphorbia hirta* in the treatment of dengue, there is a lack of precise information regarding its actual use. This study investigated the techniques used in employing this plant as a treatment for dengue from those who have had actual opportunity to use it and their perceptions on its efficacy and safety. Hopefully, the information generated herein will also facilitate more leads for others to pursue further studies in this area.

Methods

In this study, pediatric patients previously diagnosed and treated for dengue and used *Euphorbia hirta* Linn any type of preparation while confined in a private hospital were invited along with their parents to discuss their experiences. Questions were centered on their sources of information regarding *Euphorbia hirta* Linn, its preparation and use, perceived effectiveness, adverse effects, and limitations. The moderators were careful to try to elicit as much detail as possible. As there were only a handful of respondents, this author sought to understand these discussions more from a semantic perspective rather than from a mere inventory of topics. Recurring semantic themes were clustered together and examined as a way to assess views and opinions regarding their use and perceptions of *Euphorbia hirta*.

The study was approved by the Ethics Review Committee. As the patients involved in this study are minors, the researchers made sure to include the parents of these subjects.

Results

All the eight participants were from the Metro Manila area and all of them had their experiences less than two years prior to the focused group discussions which were conducted in two separate sessions. Of the eight participants in the discussions, six were adults and parents of the subjects. The transcripts were analyzed and common themes were noted and clustered together to get a better picture regarding the phenomenon of the use of *Euphorbia hirta* for dengue.

The respondents tended to get most of their information from relatives (12.5%), hospital staff such as nurses and medical interns (12.5%), doctors (6.25%), friends, neighbors, employers (31.25%) and media celebrities (6.25%). Strangers met by some of the respondents during their stay in the hospital were also significant sources of information accounting for 18.75% of the total responses. As far as media sources are concerned, television and the internet each accounted for 6.25% of total responses tallied. Print media sources of information such as newspapers and magazines were not mentioned.

In terms of grouping together only actual personal contact sources of information, all non-relatives and all non-medical personnel comprised 61.54% of these responses while relatives and medical personnel comprised only 38.46%. With regard to grouping of word-of-mouth compared with virtual endorsements such as television or internet, word-of-mouth endorsements was the overwhelming source of information (87.5%); the remaining 12.5% was attributed to non-print media.

There were two general ways in which *Euphorbia hirta* was obtained by respondents: either as a commercially prepared product or wildcrafted. Commercially prepared products were purchased at a mall near the hospital and accounted for 75% of such responses. Wildcrafted samples used by some of the respondents accounted for the remaining 25%. The commercially prepared *Euphorbia hirta* came in teabags in boxes of 20. For this presentation, infusion was the method of choice (37.5%). However, decoction was the method of choice (25%) for those who used wildcrafted preparations.

All claimed attempting to give these preparations either as a pure infusion or decoction; however, 37.5% of them complained that a disagreeable taste was a major deterrent to drink more of the concoction while 12.5% complained of its appearance. Significantly, 37.5% also reported that they used bottled tea or tea mixes to mask the taste of the *Euphorbia hirta* extracts. There were no complaints with regard to its odor.

The amount of time allotted to consume the *Euphorbia hirta* extract by the care givers of the respondents varied from 1/2 glassful consumed over one day to a total of 1.5 liters consumed over a period of 7 days. One respondent reported that 1/2 glassful of extract was given at 3 to 4 times per day for 2 days

while another reported taking 3/4 glassful 3 to 4 times per day for 2 days.

The resulting discussions with regard to the effects of treatment were divided into two categories: effects on platelet count and effects on general well-being. Of the respondents, 62.5% noted that effects of treatment were on platelet count and the remaining 37.5% were on general well-being. Of the treatment effects on platelet count, immediate improvement was noted in 12.5%, stabilization of platelet count decline with subsequent improvement was noted by 12.5% of the participants, and an initial decrease with a subsequent improvement was observed in 37.5% of responses. With regard to general well-being, 25% noted a general sense of improvement in the disposition of their patient, who seemed to have "recovered somewhat" from their illness as manifested by return to their usual behavior and the other 12.5% noted improvement in the patient's vital signs. Notably, there were no responses indicating any ill effects from consumption of the extracts.

Participants were asked to comment on whether they would personally recommend *Euphorbia hirta* to others as a treatment for dengue. Four of five participants said that they would recommend it and one chose to defer that decision to doctors. Half of those who chose to recommend said they highly would recommend it; the other two said they would recommend it as they saw nothing harmful in their earlier decision to give it to their patients. When asked to comment on whether the *Euphorbia hirta* extract would replace physician-based care, 40% responded that it would not.

Discussion

The responses of our participants show that a significant source of information on *Euphorbia hirta* as a potential treatment for dengue seemed to be from actual word-of-mouth endorsements, indicating that there may be significant public awareness. This may be viewed as a tacit acceptance of the usefulness of the herb in dengue. This also seems to be reflected by the total percentage of non-relative and non-medical personnel sources, indicating that strangers were the major source of information and supporting the contention of a significant level of public awareness.

In the preparation of herb recipes, it seemed that the manner in which the herb was sourced and its presentation or packaging greatly affected the manner of its preparation. Wildcrafted preparations were rendered exclusively by decoction while commercial preparations were exclusively rendered via infusion. There seemed to be no uniformity of the dose and duration of *Euphorbia hirta* as seen from the variations in the actual dosing. The range of the daily dose varied from 214 ml to 1500 ml per day, while the number of days in which extracts were administered ranged from 1 to 7 days. The amount of the dose, however, seemed to be a reflection of how well the patients tolerated the taste of the prepared extracts; this seemed to have been a formidable deterrent to its more liberal consumption. In the case of the commercially prepared infusion extracts, some care givers resorted to using store-bought tea drinks and mixes to mask the taste of the extracts to increase compliance. Increasing the intake of the *Euphorbia hirta* extracts achieved two things: (a) increasing the actual intake of *Euphorbia hirta* and its supposed health benefits and (b) increasing the intake of fluids to forestall the possibility of dehydration. However, it must be noted that there has been some speculation on the possibility on the non-dose dependent action of *Euphorbia hirta* in increasing platelet counts.[3]

The comments of the participants with regard to the effects of treatment were mostly centered on *Euphorbia hirta*'s ability to effect changes on the platelet count (62.5%). This may in large part be conditioned explicitly by healthcare personnel and/or by the previous experience of care givers and may account for the larger share of this facet in their recollection of their patients' illness. All the respondents claimed eventual improvement in platelet counts, although there may have been variations as to the immediateness or as to the pattern leading to this improvement. There were also no ill effects reported with the consumption of the extracts – suggesting that it may be safe to use.

One third of responses were regarding improvement in the general well-being of the patients involved. The authors believe that this is an important observation which may be overlooked in evaluating the value of *Euphorbia hirta* as a treatment for dengue. Although the nature of these observations are difficult to quantify compared to platelet counts, the ability to alleviate the general suffering from the disease be enough reason to pursue further research in this area.

Eighty percent of the participants said they would personally endorse *Euphorbia hirta* to others, and half of them said that they would highly recommend it.

The other respondent indicated that he would leave that decision for the physicians to make. This in turn may have been in deference to the lack of sanction by medical authorities which in turn may have implied illicitness in using *Euphorbia hirta* rather than any real perceived harm or ineffectiveness in their actual use of *Euphorbia hirta*.

This study showed the potential value of *Euphorbia hirta*, as perceived by the caregivers, for the treatment of dengue fever. These focused group discussions have opened possible areas for further study of *Euphorbia hirta* Linn in the treatment of dengue fever; however, the limited number of willing participants in this study was factor in obtaining a more in-depth understanding of the role of *Euphorbia hirta* in dengue.

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Laboratory observations on the use of *Diplonychus rusticus* as a potential biological control agent for Japanese encephalitis vector*

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Abstract

Introduction This study explored the practical use of the local aquatic bug *Diplonychus rusticus* in the laboratory as a potential biological control agent for the larvae of the vectors of Japanese encephalitis, *Culex tritaeniorhynchus* and *Culex vishnui*, which are commonly found in rice fields.

Methods *Diplonychus rusticus* was reared in the laboratory and its reproductive behavior and longevity were observed. Single predators consisting of III instar and adult female, respectively, were each placed in two containers with 100 Culex vishnui instars each as prey. The water bugs' feeding patterns were observed for 24 hours.

Results *Diplonychus rusticus* underwent five nymphal instar stages with an average developmental period of 50 days from egg to adult. The adult female laid six egg batches with 30 to 65 eggs per batch. The adult female lived up to 80 days while the adult male lasted 103 days. With 100 III instar and IV instar larvae of *Culex vishnui* given as prey, the III instar nymph of *Diplonychus rusticus* consumed 98% of the prey in 24 hours while the adult female consumed 96% of the prey in 12 hours and 100% at the end of 24 hours.

Conclusion *Diplonychus rusticus* is a potential biological control agent for the mosquito vector of Japanese encephalitis *Culex vishnui*.

Key words: *Diplonychus rusticus, Culex vishnui*, mosquito, instar, larvae

Japanese encephalitis virus is known to produce debilitating disease in young children in Southeast Asian and Western Pacific countries like the Philippines. In the past decade there has been an increasing incidence of Japanese encephalitis in geographic areas where it was not previously documented to occur. Reversing the trend of emerging vector-borne diseases such as Japanese encephalitis is a major challenge. In the Philippines, the more important mosquito vectors of the Japanese encephalitis virus are *Culex tritaeniorhynchus* and *Culex vishnui*.

Mosquito vector control has been identified as one method of controlling the spread of the mosquito-borne flavivirus diseases such as dengue fever, yellow fever and Japanese encephalitis. However, measures involving the use of chemicals, such as spraying insecticides to kill the adult mosquito population, pose a threat to the health and well-being of the people, since mosquitoes reside and breed near or within human habitats. Similarly, the application of

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chemical larvicides in the breeding sites of mosquitoes contaminates the environment. With the growing vigilance against the harmful effects of these methods on human health and the environment, not to mention the widespread problem of insecticide resistance, the search for an effective alternative method has become increasingly necessary.

With the advent of ecological and sustainable measures for vector control, WHO advocates the principles of integrated management,[1] which favor environment management, biological control and personal protection. In 1994, Lacey and Orr[2] presented a comprehensive approach to integrated vector control that included biological control agents for mosquitoes such as plants, predators, pathogens, and parasites as shown in Figure 1.

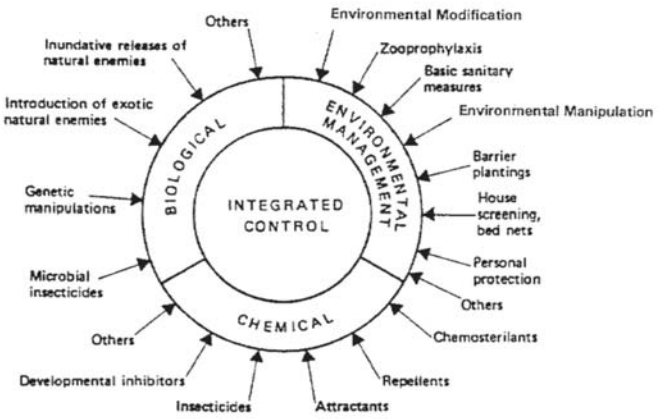

Figure 1. Diagram showing the potential components of integrated vector control, which include biological methods (Lacey & Orr, 1994).

The use of biological control is an acceptable alternative since compared to chemical insecticides; it involves reduced or no costs of labor and specialized equipment. In addition, it poses no threat to the safety of the surrounding human population and can help in restoring natural ecological conditions that have been destroyed by pollution of the environment. The WHO Manual on Environmenal Management for Mosquito Control[3] mentions several biological methods including the release of invertebrate predators. A review article by Kumar and Huwang[4] recommends biocontrol agents that are naturally found in the local environment, safe for people, and economical in their propagation.

Shalaan and Canyon[5] emphasized the renewed interest in biological control agents, particularly aquatic predaceous insects that control the number of mosquito larva in different aquatic habitats. Sivagnaname conducted a field trial in South India and found that an aquatic hemipteran bug *Diplonychus indicus*, was an efficient predator of dengue vectors in tires.[6] A recent study in the Philippines showed the predatory capability of a common rice field aquatic water bug, *Diplonychus rusticus*, on mosquito wrigglers, particularly *Aedes aegypti*,[7] an efficient vector of the dengue virus. Hemipteran bugs of the family Belostomatidae, particularly *Diplonychus rusticus*, can also be found in the Philippines specifically in rice paddies and marshes.[8] This aquatic bug is a known predator of *Culex quinquefasciatus* larva.[5]

Basic studies done in the laboratory can generate insights on natural predators. The findings on natural predators of mosquitoes, such as the *Diplonychus rusticus* in rice paddies, and the presence of vectors of Japanese encephalitis like *Culex tritaeniorhynchus* and *Culex vishnui* in rice fields highlight the significance of doing this study. No studies have been done on *Diplonychus rusticus* as predator of the vectors of Japanese encephalitis (as prey). This study supports the concept that natural enemies should play an important role in the biological control of mosquitoes in a rice field system as a part of an integrated vector control program. A natural environmental and integrated approach in the control of mosquitoes and the diseases they transmit is a more cost-efficient and sustainable program to use in developing countries like the Philippines. This study explored the practical use of this local aquatic bug in the laboratory as a potential biological control agent for the larvae of the vectors of Japanese encephalitis *Culex tritaeniorhynchus* and *Culex vishnui*.

Methods

This experimental study examined the biological characteristics and behavior of the aquatic predator bug *Diplonychus rusticus*, at both immature and adult stages, in the presence of *Culex vishnui* larvae as prey, and measured its feeding capacity under laboratory conditions. The study used constant dimensions of larval containers and room temperature. The dependent variable was the population prey with

exposure to predator as the independent variable at constant predator population.

Materials and maintenance of specimens

Adults and nymphs of the water bug *Diplonychus rusticus* Fabricus were collected from rice fields with plenty of pistia ("quiapo") in Laguna in November 2012 and propagated in a rearing laboratory. From the propagated population in the Laguna laboratory, the starting population of water bugs was transported to the study laboratory in plastic containers and consisted of 43 first instar larvae and a pair of adult female and male water bugs.

The study used a mass rearing method described by Sivagnaname[9] and Chandramohan.[10] The water bugs were maintained in 1,500cm^3 transparent plastic containers measuring 5cm x 20cm x 15cm filled with 1L of aged tap water up to 3cm in height. The containers were covered with mosquito nets to prevent oviposition of mosquitoes and other insect predators for one week prior to the start of the procedure. The aquatic bugs were provided with prey as food and some specimens of pistia. The prey was reared in the laboratory using wrigglers of *Culex quinquefasciatus*[9] and *Aedes spp.* The water in the plastic containers was changed every other day to avoid fouling.[10] Different stages of the predator were reared in separate transparent plastic containers to prevent cannibalism of the smaller nymphs or immature forms.[9] The predators were fed to satiation and then starved for 24 hours before being used for the study. This was done to standardize the hunger level of the predator.

The IV instar mosquito larvae were collected from rice fields in Bataan where migratory birds flock and where ducks and pigs are raised. These were kept in plastic containers with aerated water, and covered with mosquito nets to prevent oviposition of other mosquitoes and insect predators. The larvae were reared in the laboratory up to maturity for proper species identification. These were fed with 2g of commercial aquarium fish food to facilitate growth. The containers were cleaned regularly and the water was changed every 2 to 3 days. All larvae were reared to their adult stage. The identified adult *Culex vishnui* were reared in the laboratory for breeding and propagation. Laboratory-reared *Culex vishnui* larvae were fed with fish food. The culture of the adult *Culex vishnui* and subsequent rearing of the larvae were maintained to get a ready stock of sufficient number of III and IV instar larvae throughout the study period.

Observation of biology of Diplonychus indicus

The longevity and description of the different stages undergone by *Diplonychus rusticus* was done using acquired I instar nymphs and hatched I instar nymphs from the eggs laid by the adult female. The reproductive behavior of the adults was also observed using a pair of male and female water bugs.

Experimental method on predatory consumption

The experiment set consisted of a single predator from the III instar and adult female stages, respectively, which were released separately into round plastic containers 10cm in diameter and 4cm deep with 150ml of aged tap water. In each container with the water bug, 100 *Culex vishnui* III and IV instars were offered as prey. Every two hours starting from the first hour until the 12th hour, the number of eaten prey was obtained by subtracting the remaining live prey from the beginning count of live prey from the previous two hours. On the 12th hour, the water bugs were left in the dark to continue preying and on the 24th hour, the final count of the remaining live prey was done. The predation experiment was conducted with three replicates on three separate days, seven days apart. A control set up using round plastic containers with 100 *Culex vishnui* III and IV instars and was done every replicate. The predation experiment commenced at six o'clock in the morning and completed at six o'clock the next morning to observe the daily feeding rate. The duration of the lights-on phase was simulated by exposing the experimental set-up containers to an open window, maintaining a temperature of 30 ± 2°C; the lights-off phase was done in synchrony with the dark phase of the night by turning off tube fluorescent lights in the laboratory.

Data analysis

The fraction of prey eaten per developmental stage of the predator was subjected to an independent t-test. One-way analysis of variance (ANOVA) was used on the data on the predatorial capacity of the two stages of *Diplonychus rusticus*.

Results

Rearing of water bugs: observation of the biology of *Diplonychus rusticus*

From the starting population of 43 I instar larva, only one survived to adult stage. The larval development included five instars and lasted 54 days. The female oviposited during the night. During its 80-day lifespan, it was able to lay six batches of eggs. The eggs were laid on the dorsum of the male with 30 to 65 eggs per pad. Brooding behavior of the male lasted from 6 to 7 days until all the eggs were hatched and the I instar nymphs emerged. The eggs hatched in 1 to 3 days. The incubation period ranged from 7 to 10 days as shown in Table 1. After all the eggs had hatched, the male dropped the egg pad from its back. From each batch of eggs, 23 to 53 I instar nymphs emerged. Sixteen to 33 survivors of the first stage developed to become II instar nymphs. An average of 8.4 III instar nymphs emerged from the first to the fifth batches of eggs. An average of four IV instar nymphs emerged from the first to the fourth batches of eggs. Only the first three batches of eggs laid completed their development to the adult stage. An average of 1.6 adults emerged from three to four V instar nymphs. Among the different developmental stages, the early instars were observed to have a higher mortality as seen in Table 2. The male survived longer than the female.

Table 1. Summary of duration of each developmental stage of *Diplonychus rusticus*.

Developmental Stages of *Diplonychus rusticus*	1st batch of eggs Dec 4	2nd batch of eggs Jan 5	3rd batch of eggs Jan 25	4th batch of eggs Jan 31	5th batch of eggs Feb 14	6th batch of eggs Feb 21	Mean
Days of egg incubation	7	8	7	9	10	8	8.1
Duration to I instar	6	6	5	6	7	6	6
Duration to II instar	7	7	8	5	6	7	6.66
Duration to III instar	8	7	8	8	6	ongoing	7.4
Duration to IV instar	10	9	10	11	ongoing		10
Duration to V instar	12	13	11	ongoing			12
Days from egg to adult	52	50	49				50.33

Table 2. Summary of the mortality rate of Diplonychus indicus from one developmental stage to the next stage

Developmental stages of *Diplonychus rusticus*	1st batch of eggs Dec 4	2nd batch of eggs Jan 5	3rd batch of eggs Jan 25	4th batch of eggs Jan 31	5th batch of eggs Feb 14	6th batch of eggs Feb 21	Mean
Number of eggs laid	30	53	57	35	65	41	46.83
Number survived to I instar (% mortality of eggs)	23 (23.33)	49 (7.54)	50 (12.28)	32 (8.57)	53 (22.64)	28 (31.17)	39.16 (17.58)
Number survived to II instar (% mortality of I instar)	16 (30.43)	20 (59.18)	33 (34.00)	22 (33.33)	29 (45.28)	10 (64.28)	21.66 (49.48)
Number survived to III instar (% mortality of II instar)	7 (56.25)	9 (55.00)	10 (69.69)	11 (50.00)	5 (82.75)	ongoing	8.4 (62.74)
Number survived to IV instar (% mortality of III instar)	4 (42.86)	3 (66.66)	5 (50.00)	4 (63.63)	ongoing		4 (55.79)
Number survived to V instar (% mortality of IV instar)	2 (50.00)	3 (0)	2 (60.00)	ongoing			2.333 (36.66)
Number survived to adults (% mortality of V instar)	1 (50.00)	2 (33.33)	2 (0)				1.66 (27.77)

Predatory Behavior of Diplonychus rusticus

The *Diplonychus rusticus* preyed on the available live wrigglers when offered *Culex vishnui* III and IV instar larvae as shown in Figures 2 and 3. The number of live prey available to the adult female water bug decreased every two hours and was nearly totally consumed after 24 hours. The III instar nymph consumed 98 wrigglers, whereas the adult female which consumed all of the 100 wrigglers, in the 24-hour observation period. For hour 2 and hour 4, the adult female predator consumed more prey than the III instar nymph. However for hour 6 and hour 8, the III instar nymph consumed more prey than the adult female and the difference was significant. For hour 10 to hour 12, both adult and nymph consumed a comparable number of prey.

Discussion

The female *Diplonychus rusticus* was able to lay six batches of eggs during its lifespan of 80 days, exceeding the observations made by Sivagnaname that female *Diplonychus indicus* could lay four to five times only in its lifetime.[9] This is an indication that *Diplonychus rusticus* has a better reproductive potential. Having a high reproductive capacity is a good attribute of an effective biological control agent. The production of more offspring by a single female in several egg-laying batches would allow self-propagation in the field by the predator and limit reintroduction or continuous application. These are the disadvantages of other biological control agents for mosquitoes that are currently used or have been used in the past. Only a pair of reproductive adult water bugs was used. More accurate observations on the life cycle and reproductive capabilities could have been done if more subjects had been observed.

The adult female in this study survived for 80 days and laid six egg batches; the adult male was still alive on the 103rd day. The longevity of the adults is comparable to that of *Diplonychus indicus* studied by Sivagnaname.[9] As a biological control agent, longevity is a crucial factor for the maintenance of the predatory population in the field as well as in its use for developing a mass rearing technique in the laboratory.

This study noted a high mortality among early stages, particularly I and II instar, attributed to cannibalism, similar to the observations by Sivagnaname.[9] Cannibalism is observed especially in an environment where there is limited number of prey available. Moreover, cannibalism also occurs in the older stages, but to a lesser degree and among newly molted individuals, and likewise occurs in an environment with limited availability of prey. Cannibalism in this species limits the size of the predator population in the field especially when heterogenous stages of development coexist in the natural habitat like rice fields and marshes. On the other hand, cannibalism is a desirable characteristic of a predator for this allows natural selection of the species and control of the population in the wild.

In this study, *Diplonychus rusticus* was documented to prey on III and IV instar larvae of *Culex vishnui*, a known vector of Japanese encephalitis. It is evident that III instar nymph and adult female water bug can kill a good number of older and larger *Culex vishnui* larvae. The rate of predation depends on factors such as the age of the predator and the density of the prey.[11] The adult water bug can kill more than the younger III instar nymph.

The adult female *Diplonychus rusticus*, consumed less prey per hour after the fourth hour because by that time there was less prey available as shown in Figure 2. Another possible explanation for this is that the female water bug may have been satiated immediately after the fourth hour, after consuming almost 75% of the total available prey. On the other hand, the III instar nymph reached satiety after the eighth hour when it had already consumed 71% of the prey. The adult female consumed all the prey by the 24th hour and may have still been able to consume more prey if these were available. The III instar nymph had more prey to consume within the remaining 12 hours compared with the adult (Figure 3).

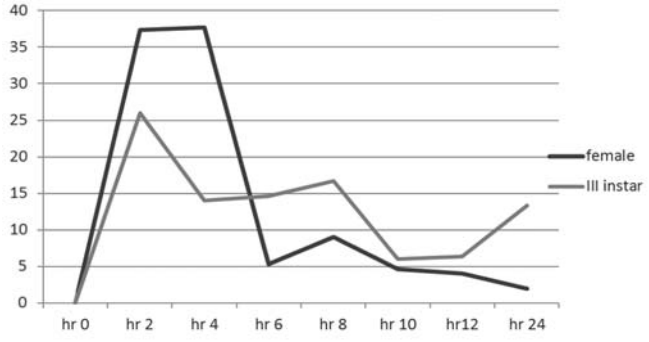

Figure 2. Mean number of III and IV instar larvae of *Culex vishnui* consumed by the adult female and III nymphal instar of *Diplonychus rusticus*.

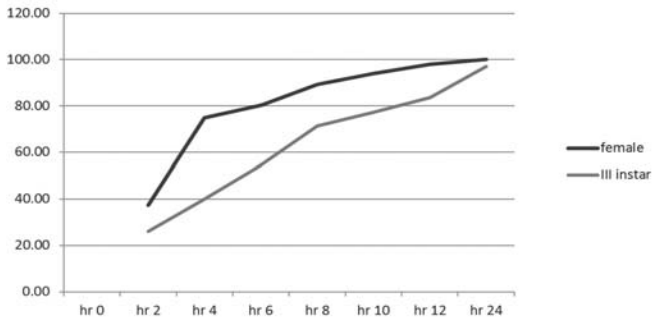

Figure 3. Cumulative number of III and IV instar larvae of *Culex vishnui* consumed by the adult female and III nymphal instar of *Diplonychus rusticus*.

The finding that the nymphal and adult stages preyed on mosquito larvae strengthens the premise that *Diplonychus rusticus* may be a promising biological control agent. Because of its preference for larger larvae, it prevents wrigglers from pupating and becoming adults, thereby decreasing the mosquito vector population. The study also showed some of the desirable characteristics of the water bug such as its longevity and reproductive capability.

The presence of *Diplonychus rusticus*, a natural predator of a vector of Japanese encephalitis, in rice fields and semi-permanent water habitats in the Philippines opens a new possibility for its use in controlling mosquitoes, such as the Japanese encephalitis vector *Culex vishnui*, found in rice fields. *Diplonychus rusticus* was observed to be long-lived, exhibited a high reproductive capacity and was a voracious feeder of *Culex vishnui* wrigglers. These are important attributes of an efficient biological control agent of mosquito-borne diseases such as Japanese encephalitis. However further studies on its characteristics as a predator, such as the functional response and frequency-dependent prey selection,[9] need to be further established to determine the efficiency of the water bug as a biological control agent. Ultimately, field studies are needed to explore its practical application as part of an integrated vector control program.

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Randomized controlled trial on the effect of pre-operative gum chewing on the level of postoperative anxiety among boys undergoing circumcision*

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Abstract

Introduction Circumcision is a Filipino tradition that persists today as a pre-adolescent coming-of-age surgery that, without sufficient mental preparation, may confer some level of psychological trauma. The procedure is made difficult by the anxiety of young boys prior to the operation. This study aimed to determine the effects of gum chewing in the pre- and post-circumcision anxiety of school-aged boys.

Methods Data were gathered from 241 boys, aged 7 to 13 years, who were present in medical missions in Quezon City in April 2013. The anxiety of the participants was assessed using the Yale Preoperative Anxiety Scale. Data were encoded and analyzed using EpiInfo™ 3.5.4. Percent change in mean anxiety scores during pre-intervention, post-intervention, and post-circumcision were compared between and within the group using T-test.

Results A 7.6% decrease in anxiety score was seen in the experimental group, while a 34.4% increase was seen in the control group.

Conclusion This study shows that gum chewing has an immediate anxiety-relieving effect which is carried over until the postoperative period in boys undergoing circumcision.

Key words: anxiety, circumcision, gum chewing, postoperative anxiety

Circumcision, as culturally dictated in the country, is one of the anxiety-provoking milestones in a school-age boy's life. Boys are circumcised as an act of religious dedication and mark of cultural identity. The procedure is usually made difficult by the anxiety of the young boys about to undergo the operation, identified in this study as pre-operative anxiety. In the study of Mahmoudi-gharaei and colleagues,[1] postoperative distress and the level of anxiety in the operative period were shown to be associated with the level of anxiety in pre-operative period. This anxiety, experienced in anticipation of the procedure, manifests as restlessness, irritability, aggression or depression and often leads to uncooperative behavior, emotional outbursts, and combative behavior peri-operatively. This eventually leads to negative postoperative outcomes including physical injury or emotional and psychological trauma to the child.[2] Menage,[3] Boyle[4] and Freud[5]

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viewed circumcision as an act of bodily mutilation; thus, without sufficient mental preparation, it may confer some level of psychological trauma, which may sometimes lead to development of aggressive behavior and nightmares. Further studies[6,7] also reported a direct causal relationship between circumcision and the subsequent development of post-traumatic stress disorder (PTSD) among young boys, since the experience involves extreme physiological and behavioral responses, which cause trauma to the patient.[8]

In the Philippines, where the population of males aged 5 to 14 years is more than 10 million,[9] circumcision can be considered to be one of the surgical procedures done mostly among the male pediatric population. In 2007, 42% of boys circumcised were under 10 years, 52% 10 to 14 years, and 5% 15 to 18 years.[6] The high demand for this outpatient minor surgical procedure has been addressed by the medical community in the form of medical missions or "tuli missions" done in the communities. These missions, however, because of the makeshift nature of recreating a medical set-up in the community setting, are often lacking in facilities for comfort and health education; thus, adding to the anxiety experienced by the patients. Both external and internal factors could aggravate the anxiety of the child.[6] No measures are currently offered to the children to address the anxiety they feel prior to the procedure.

A proposed anxiety-distraction[10] measure to decrease pre-operative anxiety associated with circumcision is gum chewing. Kamiya,[11] Young[8] and Scholey[12] stated in three different researches that gum chewing decreases pain response, enhances serotonin release and helps in pre-operative relaxation, and decreases self-rated task-induced anxiety. Thus, this study aimed to determine the effectiveness of gum chewing in reducing the pre-operative and post-operative anxiety of school-age boys undergoing circumcision.

Methods

This was a randomized controlled trial involving boys for circumcision from three barangays in Quezon City last April 2013. Postoperative anxiety levels were compared between the experimental group who were iven chewing gum before circumcision and a control group.

Boys present at medical missions in Barangays San Perfecto, Doña Imelda and Doña Aurora in Quezon City who satisfied the following inclusion criteria were recruited: 7 to 13 years old, a resident of Quezon City, able to read and write in Filipino, without any co-morbidities, and accompanied by a parent or guardian. The computed sample size was 256 subjects. Assent and consent were both obtained from the boys and parents, respectively. Participation was voluntary and anonymous. Ethical approval for the study was granted by the Ethics Review Committee of the institution.

The anxiety level of the participants was assessed using the Yale Preoperative Anxiety Scale (YPAS), an observational instrument which measured the children's anxiety during preoperative and postoperative periods. It consisted of 22 items in five categories: activity, emotional expressivity, state of arousal, localization, and use of parents. The highest behavioral level/number observed in each category was the score for that particular category. Since each category had a different number of items, partial weights were calculated by dividing the observed level with the total number of items in that category. The partial weights were then added; the sum of the partial weights was multiplied by 100 to get the percentage and then divided by five, yielding the total adjusted score which ranged from 23 to 100. The total adjusted score or the YPAS score was classified into low-anxiety level (23 to 48), moderate-anxiety level (49 to 74), and high-anxiety level (75 to 100). YPAS was used to measure the baseline, post-intervention and post-circumcision anxiety scores of the subjects. Researchers underwent standardized training prior to the implementation of the study in order to prevent inter-observer bias. The designated observers were blinded to prevent subjective bias in the YPAS scoring.

Subjects were randomly allocated to either experimental or control group by drawing lots. The baseline anxiety levels were determined for both groups. The experimental group was then asked to chew sugar-free gum (Orbit®) for five minutes, 5 to 10 minutes before circumcision. The post-intervention anxiety level was then determined. The post-circumcision anxiety levels were determined.

Baseline, post-intervention, and post-circumcision anxiety scores for both control and treatment groups

were encoded using EpiInfo™ 3.5.4; data was analyzed using EpiInfo™ 3.5.4 and OpenEpi 3.01. An independent T-test used to compare the scores between groups and a paired T-test was used to compare the data within the same group. A chi-square test was used to compare the change in anxiety level between and within both groups.

Results

A total of 261 participants qualified for the study; of these, 20 (7.66%) were excluded due to 1) sudden withdrawal, either before or while the participant was on the operating table and 2) failure to pass the medical criteria for circumcision as assessed by physician volunteers but in whom chewing gum had already been given. The remaining 241 participants consisted of the experimental group with a mean age of 10.7 years comprising 46.9% of the subjects, and the control group with a mean age of 10.8 years comprising 53.1% of the subjects. Table 1 shows that the two groups are comparable in terms of age, caregiver and previous hospitalization.

Table 2 shows that the baseline mean anxiety scores of the chewing gum group were significantly higher than that of the control. After being given chewing gum, the experimental group's mean anxiety score decreased by 1.4% while the control group's score increased by 20% pre-operatively. The difference in percentage change from baseline to post-intervention between the two groups was significant. The percent change from post-intervention to post-circumcision score was then compared between and within the groups. In the control group, a significant 12% increase in anxiety score was seen, while a 6.2% decrease was observed in the experimental group. A net increase in anxiety score was observed in the control group, while a net decrease in anxiety score was observed in the experimental group as seen in Table 2. The differences were significant. Figure 1 shows a decreasing trend in the anxiety scores in the experimental group from baseline to the post-circumcision period (7.6% decrease) while an increasing trend is seen in the control group (34.4% increase).

Table 1. Demographic characteristics of participants.

	E n = 113	C n = 128	p-value
Age (years)			
7	1 (0.9%)	3 (2.3%)	0.99
8	2 (1.8%)	6 (4.7%)	0.98
9	15 (13.3%)	14 (10.9%)	0.99
10	28 (24.8%)	32 (25.0%)	1.0
11	31 (27.4%)	33 (25.8%)	0.99
12	28 (24.8%)	27 (21.1%)	0.98
13	8 (7.1%)	13 (10.2%)	0.98
Caregiver			
Mother	85 (75.2%)	96 (75.0%)	1.0
Father	23 (20.4%)	25 (19.5%)	0.99
Both	5 (4.4%)	5 (3.9%)	1.0
Others	0	2 (1.6%)	0.99
Previous Hospitalization			
With	69 (61.1%)	85 (66.4%)	1.0
Without	44 (38.9%)	43 (33.6%)	0.99

E: experimental; C: Control

Table 2. Mean anxiety scores and corresponding percent changes in different periods of anxiety: experimental vs. control.

Period of Anxiety	E	C	Statistical test
	Mean	Anxiety Scores	P-value
B	32.7	29.9	0.044
PI	32.2	35.9	0.014
PC	30.2	40.2	$<1x10^{-7}$
% Change in	Mean	Anxiety Score	T-test
B to PI	1.4% ↓	20% ↑	14.30
PI to PC	6.2% ↓	12% ↑	17.57
B to PC (Net Change)	7.6% ↓	34.4% ↑	23.93

E: Experimental; C: Control; B: Baseline; PI: Post-intervention; PC: Post-circumcision

Table 3 shows the proportion of participants in each anxiety level. In all three assessment periods, most participants were in the low-anxiety group. Among the control, there was a decreasing number in the low-anxiety group and an increasing number in the moderate-anxiety group, while the reverse was observed among the experimental group. A comparison of the two groups' composition showed

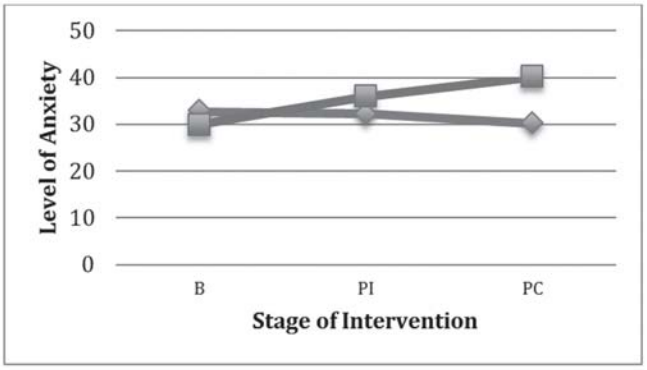

Figure 1. Comparison of the trend in the mean YPAS scores from baseline/pre-intervention (B), post-intervention (PI), to post-circumcision (PC).

that baseline distribution of the subjects was similar (P = 0.11), while both post-intervention and post-circumcision were significantly different (P < 0.05). Within the group, chi square test showed no significant changes in the distribution of subjects in the experimental group from baseline to post-intervention (P = 0.21) and from baseline to post-circumcision (P = 0.13); however, the opposite was noted in the control group. Significant increases in the proportion of moderate/high anxiety subjects in the control group were seen from baseline to post-intervention (P = 0.004), and from post-intervention to post-circumcision (P = 0.02).

Discussion

The current study evaluated the effectiveness of preoperative gum chewing on postoperative anxiety among boys undergoing circumcision. Baseline YPAS scores obtained in both arms indicated some level of anxiety among the subjects. This anxiety may be attributed to the extreme physiological and behavioral responses brought about by circumcision.[12] According to Cansever, the experience of circumcision lends a feeling of reality to the repressed fantasies, which amplifies the anxiety associated with it.[13] Apart from the external threatening situation, and an internal anxiety is presented for the child's ego to face.

Table 3. Percent distribution of subjects according to level of anxiety.

	Level of Anxiety					
	Experimental			Control		
	L	M	H	L	M	H
B	85.8	13.3	0.9	92.2	7.8	0
PI	91.2	8.0	0.9	79.7	19.5	0.8
PC	92.0	6.2	1.8	67.2	30.5	2.3

*B - Baseline; PI - Post-intervention; PC - Post-circumcision; L - Low Anxiety; M - Moderate Anxiety; H - High Anxiety

The increasing trend in the mean anxiety scores of the control group immediately before and after the procedure exemplifies the natural course of a child undergoing circumcision based on its accompanying psychological effects – one of which is castration-anxiety.[12] In a study by Yilmaz, several forms of anxiety disorders such as multiple anxieties, overanxious disorder, simple and social phobias, and separation anxiety were observed to be remarkably high in their subjects undergoing circumcision.[6]

A decrease in mean YPAS score in the experimental group between baseline and post-intervention period and an increase in the control group suggests an immediate anxiety-decreasing effect in the experimental group which may be attributed to gum chewing. This is supported by Menage, who found that regular gum chewing may decrease anxiety in the healthy young adult population.[3] Another study by Scholey reports a decrease in self-rated anxiety and stress associated with gum chewing on task-induced stress.[12] Lastly, a study by Otomaru states that regular gum chewing improves anxiety, mood and fatigue.[14]

Post-operative distress and the level of anxiety in the operative period are associated with the level of anxiety in pre-operative period.[1] Thus, the researchers wanted to know if the effect of the gum-chewing would persist even after the circumcision. The further decrease in the post-circumcision YPAS score in the experimental group suggests a lasting effect of gum chewing on anxiety until the post-circumcision period.

Since there were no other interventions made to this arm aside from gum administration and care was done to ensure an equal setting among arms, it its highly likely that the decreasing trend in anxiety level in the experimental group effect came from the gum chewing. This is consistent with the study of Kamiya that prolonged rhythmic gum chewing activates the

ventral part of prefrontal cortex augmenting activity of 5-HT neurons in the dorsal raphe nucleus, which in turn suppresses nociceptive responses.[11]

The classifications of subjects according to their levels of anxiety - low, moderate and high - were analyzed for a more clinical application. Chi-square was used to determine the significance in the change in distribution in these classifications, from baseline to post-circumcision, between groups and within groups. Baseline data showed that both groups had similar distributions initially. Within the experimental group, even though there was an increase in the number of subjects in the low-anxiety group from baseline to post-circumcision, this change in the distribution of subjects across levels of anxiety was not significant. However, the fact that there was a decreasing trend is significant because previous studies[1] showed that even experimental groups would have an increasing trend in anxiety score and that intervention would just decrease the increment in the postoperative anxiety. On the other hand, more subjects changed from low-level to moderate-high level anxiety in the control group as the circumcision neared, with a significant change from baseline to post-intervention and from post-intervention to post-circumcision. This increasing trend in anxiety would be expected from anyone undergoing a surgical operation. This means that the administration of gum chewing to the experimental group prevented an increase in anxiety level, contrary to what was experienced by the controls.

However, the presence of other factors may have contributed to the aforementioned results. This includes the lack of blinding among subjects which may have rendered subjective bias. Gum chewing in the experimental group might have caused a placebo effect and a counterpart increase in the anxiety levels of those who were not given chewing gums. Another factor may have been inter-observer bias. Individual differences among the observers in terms of observational skills possibly caused variation in scoring of the subjects despite training prior to data gathering. The duration of the procedure may also have contributed to variations in anxiety level carryover. A short procedure might have reflected a carry-over in post-circumcision anxiety, whereas, a long procedure may have exhausted the low anxiety carry-over of the gum chewed. The surgical difficulty of the procedure based on individual physical differences of each participant or intra-operative complications were not taken into account. These differences may have led to variations in anxiety levels intra-operatively which subsequently led to variations in anxiety level postoperatively apart from the effects of gum chewing. Furthermore, there was a dropout of 20 participants which might have increased the P-value.

With results suggesting reduced state anxiety[5] and reduced perceived levels of stress,[6] therapeutic use of chewing gum as pre-circumcision modality seems promising as the method is simple, inexpensive and easy to administer. Since circumcision is a prevalent practice in our country[15] then its implications are highly favorable for this nation's youth. However, further studies that serve to eliminate limitations mentioned are encouraged to further investigate the role of gum chewing in circumcision anxiety level reduction.

This study showed that gum chewing has an immediate anxiety-relieving effect pre-operatively in boys undergoing circumcision; this effect was carried over until the post-operative period. Thus, the researchers recommend the inclusion of a pre-operative anxiety assessment in the surgical checklist of pediatric patients. Anxiety should be considered as a fifth vital sign that assesses the psychological readiness of the child and thus should be addressed through anxiety-reducing modalities such as gum chewing.

Implications of the study as a possible modality in improving preoperative patient care in relation to development of postoperative psychological trauma, aggressive behavior, nightmares and later on post-traumatic stress disorder should be considered. Thus, an immediate and accessible anxiety control modality such as gum chewing should be advocated by mental health practitioners for those who have poor or immature psychological defense mechanisms such as children and those with anxiety disorders as an emergent therapy for anxiety-provoking situations to allay symptoms and prevent further morbidities.

A follow-up study may be done on the effects of gum chewing on circumcision. To improve validity, points for improvement would be employment of stratification to ensure comparable baseline values, participants should be blinded to prevent a placebo effect and subjective bias, parents or guardians should be instructed not to give their children gums to avoid contamination of controls, and surgical factors should be considered in evaluating carry-over of the

decrease in anxiety level. Furthermore, a separate study may be conducted to modify the YPAS scale to include assessment for intraoperative anxiety and to tailor the assessment tool to the specific age group being assessed. The modified tool would be beneficial for parallel studies conducted including minor surgery postoperative anxiety assessment for both pediatric and adult age groups.

The results of our study show that gum chewing - a simple, inexpensive and accessible non-pharmacologic modality - can decrease the pre-circumcision anxiety, an effect carried over until the post-circumcision period; the researchers strongly recommend the provision of gum among boys prior to circumcision. Additional studies involving other minor surgical procedures conducted not just among the pediatric group but also among adults are also recommended.

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Effectiveness of *Cananga odorata* (ylang-ylang) vapor aromatherapy in chemotherapy-induced state anxiety reduction among breast cancer patients: a randomized controlled trial*

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Abstract

Introduction Vapor aromatherapy is one of the complementary therapies offered to address anxiety, the most common psychological effect of chemotherapy. It has been consistently reported to reduce anxiety among cancer patients and as such, vapor aromatherapy using the indigenous plant *Cananga odorata* (ylang-ylang) was investigated for its short-term, immediate effect in reducing chemotherapy-induced state anxiety.

Methods Thirty four stage II and III female breast cancer patients aged 25 years and above with low to moderate trait anxiety were randomly allocated to receive vapor aromatherapy using either *Cananga odorata* (experimental group) or virgin coconut oil (control group), given once for 30 minutes after their chemotherapy session. State anxiety was measured using the State-Trait Anxiety Inventory Form Y before and after vapor aromatherapy.

Results No significant anxiety reduction was observed after vapor aromatherapy among participants with low and moderate anxiety in both treatment groups. However, the state anxiety scores were significantly lower in the experimental group when the low and moderate anxiety groups were combined. The difference in anxiety reduction between the treatment and control arms was significant in the moderate anxiety group but not in the low anxiety group.

Conclusion *Cananga odorata* VA conferred state anxiety reduction among Stage II and III breast cancer patients undergoing chemotherapy who had moderate anxiety.

Key words: anxiety reduction, vapor aromatherapy, breast cancer, *Cananga odorata*, ylang-ylang

Anxiety is the most common psychological effect of chemotherapy. Studies have shown that patients undergoing chemotherapy have high anxiety at the beginning and higher anxiety at the end.[1] Anxiety may take form as situation anxiety, disease-related anxiety, treatment-related anxiety, or as an aggravation of pre-treatment diagnosis. It is associated with physical problems and may manifest as eating difficulties, fatigue, indigestion, nausea or sleeping difficulties.[2]

Aromatherapy is one of the complementary therapies offered to patients in cancer clinics and

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hospitals to address anxiety. One of its forms, vapor aromatherapy (VA), is said to be an effective method in managing stress and depression.[3] In VA, inhaling essential oil molecules may influence certain neurotransmitters and brain chemicals that are involved in the regulation of mood and anxiety.[4] A number of studies have revealed that VA consistently reduced self-reported anxiety among cancer patients[5,6] as inhaled essential oils are rapidly absorbed thereby altering brain function fast.[7] An example of an inhaled essential oil is *Cananga odorata* (*ylang-ylang*), an indigenous plant used prominently in the perfume industry and in aromatherapy.[8] It is often used as a stress reliever and as such, was investigated for its effectiveness in reducing chemotherapy-induced state anxiety.

Methods

Thirty four female breast cancer patients were randomly allocated to either *Cananga odorata* VA or virgin coconut oil VA groups after preliminary screening and baseline trait anxiety determination. Their state anxiety was determined before and after VA and their scores were compared. Ethics approval was obtained prior to the study.

Women aged 25 years or older with stage II or III breast cancer who had reached at least grade school, who could read and write, who were to undergo chemotherapy, and who were conscious and coherent at the time of the trial were considered for inclusion in this study. The following were excluded: those with diagnosed psychiatric problems or took anxiolytic medications prior to the trial; those who had nasal infections and/or allergies which may affect the inhalation of VA; those who underwent VA prior to this study.

Informed consent was obtained from the women who passed the preliminary screening. The participants' demographic and clinical characteristics were collected at baseline. Form Y-2 of the State-Trait Anxiety Inventory Form Y (STAI-Y) was administered to obtain the participants' baseline trait anxiety. This served as a second screening. STAI-Y is a self-report instrument used to quantify anxiety. It consists of two, 20-item subscales measuring state anxiety and trait anxiety. State anxiety is, "a transitory emotional state or condition of the human organism that is characterized by subjective, consciously perceived feelings of tension and apprehension, and heightened autonomic nervous system" whereas trait anxiety refers to, "relatively stable individual differences in anxiety proneness, that is, to differences between people in the tendency to respond to situations perceived as threatening with elevations in state anxiety intensity".[9] The English version, accompanied by a Filipino translation of the subscale items, was used in this study. A number of reliability and validity studies have provided satisfactory evidence recommending the STAI-Y as an instrument for studying anxiety in research and clinical settings.

A score ranging from a minimum of 20 to a maximum of 80 may be obtained from the STAI-Y, with higher scores indicating greater anxiety. Trait anxiety was classified as low (20-34 points), moderate (35-49 points), high (50-64 points), or very high (65-80 points) as patterned in a study.[10] Participants classified to have high or very high anxiety were not considered for this study since people with high trait anxiety exhibit state anxiety elevations more frequently than people with low trait anxiety because they consider a wider range of situations as dangerous and/or threatening.[9] State anxiety fluctuates as a function of the intensity of a present stress, but the perception of stress may greatly skew the state anxiety appropriately associated with the given situation.[9] These observations were considered to ensure that the participants' anxiety were due to chemotherapy alone.

Women with low to moderate anxiety were then assigned by unequal randomization (the most statistically efficient randomization ratio for this study as it maximized the statistical power for a given total sample size) to either *Cananga odorata* VA (experimental group) or virgin coconut oil VA (control group). The essential oils were administered through diffuser for 30 minutes post-chemotherapy with the participants comfortably seated inside a closed, 3 x 4 m air-conditioned room. Coffee was used to eliminate the scent of either of the oils in preparation for the subsequent sessions.

Form Y-1 was administered to the participants after their respective chemotherapy sessions to obtain their pre-VA state anxiety. Another Form Y-1 was administered after their randomly assigned VA sessions to obtain their post-VA state anxiety. Participants were debriefed on the nature of the study right after their respective trials. The STAI-Y was the primary validated instrument used in this study. Form Y-2 used a four-point Likert scale with the following

categories: 1) almost never; 2) sometimes; 3) often, and; 4) almost always. Form Y-1 also used a four-point Likert scale with the following categories: 1) not at all; 2) somewhat; 3) moderately so, and; 4) very much so. Scores on both forms were obtained by manual hand scoring as specified in its manual. Scores obtained from Form Y-1 were used to compute for the means and standard deviations of the two treatment groups.

Means and standard deviations pre- and post-VA were analyzed using a two-tailed t-test to determine if there was a reduction in state anxiety that may be attributed to VA. All outcome analyses were carried out on an intention-to-treat basis.

Results

Table 1 presents the distribution of the participants according to demographic variables. No significant differences in age, chemotherapy cycle, cancer stage, and educational attainment were noted between the experimental *Cananga odorata* and virgin coconut oil control group. Table 2 shows that the baseline anxiety of the two study groups, as measured by STAI-Y Form Y-2, was comparable.

No significant state anxiety reduction was observed after vapor aromatherapy among participants with low and moderate anxiety, respectively, in both treatment groups as shown in Table 3. However, a significant reduction in state anxiety was noted in the *Cananga odorata* group when low and moderate anxiety scores were combined (P = 0.04). Table 4 shows a significant difference in the reduction of state anxiety between the two study arms among participants with moderate anxiety (P = 0.05) but not among participants with low anxiety (P = 0.58).

Discussion

This study highlighted the effectiveness of *Cananga odorata* vapor aromatherapy in the reduction of state anxiety when administered immediately after chemotherapy.

State anxiety was observed to be reduced after a 30-minute period of VA in the experimental group. In this regard, it can then be inferred that *Cananga odorata* VA is effective in reducing chemotherapy-induced state anxiety on a short-term, immediate basis. This finding is in line with a study wherein short sessions of aromatherapy improved clinical anxiety and/or depression experienced by cancer patients.[6] Moreover, this study further supported the claim that one of the many benefits of aromatherapy is anxiety reduction.[11] The reduction offered by aromatherapy was likewise exhibited in other studies involving cancer patients in a palliative care setting[4,12] and in a systemic review on aromatherapy and symptom relief among cancer patients.[5] A proposed explanation was aromatherapy improved circulation, relaxed muscular and nervous tissue, and sped up waste product elimination.[13]

Table 1. Demographic profile of participants.

Demographic Variable	*Cananga odorata* (n = 17)	VCO (n = 17)	p-value
Age			
25-35	0	3	
36-45	4	3	
46-55	9	8	0.26
56-65	3	2	
66 above	1	1	
Chemotherapy cycle			
1	2	4	
2	2	3	
3	5	1	0.86
4	5	5	
5	1	1	
6	2	1	
Cancer Stage			
IIA	0	1	
IIB	4	4	
*IIIA	9	7	0.85
IIIB	3	3	
IIIC	1	2	
Educational Attainment			
Elementary	0	2	
HS	3	3	
*College	10	12	0.82
Post Grad	2	0	
Vocational	2	0	

*Majority Group

Table 2. Baseline anxiety assessment of participants based on Form Y-2.

Anxiety Level	*Cananga odorata* (n = 17)	VCO (n = 17)	p-value
Low	7	6	0.50
Moderate	10	11	0.91
Total	17	17	

Table 3. State anxiety levels among participants pre- and post-vapor aromatherapy

Groups	Pre-VA	Post-VA	Mean Difference	P-value
Cananga odorata				
Low	39.07	36.33	2.74	0.23
Moderate	55.03	45.52	9.52	0.09
Total	47.11	40.12	7.02	0.04
VCO				
Low	45.66	42.59	3.07	0.56
Moderate	52.44	47.62	4.82	0.22
Total	51.9	45.26	6.07	0.08

Table 4. State anxiety reduction between treatment groups.

Anxiety Reduction	*Cananga odorata* (n = 17)	VCO (n = 17)	P-value
Low			
n	11	6	
Mean	2.08	3.08	0.58
SD	3.66	3.26	
Moderate			
n	6	11	
Mean	16.06	6.31	0.05
SD	9.18	6.22	

The widely regarded calmative properties of *Cananga odorata* may also have contributed to the state anxiety reduction among the participants in the experimental group.[14] Essential oils may exert positive influences on a patient's well-being upon entering the body through the olfactory system or through absorption via the skin. However, intervention through massage aromatherapy alone and/or control of breathing patterns may also account for its anxiolytic affects.[15]

The *Cananga odorata* VA mean state anxiety reduction, however, showed no significant difference compared to that of the control group. This could mean that the state anxiety reduction in the experimental group was probably due to a placebo effect. The state anxiety scores may have diminished while focusing on the *Cananga odorata* scent, or perhaps, the non-fragrant control, virgin coconut oil, may have been relaxing in itself. The observed state anxiety reduction could have been a result of the participants' expectations regarding the pleasant effect of VA. Cognitive or psychological mechanisms of odor transduction may confound pharmacological effects of aromatherapy in humans.

The investigators also made use of only one closed room alternated daily for both study groups. The essential oil was applied to the diffuser only once before VA sessions began. It is possible that the largest effects were seen on the first batch of participants undergoing VA where the concentration of the essential oil was at its highest. Room structure and design was not controlled, nor modifiable by the investigators.

When subgroup analysis was obtained, data showed a significant state anxiety reduction among those with moderate anxiety in the experimental group. This is incongruent with a study on lavender aromatherapy where the authors concluded that lavender had anxiolytic effects in humans under conditions of low anxiety. No conclusions were drawn for high anxiety or clinical anxiety disorders.[16] The absence of significant state anxiety reduction in participants with low pre-VA state anxiety may be explained by very small changes in state anxiety that cannot be measured using the STAI-Y.[17] The minimum score that can be obtained is 20. Those participants with pre-VA state anxiety score of 20 will not yield a lower score even if there was a greater reduction value expected.

Safety testing on essential oils has shown minimal adverse effects.[18,19] Only two out of the 17 participants in the experimental group and one out of 17 in the control group complained of having headache after the VA session. No other side effects were noted. According to the majority of participants, the odor of the VA was pleasant.

As supported by the data presented, it is therefore concluded that *Cananga odorata* VA conferred state anxiety reduction among Stage II and III breast cancer patients undergoing chemotherapy who had moderate anxiety.

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Effect of zinc supplementation as an adjunct in the treatment of pneumonia in children: a meta-analysis

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Abstract

Introduction This meta-analysis was done to evaluate the efficacy of zinc supplementation as adjunct to antibiotics in decreasing the duration of resolution of tachypnea, time to recovery, and length of hospital stay of pneumonia in children 2 to 59 months.

Methods This was a meta-analysis of randomized, blinded, placebo-controlled intervention trials. The studies for inclusion were identified from several international and local search engines and journal hand search. Included studies were appraised for validity. Statistical analysis of the data was done using Review Manager Version 5.2. Summary of mean difference and 95% confidence interval for each outcome variable were estimated using a random-effects model in the presence of significant heterogeneity. Tests for heterogeneity were computed using tau-square and chi-square.

Results There was no difference in children who received elemental zinc as adjunct to antibiotics in terms of decreasing the duration of resolution of tachypnea (P = 0.48, 95% CI -13.76, 6.41), time to recovery (P = 0.11, 95% CI -10.18, 1.00) and length of hospital stay (P = 0.05, 95% CI -11.54, 0.04).

Conclusion This study showed that there was no significant evidence for the efficacy of zinc supplementation as an adjunct in the treatment of pneumonia in children despite including newer studies for analysis.

Key words: pneumonia, zinc

The worldwide incidence of pneumonia has been estimated to be 156 million cases each year in children less than five years, of which approximately 20 million cases were severe enough to require hospital admission.[1] More than 95% of all episodes of clinical pneumonia in young children globally occur in developing countries.[1] In the Philippines, pneumonia remains in the list of the leading causes of morbidity and mortality. Data gathered by the Department of Health in 2005 showed pneumonia as the top cause of morbidity in all ages.[2] It ranked third among the leading causes of infant mortality accounting for 8.9 per 1000 population, and first among the leading causes of child mortality in children aged 1 to 4 years, equivalent to about 23.2 per 100,000 population.[2]

Zinc deficiency in the developing countries places children at increased risk of illness and death from infectious diseases.[3] Several well-designed, randomized, and controlled trials on zinc supplementation in young children have been shown to lower mortality and morbidity due to infectious disease. Reported studies on zinc have been shown to prevent pneumonia,[4-7] and prevent and treat diarrhea in children.[6,8-10] Based on the Philippine

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Pediatric Society 2004 Clinical Practice Guidelines in Pediatric Community-Acquired Pneumonia, zinc supplementation (10 mg for infants and 20 mg for children beyond two years of age given for a total of 4 to 6 months) may be administered to prevent pneumonia; this is a Grade A recommendation.[11]

Studies appraising the impact on zinc supplementation as an adjunct in the treatment of pneumonia have shown variable results. Two of the published meta-analysis[12,13] on the efficacy of zinc as an adjunct in treatment of pneumonia concluded that zinc is not effective in the outcomes they measured. This necessitated a systematic review that would include available, newer relevant studies in addition to those in the previous meta-analyses[12,13] to determine the role of zinc as an adjunct treatment for pneumonia in pediatric patients aged 2 to 59 months.

The objective of this study was to evaluate the efficacy of zinc supplementation, as an adjunct to antibiotics, in decreasing the duration of tachypnea, period of time to recovery, and length of hospital stay of pneumonia in children aged 2 to 59 months.

Methods

This is a meta-analysis of published and unpublished studies on the efficacy of zinc supplementation, as an adjunct to antibiotics, in decreasing the duration of tachypnea, period of time to recovery, and length of hospital stay of pneumonia in children aged 2 to 59 months.

Identification of studies

A comprehensive literature search was conducted using electronic bibliographic databases - PubMed and the Cochrane Library - including articles cited from 1981 up to November 25, 2012. The studies considered for possible inclusion in this meta-analysis were identified using the following MeSH terms: ("pneumonia"[MeSH Terms] OR "pneumonia"[All Fields]) AND (("zinc"[MeSH Terms] OR "zinc"[All Fields]) AND ("therapy"[Subheading] OR "therapy"[All Fields] OR "treatment" [All Fields] OR "therapeutics"[MeSH Terms] OR "therapeutics" [All Fields)) AND Randomized Controlled Trial[ptyp]. Additional studies were obtained through hand search of bibliographic citations of all the articles selected for inclusion in the analysis. A search of locally published clinical researches was also conducted using the Herdin Neon Database from 1999 to 2006 and PIMEDICUS database of the University of the Philippines College of Medicine Library from 2001 to 2010. Search for unpublished local trials was also done by inquiring from the department's pediatric pulmonologist. Two authors reviewed the titles and abstracts independently to identify controlled studies conducted in developing countries in which supplemental zinc was administered, and its effect on the recovery from pneumonia.

Inclusion criteria

Studies were considered for inclusion in the meta-analysis if they met the following criteria:

1. The study was a randomized, placebo-controlled trial in which the treatment and control groups were enrolled concurrently in a hospital-based setting;
2. The subjects were children between 2 to 59 months of age diagnosed with severe pneumonia with no co-morbidities;
3. The subjects were given antibiotics of any class along with daily supplemental zinc during the acute phase of pneumonia;
4. The outcomes measured were duration of tachypnea until resolution, period of time to recovery, and length of hospital stay.

Review of studies and extraction of summary data

Once the final set of studies for inclusion in the analyses was established the authors independently appraised the included studies for validity using "Evidence-Based Medicine User's Guide for Appraisal of an Article on Therapy" (www.apebm.com). The randomized controlled trials were scored based on the questions as follows:

1. Was the study randomized?
2. Was follow-up of subjects adequate and complete?
3. Was there an intention to treat analysis?
4. Was there blinding?
5. Were baseline characteristics similar?
6. Were groups treated equally?

The article was classified as "A" if numbers 1 to 6 were all answered "yes"; "B" if numbers 1, 2, 3,

were answered "yes" but there was at least one "no" for numbers 4, 5 , 6; and "C" if there was at least one "no" numbers 1, 2, 3. Data extraction was done independently and any conflicts were resolved by a third party.

Analysis of data

Statistical analysis of the data was done using Review Manager Version 5.2. The summary of mean difference and 95% confidence interval for each outcome variable were estimated using a random-effects model when there was significant heterogeneity. The tests for heterogeneity were computed using tau-square and chi-square.

Results

The search of PubMed and the Cochrane Library yielded 27 studies; two more were obtained by hand search. From the PIMEDICUS database, two studies were identified while none was found from Herdin Neon Database. The results of the search are summarized in Figure 1.

Description of studies

Seven studies up to 2000 were included in the analysis. The characteristics of these studies, including their appraisal, are shown in Table 1. They had the same study design. Six studies[14-19] included only patients with severe pneumonia, while one study[20] included non-severe pneumonia. Six[14-19] out of the seven studies, defined severe pneumonia as respiratory rate of more than 50 breaths per minute for children less than 12 months or more than 40 breaths per minute for children older than 12 months, or with/without lower chest indrawing (LCI) and associated with either crepitations upon auscultation and/or presence of one or more of the following danger signs: inability to drink or feed, lethargy or marked irritability or central cyanosis. One study[20] defined it as a child with LCI but without general danger signs including inability to drink/breastfeed, persistent vomiting, convulsions, lethargy, or unconsciousness. Zinc sulfate was administered in four studies[15,18-20] while two studies[16,17] used zinc acetate and one[14] used zinc gluconate. A maximum of 20 mg/day was given from the time of enrollment. Duration of intervention varied in seven studies. Blinding was done in all of the trials.

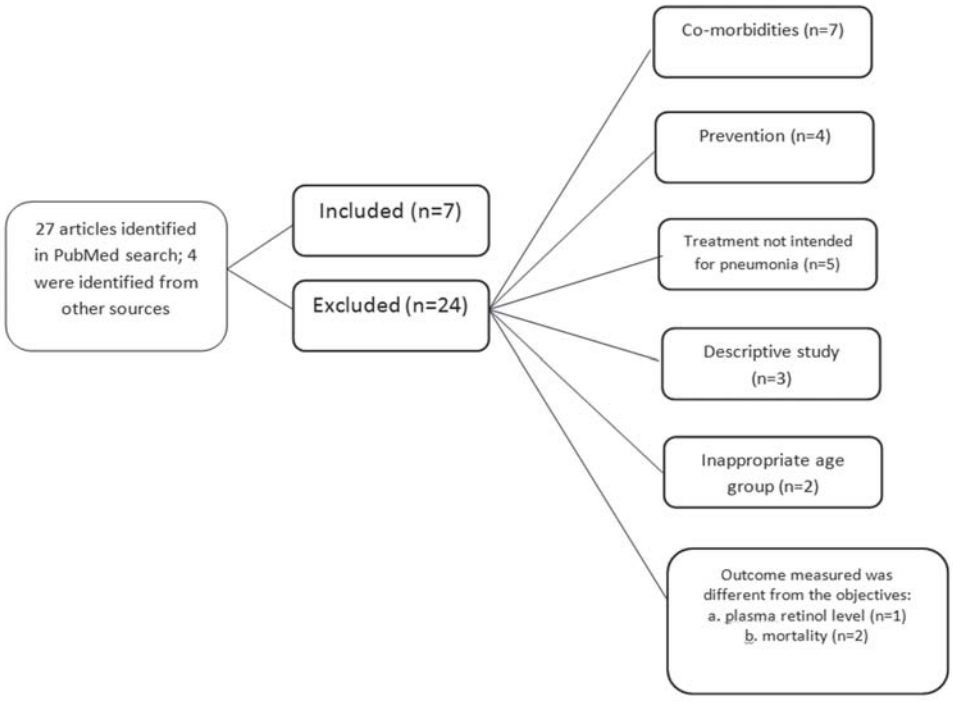

Figure 1. Studies excluded and included in the meta-analysis.

Table 1. Characteristics of included studies.

Authors	Patients	Intervention	Control	Outcome	Method	Validity Score
Bansal, et al. 2011	2-24 months With severe pneumonia Consent	Zinc 20mg OD For 5 days	Placebo	Duration of symptoms, length of hospitalization	RCT Triple-blind	A
Bose, et al. 2006	2-23 months With severe pneumonia	Consent Zinc 10mg BID while admitted	Placebo	Duration of symptoms, length of hospitalization	RCT Double-blind	A
Brooks, et al. 2004	2-23 months With severe pneumonia Consent	Zinc 10mg BID until discharge	Placebo	Duration of symptoms, length of hospitalization	RCT Double-blind	A
Mahalanabis, et al. 2004	2-24 months With severe pneumonia Consent	Zinc 10mg BID for 5 days	Placebo	Time to recovery	RCT Double-blind	A
Valavi, et al. 2011	Between 3-60 months With severe pneumonia Consent	2mg/kg/day in 2 divided doses max of 20mg/day for 5 days	Placebo	Duration of symptoms, length of hospitalization, Time to recovery	RCT Double-blind	A
Valentiner-Branth, et al. 2010	2-35 months With severe and nonsevere pneumonia Consent	10mg per day - 2 to 11 months 20mg per day - >12 months for 14 days	Placebo	Duration of symptoms, Time to recovery, Treatment failure	RCT Double-blind	A
Basnet, et al. 2012	2-35 months With severe pneumonia Consent	10mg per day - 2 to 11 months 20mg per day - >12 mo for 14 days	Placebo	Duration of symptoms, Time to recovery, Treatment failure	RCT Double-blind	A

Effect of zinc on duration of resolution of tachypnea

Zinc supplementation to standard antibiotic therapy in children with severe pneumonia did not show a significant effect on the duration of resolution of tachypnea compared to the control group. The pooled mean difference of 4 studies with 663 subjects was -3.67 (95% CI -13.76, 6.41). There was significant heterogeneity; use of a random-effects model showed no significance (P = 0.48) as seen in Figure 2.

Effect of zinc on period of time to recovery

In children with severe pneumonia, zinc supplementation did not show any significant effect in the time to clinical recovery compared to control. The pooled mean difference of three studies with 885 subjects was -4.59 (95% CI -10.18, 1.00). There was significant heterogeneity; a random effects model showed no significance (P = 0.11) as seen in Figure 3.

Effect of zinc on the length of hospital stay

Valavi showed that there was a shorter hospital stay in the zinc-treated group than the placebo group with a mean hospital stay of 126.7 hours versus 137.7 ($P < 0.001$). The study of Brooks also showed decreased duration of hospital stay - 5 (4.8 to 5.5) and 6 (5.1 to 6.1) days for the zinc and placebo groups, respectively. However, the studies of Bansal, Bose and Valentiner–Branth found that there was no difference in the length of hospital stay. The pooled mean difference for five studies with 835 subjects was -.5.75 (95% CI -11.54, 0.04). There was significant heterogeneity; a random effects model showed no significance ($P = 0.05$) as seen in Figure 4.

Discussion

Zinc plays an important role in the development and maintenance of host defense against infections.[21,22] It is an essential micronutrient for human growth and immune function. Zinc deficiency impairs overall immune function and resistance to infection.[23] Therefore, zinc supplementation may have a positive impact on the outcome of infectious diseases. Its therapeutic benefit in diarrhea has been well-documented in a Cochrane review of 24 trials which showed that it shortened the recovery time in children with acute or persistent diarrhea in ages one month to five years.[24] Furthermore, it has been shown that routine zinc supplementation lowers the risk of acute respiratory infections and clinical pneumonia in children.[25]

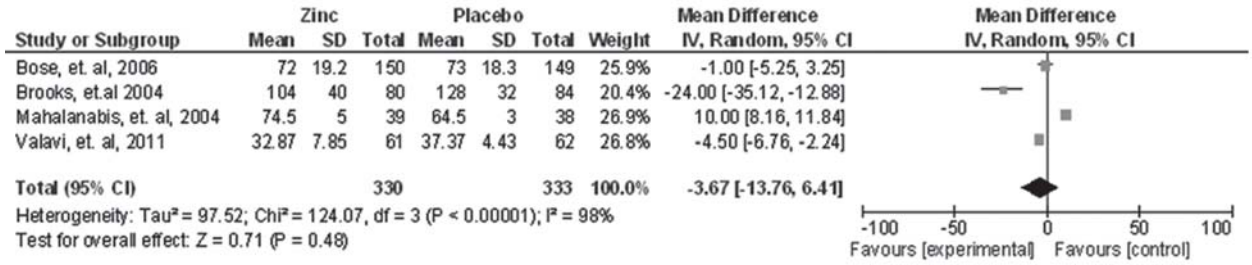

Figure 2. Forest plot of Comparison: I. Zinc Supplementation versus placebo, outcome: Duration of resolution of tachypnea (respiratory rate of > 50 breaths per minute) in pneumonia.

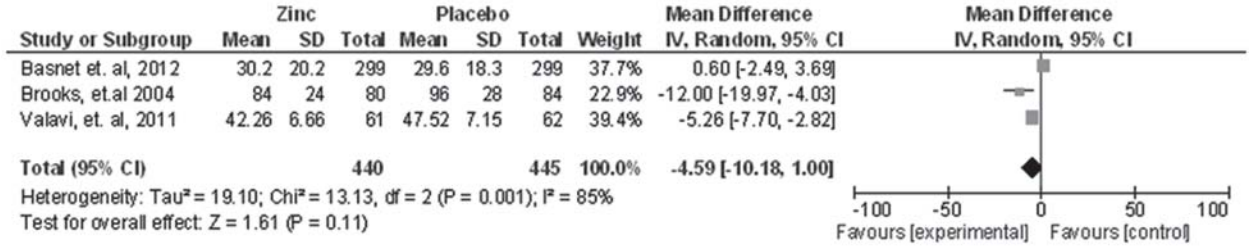

Figure 3. Forest plot of Comparison: I. Zinc Supplementation versus placebo, outcome: period of time to recovery in pneumonia.

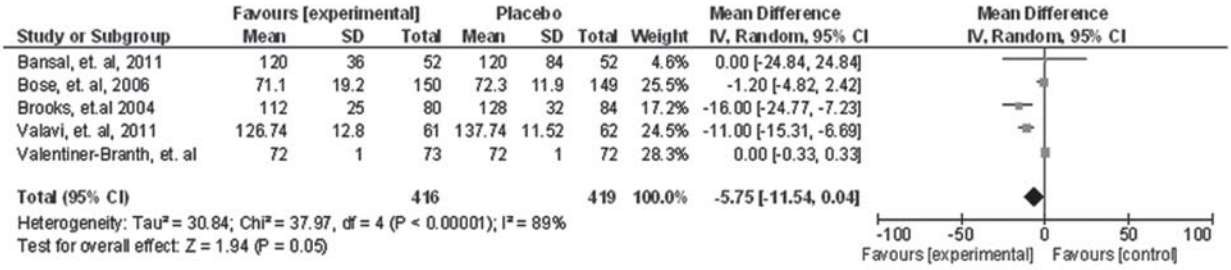

Figure 4. Forest plot of Comparison: I. Zinc Supplementation versus placebo, outcome: length of hospital stay in pneumonia.

Baseline serum zinc levels in children with pneumonia were measured at the time of recruitment in the studies. Since serum zinc values decrease during an acute phase of infection, the baseline levels taken at the time of recruitment may not reflect the true underlying zinc status while they do not have the illness. Differences in the prevalence of zinc deficiency in the study populations may have implications on the role of adjuvant zinc therapy in the treatment of pneumonia.[12]

The results of this meta-analysis indicate that zinc supplementation as an adjunct to standard antibiotics has no significant effect on clinical recovery in children with severe pneumonia in general. Analysis of data from the studies gathered does not show any significant impact of the intervention on the duration of tachypnea, time of recovery and length of hospitalization. Moreover, the results showed significant heterogeneity.

Although this meta-analysis achieved its aims, there were some unavoidable limitations. This meta-analysis focused on four databases and a hand search of all bibliographic citations to identify studies to be included in the analysis.

This meta-analysis shows that there is no significant evidence for the efficacy of zinc supplementation as an adjunct in the treatment of acute pneumonia in children by reducing the duration of tachypnea, time to recovery of and length of hospital stay despite including newer studies for analysis. Since there are ongoing trials[12] which will be available in the near future, these results will contribute in eliciting the efficacy of zinc supplementation as an adjuvant treatment in childhood pneumonia. A large scale randomized, placebo-controlled trial must be conducted to evaluate the efficacy of zinc supplementation in the treatment of acute pneumonia in children less than five years of age especially in developing countries where zinc deficiency is common.

Acknowledgments

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Conflict of Interest Declaration

The authors have no affiliation or financial interest that may benefit, directly or indirectly, from dissemination of this research results in any particular way (including any unwarranted delay in or restriction upon publication of results).

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A cross sectional study to determine the risk factors of work-related musculoskeletal disorders among physical therapists in Metro Manila*

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Abstract

Objective This study aimed to determine the factors that contribute to the occurrence of the work-related musculoskeletal disorders among physical therapists in Metro Manila.

Methods This cross-sectional study was done in selected hospitals in Metro Manila with the approval of the Ethics Review Committee. Licensed physical therapists were recruited using a purposive sampling technique. The Personal Demographic Sheet, Ovako Work Posture Analyzing System, MM040 Questionnaire were used to determine, assess and describe the participants' demographic profile, working conditions and environment, respectively. The Standard Nordic Musculoskeletal Questionnaire was used to determine the presence of work-related musculoskeletal disorders among the respondents. A Marascuilo table of statistics was used to determine the person-tasks-environment relationship to work-related musculoskeletal disorders.

Results Twenty-eight physical therapists participated in the study. Subjects perceived their working environment as good to very good in terms of temperature, cleanliness, noise and light. The common work-related musculoskeletal disorders involved the upper extremities and back. Gender, number of co-workers, years of service, BMI, and category 2 and 4 postures were associated with work-related musculoskeletal disorders among the subjects in the last 12 months. Years of service, number of co-workers and cleanliness were associated with symptoms in the last 7 days. Gender, BMI, temperature, and category 2 and 4 postures prevented the subjects from working.

Conclusion The factors commonly associated with work-related musculoskeletal disorders involving mostly the upper extremities and back are gender, BMI, number of co-workers and years of service. Work-related musculoskeletal disorders involving the neck, shoulder, upper and lower back prevented the therapists from doing their work.

Key words: Ergonomics, Nordic Musculoskeletal Questionnaire, physical therapy, work-related musculoskeletal disorders

Musculoskeletal disorders (MSDs) are common among workers. Data from the United States Department of Labor Occupational Safety and Health Administration show that MSDs affect workers in almost every occupation and industry,[1] including physical therapists.[2] Physical therapists have a moderately high prevalence of occupational low-back pain due to the tasks they carry out in the clinics. Such activities include lifting, bending, twisting, reaching, performing manual therapy, and maintaining awkward positions for prolonged periods of time. This makes physical therapists (PTs) and physical therapist assistants (PTAs) susceptible to musculoskeletal injuries.[3]

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According to the Occupational Safety and Health Administration (OSHA 3125),[1] work-related musculoskeletal disorders (WRMSDs) occur when the physical capabilities of the worker do not match the physical requirements of the job; thus prolonged exposure to ergonomic risk factors such as force, repetition, awkward postures, static postures, quick motions, compression or contact stress, vibration and cold temperatures can cause damage to a worker's body and lead to MSDs.

Many factors have been shown to cause MSDs, but more studies should be conducted to further explain the prevalence of these disorders among different workers, especially physical therapists, who are professionals that are mainly concerned with proper body mechanics. Concrete risk factors should be determined to help prevent WRMSDs as well as aid the physical therapists to maximize their capabilities without physical hindrances. Hence, this study was done to determine the most common risk factors contributing to the development of WRMSDs among physical therapists.

Methods

This was a cross-sectional study involving physical therapists from selected hospitals in Metro Manila. The study was approved by the Ethics Review Committee.

A sample size of 33 was computed using a sample size calculator from National Statistical Service of Australia. Out of 10,600 physical therapists in Metro Manila, based on 95% confidence interval, 0.05 standard error and 91% proportion of WRMSDs as stated in the study of Cromie.[4] Licensed physical therapists were recruited from the different rehabilitation departments in Metro Manila by purposive sampling.

The following instruments were utilized in the study: Personal Demographic Sheet (PDS), Ovako Work Posture Analyzing System (OWAS), MM 040 Office Questionnaire, and the Standard Nordic Musculoskeletal Questionnaire (SNMQ). The PDS was a one page questionnaire used to determine the demographic profile of the participants; it asked the age, gender, handedness, length of service, civil status and average number of patients per day. The OWAS is a computer system used to estimate the extent of a workload at the workstation by analyzing worker body's posture.[5] It analyzes the different positions of the back, shoulders and legs; each position is encoded and arranged in four risk groups of static injuries. This method takes into consideration the weight lifted by a worker and analyzes the force exerted during work as well as the time of force in a given position. OWAS method classifies standard postures of back, forearms, legs and involved external load. OWAS has an intra-rater reliability of 95.0%,[6] inter-rater repeatability of > 85%[7] and intra-rater reliability coefficient of > 97%.[8]

The MM 040 Office Questionnaire is a standardized tool used to assess the different factors of the indoor environment such as temperature, cleanliness, noise, light and others. Validity and reliability of the modified questionnaire was tested by Lanhtinen[9] and Reijula.[10] The SNMQ was used to identify the symptoms of the subjects' WRMSDs. SNMQ was found to be valid and reliable. It is answerable by yes or no if they had trouble like ache, pain and discomfort during the last 12 and 7 months or whenever it hindered work at home or away from home. It has a negative predictive value of 64.5% to 100%, specificity of 82.4% and sensitivity of 82.3%.[11] The PDS, MM 040 and SNMQ were pilot tested among five physical therapy interns. Each was asked to answer the questions and give feedback thereafter. According to the pilot test participants, the version of PDS, MM040 and SNMQ were easy to understand. Reliability of the study's OWAS assessor was also determined in a pilot testing. Two licensed physical therapists were asked to evaluate a twenty minute-video work sample twice, randomly and independently. The test was repeated after a week. The results revealed that both assessors had a high reliability given the intra-class correlation coefficient of 0.80.

After approval was obtained from various hospitals, the researchers discussed the objectives of the study and had the respondents sign the informed consent. Questionnaires were then distributed and answered by the subjects. A video of the tasks and task objects of the subjects was taken and sent to the study's OWAS assessors for the analysis. After obtaining all necessary data from the subjects and assessors, raw data were tabulated and tallied.

Descriptive statistics such as mean, median, mode and standard deviation were used to describe the subjects' profile, task posture, working environment

and WRMSD symptom duration and location affected by work-related musculoskeletal disorders. A Marascuilo table of statistics was used to determine the relationship of the PTE and WRMSD symptoms, duration and location of the subjects. The level of significance was set at $P < 0.05$.

Results

Out of 44 hospitals contacted, eight allowed their physical therapists to participate in the study. Out of 33 physical therapists, 28 were qualified based on the inclusion and exclusion criteria and consented to participate.

The characteristics of the 28 subjects are summarized in Table 1. Majority of the participants were 24 to 26 years old, with a BMI of 18.5 to 24.5, had 5 to 10 co-workers, had 5 to 10 patients a day and had 1 to 3 years of service. Equal numbers of male and female were noted. The subjects usually worked under category 2 posture based on OWAS category - described as requiring corrective measures in the near future. The subjects' working environment good, good, acceptable and very good, as to temperature, cleanliness, noise and light was rated, respectively.

The subjects' work-related musculoskeletal disorder symptoms are shown in Table 2. Neck, both shoulder, right elbow and wrist and hand, upper back and lower back were the most common WRMSDs symptom locations during the past 12 months while the neck, upper and lower back were the symptom locations of WRMSDs within the past 7 days. Symptoms in the neck, both shoulder, upper and lower back prevented the subjects from doing their respective jobs.

The correlation between person-task-environment (PTE) factors and WRMSD symptoms in the past 12 months is illustrated in Figure 1. Among the risks factors for WRMSDs, gender was found to have a significant correlation with WRMSDs of the neck ($P = 0.007$) and low back ($P = 0.031$). The number of co-workers was found to have to have significant correlation with WRMSDs of the neck ($P = 0.006$) and upper back ($P = 0.036$). WRMSDs symptoms of the elbow showed a significant correlation with years of service ($P = 0.049$) and category 2 posture ($P = 0.007$). Moreover, BMI ($P = 0.04$) and category 4 posture ($P = 0.46$) were found to have significant correlation with the WRMSDs of the hips/thighs, and ankle and foot, respectively.

The correlation between PTE and WRMSDs in the past 7 days is illustrated in Figure 2. WRMSDs symptoms of the elbow were found to have significant correlation with years of service ($P = 0.002$), number of co-workers ($P = 0.02$) and cleanliness ($P = 0.03$). Likewise, years of service ($P = 0.05$) showed a significant correlation with WRMSDs symptoms of the knee.

Table 1. Characteristics of the subjects.

Variable	Sample n = 28
A. Person	
1. Gender (# of males)	14
2. Age years)	
21- 23	8
24- 26	9
27- 29	5
30- 32	1
33- 35	5
3. Body Mass Index	
< 18.5	1
18.5 - 24. 9	15
25- 29.9	8
>30	4
4. Number of co-workers	
1-3	1
3-5	3
5-10	24
5. Number of patients per day	
< 5	5
5-10	20
>10	3
6. Number of years in service	
<1	9
1-3	9
4-6	3
6-10	6
>10	1
B. Task Factor	
1. No Corrective measures	61%
2. Corrective measures in the near future	34%
3. Corrective measures as soon as possible	3%
4. Corrective measures immediately	2%
C. Environment	
1. Temperature (# of good)	10
2. Cleanliness (# of good)	15
3. Noise (# of acceptable)	19
4. Light (# of very good)	12

Table 2. Symptoms of WRMSDs.

Body Regions	Work-related Musculoskeletal Disorders 12 month	Preventing to work	7 days
1. Neck	21	8	14
2. Shoulder			
a. Both	6	3	2
b. Left Shoulder	2	0	1
c. Right Shoulder	4	2	5
3. Elbow			
a. Both	1	1	3
b. Left Elbow	1	0	0
c. Right Elbow	4	1	1
4. Wrist/ Hands			
a. Both	4	1	1
b. Left Wrist/ Hands	1	1	3
c. Right Wrist/ Hands	11	5	4
5. Upper Back	21	11	13
6. Lower Back	24	13	13
7. One or Both Thighs	6	6	7
8. One or Both knees	8	5	4
9. One or Both Ankles/ Feet	7	4	6

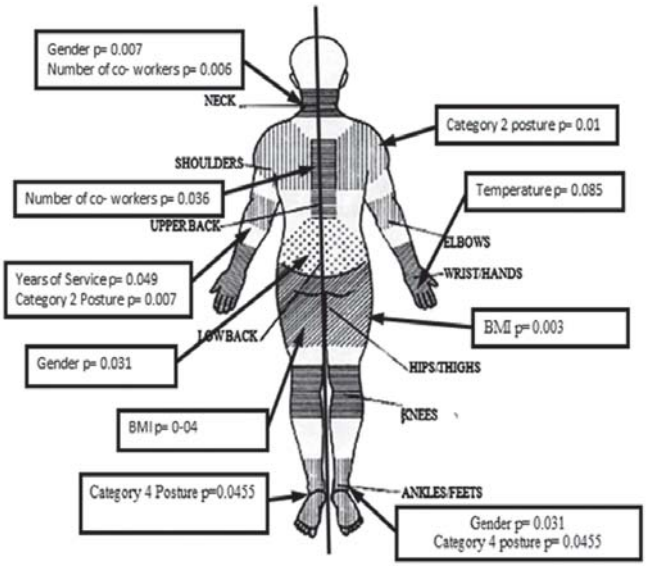

Figure 1. Correlations between person-tasks-environment factors and work-related musculoskeletal disorders (Legend: Left side - 12 months; Right side: Problems that prevent the subjects in doing work).

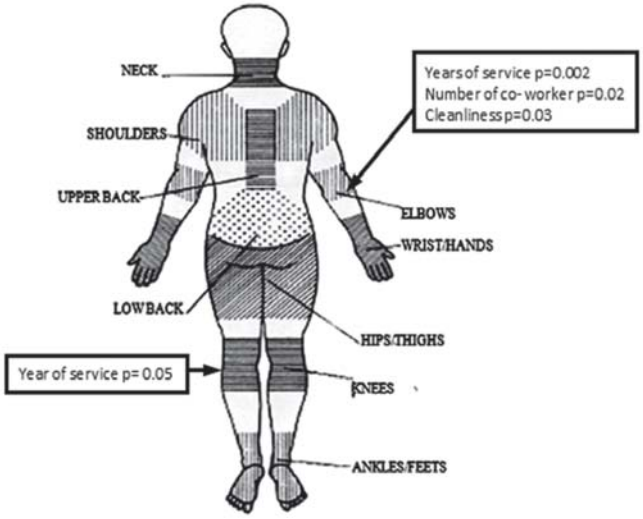

Figure 2. Correlations between person-tasks-environment factors and work-related musculoskeletal disorders in the past 7 days.

Category 2 posture showed a significant correlation with WRMSDs of the shoulder (P = 0.01) while category 4 posture showed a significant correlation with the WRMSDs of ankle and foot (P = 0.0455) which prevented the subjects from doing their job. Temperature likewise showed a significant correlation with WRMSDs of the wrist and hand (P = 0.035), BMI with the hips/thighs (P = 0.003), gender with the WRMSDs of the ankle and foot (P = 0.031).

Discussion

The results show that the factors associated with WRMSDs involving mostly the upper extremities and back are gender, BMI, number of co-workers, years of service, posture, temperature and cleanliness.

Our results regarding gender are similar to the findings of other studies. Cromie[4] found that more male therapists reported neck, wrist and thumb symptoms than the female therapists. This may be because of male therapists perform mobilization and manipulation more than the females. According to Campo[12] and Alrowayeh[13] female therapists were more prone to have WRMSDs because males have a better body build than female.

According to Nordin,[14] therapists with BMI more than 25 were more prone to WRMSDs. Workers who were overweight may not have been physically active, making them susceptible to WRMSDs.[15] Other studies

found out a higher prevalence of WRMSDs in those with lower BMI. With regard to years of service, the results are consistent with those of Cromie[4] who found that therapists who had worked longer had a higher possibility of acquiring WRMSDs. This was also shown by Erick.[16] Posture was another risk factor identified; this was consistent with Salik's study which showed that transferring patients (15%), performing repetitive tasks (14%), and lifting (14%) were associated with WRMSDs.[3] Our results were similar to those of Cromie[4] and Campo.[12]

The results showed that high environmental temperature was also associated with WRMSDs. Chad and Brown showed that environmental heat significantly influenced the cardiovascular and thermoregulatory systems in workers performing both light and heavy work tasks. In addition, environmental heat was shown to influence the magnitude of the frequency shift during the fatiguing muscle contraction in the typists rather than the lifters. These results suggest that: (1) performance of even sedentary workers performing a light manual task may be deleteriously affected by environmental heat; and (2) environmental heat may be of secondary importance to the nature of the task investigated on the level of muscle fatigue.[17] Magnavita hypothesized that heat —through sweat — caused salt depletion and water and electrolyte imbalance resulting in muscle cramps followed by muscle soreness, stiffness and reduced mobility.[18] Moreover, air draughts in a hot environment could be a factor to MSDs causing painful muscle contractures and reduced mobility.

The results show that the factors commonly associated with WRMSDs involving mostly the upper extremities and back are gender, BMI, number of co-workers and years of service; the other factors are posture, temperature and cleanliness. WRMSDs involving the neck, shoulder, upper and lower back prevented the therapists from doing their work.

The researchers recommend that therapists must be conscious of the proper posture, minimize twisting and bending during work, and reposition themselves from a prolonged awkward posture to prevent WRMSDs. Based on the study's limitations, doing the study using a larger sample may produce a more significant result. It is also recommended to utilize a CCTV camera in assessing posture, instead of using a video camera in front of the subject, in order to limit the Hawthorne effect. The researchers also recommend a study with the subjects in specialized work phases.

Declaration of Conflict of Interest

The researchers declare that there is no conflict of interest during the conceptualization and implementation of the study.

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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: 101-106.

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