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TABLE OF CONTENTS

EDITORIAL PREFACE

With much pleasure, we would like to present the inaugural issue of the Health Sciences Mindanao Journal (HSM)- the official scientific peer-reviewed journal of Davao Medical School Foundation Inc. (DMSFI). In pursuit of the DMSFI's vision of "Healthy Communities Enjoying Quality Life", this journal presents researches from basic to translational sciences that contribute towards understanding the factors that influence human health and disease.

We are certain that this will be the first of many others that will follow, giving an account to new knowledge and developments of its area of interest. This issue would not have been possible without the support of the Editorial Board members and staff. Our sincere thanks to all of you.

It is our hope that this compilation of articles would be a valuable resource for readers both from the medical and the non-medical field and will encourage further research into this dynamic area of human health and disease.

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Safety and Efficacy of Pomelo *(Citrus grandis (L.) Osbeck)* Peel Methanolic Extract in Prolonging Bleeding and Clotting Time in Albino Rats *(Rattus norvegicus)*

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RESEARCH ARTICLE

Abstract

Thrombus formation is the most common underlying pathology of cardiovascular diseases. Antiplatelet drugs are costly and pose serious side effects, hence, warranting an effective natural alternative. This study aimed to determine the safety and efficacy of pomelo *(Citrus grandis (L.) Osbeck)* peel methanolic extract in prolonging bleeding and clotting time in albino rats *(Rattus norvegicus)*. Safety was assessed by subjecting three rats to acute oral toxicity testing. Efficacy was determined by conducting a randomized, controlled, preclinical trial to thirty male rats divided into five groups: the negative control (0.2 mL saline solution), positive control (Clopidogrel 1mg/kg) and experimental control groups using low (15mg/kg), medium (30mg/kg) and high dose (60mg/kg) pomelo peel methanolic extracts. Extract activity was measured through bleeding and clotting time. Baseline bleeding and clotting time were determined. Once daily dosing of the intervention was administered for fourteen days. Post-intervention bleeding and clotting time were recorded. Results showed significant prolongation of bleeding and clotting time of rats in a dose dependent manner. The high dose pomelo peel extract showed significantly better results than Clopidogrel.

Keywords: *Citrus grandis, safety, efficacy, bleeding time, clotting time, rats*

INTRODUCTION

Cardiovascular diseases are the top causes of death globally. In 2012, 17.5 million cardiovascular related deaths were reported constituting 31% of all reported deaths. Approximately 7.4 million deaths were due to ischemic heart disease and 6.7 million deaths due to stroke.[1,2,3] The 2016 Department of Health (DOH) annual census reported that 118,740 Filipinos died of cardiac diseases and 68,325 of vascular diseases.[4] Cardiovascular diseases kill more people annually than any other cause with a rate of three in every ten deaths.[3]

Thrombus formation, composed mainly of platelets and fibrin, is the most common underlying pathology of cardiovascular diseases. Platelets function in the initial hemostatic plug that can vastly aggregate and block blood circulation in pathologic thrombosis, eventually causing ischemic heart disease and stroke.[5,6] Potent platelet function inhibitors have been developed into antiplatelet drugs.[7]

Antiplatelet drugs are proven effective in inhibiting platelet aggregation but pose serious side effects. These include gastrointestinal discomfort, central nervous system disturbances, severe neutropenia, nausea, vomiting, diarrhea, and even thrombocytopenic purpura.[8] Issues on treatment costs and side effects prompted the researchers to look for a potentially safe, effective and natural alternative antiplatelet agent.

Citrus fruits contain flavonoids known to exhibit effects on platelet function thus, making it a potentially good pharmacologic alternative. Pomelo, a local variety of citrus fruits, is known to contain flavonoids such as hesperidin and naringin in its peels and have demonstrated antiplatelet activity.[9] Hesperidin blocks the collagen-mediated phospholipase C (PLCγ2) phosphorylation causing concentration - dependent decreases of cytosolic calcium mobilization.[8] Naringin reduces intracellular calcium concentration resulting to inhibition of the myosin light chain phosphorylation and platelet activation and aggregation.[10]

The researchers aimed to determine the safety and efficacy of pomelo *(Citrus grandis (L.) Osbeck)* peel methanolic extract in prolonging bleeding and clotting time in albino rats *(Rattus norvegicus).* Success of this study may support the potential of pomelo as a future source of an alternative antiplatelet agent.

METHODOLOGY

Research Design

A randomized-controlled experimental study design was utilized. Potential antiplatelet activity of prepared doses of the flavonoid fraction from pomelo peel was measured through bleeding and clotting time. The results were then compared to the negative control (water) and positive control (Clopidogrel).

Research Locale

The extraction process was performed at Davao Medical School Foundation Incorporated (DMSFI) Research Laboratory. The test animals, *Rattus Norvegicus* were procured from a certified breeder and housed in the DMSFI animal house facility. Animal certification was done in a local veterinary clinic.

Plant Collection and Authentication

The pomelo fruit was obtained from a local farm in Davao City. The fruit and stem with leaf samples were verified and authenticated by a certified taxonomist as *Citrus grandis (L.) Osbeck.*

Fruit Peel Extraction

The method of extraction was patterned after a combination of similar methods by Baniya et al,[11] and Zakaria et al.[12] The albedo of the fruit was used for the experiment. The albedo was dried in an oven at 60°C overnight and homogenized using a blender.[11] Two hundred fifty grams (250g) of powdered albedo samples were placed in a beaker soaked in 2500mL of 80% methanol (1:10 ratio w/v) for 24 hours. The mixture was filtered, and the filtrate was subjected to rotary evaporation at 50°C to remove the solvent. The obtained extract was a yellow-brown viscous fluid.[11,12]

Oxidation Test to Detect Presence of Methanol

Oxidation test using sodium dichromate was used to test for the presence or absence of methanol in the extract. Sodium dichromate and sulfuric acid were added in a test tube containing an aliquot of the extract. The presence of methanol would turn an initial orange solution into green. There was no color change observed in the extract which supported the absence of methanol.

Animal Rearing

This study procured male rats aged eight to twelve weeks and weighed 150-300 grams. The rats were obtained from a certified breeder pathogen free and authenticated by a veterinarian. The experimental rats were housed in glass cages fed with rat food and ad libitum access to water. The living room conditions were set on the following specifications: room temperature at 25^{0}C and room humidity at 30-70%. Rats were allowed to acclimatize in the grouped cage for five days. Institutional Animal Care and Use Committee (IACUC) clearance was acquired prior to experimentation.

Determination of Doses

The determination of pomelo extract doses was based on the study of Xiao et al.[10] The doses were 15mg/kg, 30mg/kg, and 60mg/kg for the low, medium and high doses of pomelo peel methanolic extract respectively. The administered doses to the rat were calculated using the 10.07 mg of naringin/g of pomelo peel extract quantified by similar processes in a study by Pichaiyongvongdee and Haruenkit.[13] The weight of extract to be administered utilized Equation 1.

Equation 1: *Formula for calculating the grams of naringin to be administered to rats.*

$$\text{Grams of dose to be administered} = \frac{\text{weight of rat (in kg)} * \text{dose of naringin (in } {}^{mg}/_{kg})}{10.07\ {}^{mg}/_{kg}\ \text{of extract}}$$

The yielded amount of extract (in g/mL) were divided by result in Equation 1. The result was the dose of the extract administered onto the rat. The amount of oral gavage solution or mixture was determined by the amount yield on the extraction process.[13]

Administration of Methanolic Extract on Rats

Method of administration was patterned after Akoma et al.[14] and the Oakland University Training

and Information Manual Animal Care and Use Committee (IACUC) guidelines.[15] The undiluted crude methanolic extract was administered through oral gavage. Prior to drug administration, proper positioning of the rat was achieved, with the tube measured exactly from the rat's nose tip to the last rib, for it to pass through the esophagus and stomach. With the rat's head tilted back and the neck on a straight line, the gavage needle was inserted on one side of the mouth in a forty-five degree angle. The gavage needle passed down the esophagus without resistance. A noted struggle would require the procedure to be restarted. After administration of intervention, the rats were observed for any signs of distress and of any fluid coming from the mouth or nose.[14, 15]

Determination of Acute Toxicity

Several studies have deemed *Citrus grandis L. Osbeck* as potentially non-toxic at a dose of 2000 mg/kg. In a study by Sheik et al.,[16] no behavioral changes, toxicity nor mortality were seen at a dose of 2000mg/kg pomelo extract. This information was the guide to produce a limit test dose of 5000 mg/kg pomelo peel methanolic extract carried out in accordance with Organization for Economic Co-operation and Development (OECD) Guideline 423.

The study used three male albino rats to determine the acute toxicity of the pomelo peel methanolic extract. The rats were observed for forty-eight hours for the presence of any behavioral changes, nervous manifestations and physical discomfort.

Determination of Bleeding Time and Clotting Time

Thirty male albino rats were randomly divided into five groups. Each group had six rats placed in an individual cage. Group I was designated as the negative control and received 0.2 mL normal saline solution. Group II was designated as the positive control and received clopidogrel (1 mg/kg). For the experimental groups: Group III received low dose (15 mg/kg) pomelo peel extract, Group IV received the medium dose (30 mg/kg) pomelo peel extract and Group V had the high dose (60mg/kg) pomelo peel extract.[9,10] Baseline determination of bleeding time and clotting time was done. The extract was given once daily for fourteen days and post-intervention bleeding and clotting time were measured.[17]

Bleeding Time (Duke's Method)

Bleeding time was determined from the time taken between the emergence of the blood to the termination of bleeding expressed in minutes and seconds.[17] This was done by cutting the rats tail using a scalpel about one to two cm proximal to the tail end.

Clotting Time (Slide Method)

Clotting time was determined using the Ivy slide method described by Akomas and Ijioma.[14] A drop of blood collected from the bleeding tail of each rat was placed on a clean glass slide. A pin was passed across the drop of blood. The endpoint was the appearance of fibrin strands. A stopwatch was used to record the time (in minutes and seconds). The time between the placement of the blood on the slide and the visualization of fibrin strands was denoted as the clotting time. Both the procedure and the recording of bleeding and clotting times were done respectively by different assigned personnel.

Ethical Considerations

Treatment, acquisitions, care and use of test animals were in compliance to existing state and local laws as well as the institution's IACUC guidelines. Only trained handlers/researchers performed and supervised in the animal administration and made sure the experimental rats lived comfortably. Discomfort, pain, and illness among the rats were minimized during the study period.

Analysis of Data

Data were presented as means and mean differences. Multivariate Analysis of Variance (MANOVA) was used to determine significant differences in the baseline and post-intervention bleeding and clotting time. MANOVA showed the presence of a significant difference on results, thus, post hoc analysis was carried out using Duncan's Multiple Range Test (DMRT) to determine the differences that occurred between the groups.

RESULTS

Acute Oral Toxicity Determination

There were no physical and behavioral changes noted in the rats within forty-eight hours after administration of a single oral 5000mg/kg dose pomelo peel extract. All experimental subjects survived for two weeks. The researchers postulated that the toxic dose is deemed to be greater than 5000mg/kg.

Determination of Mean Bleeding and Clotting Time

Prior to start of experimentation, Shapiro-Wilk test was conducted on the baseline weight, bleeding and clotting time to assess for normality of data. The rats across all groups had similar physiological and hematologic parameters at baseline.

Figures 1 and 2 show the baseline and post-intervention mean bleeding and clotting time

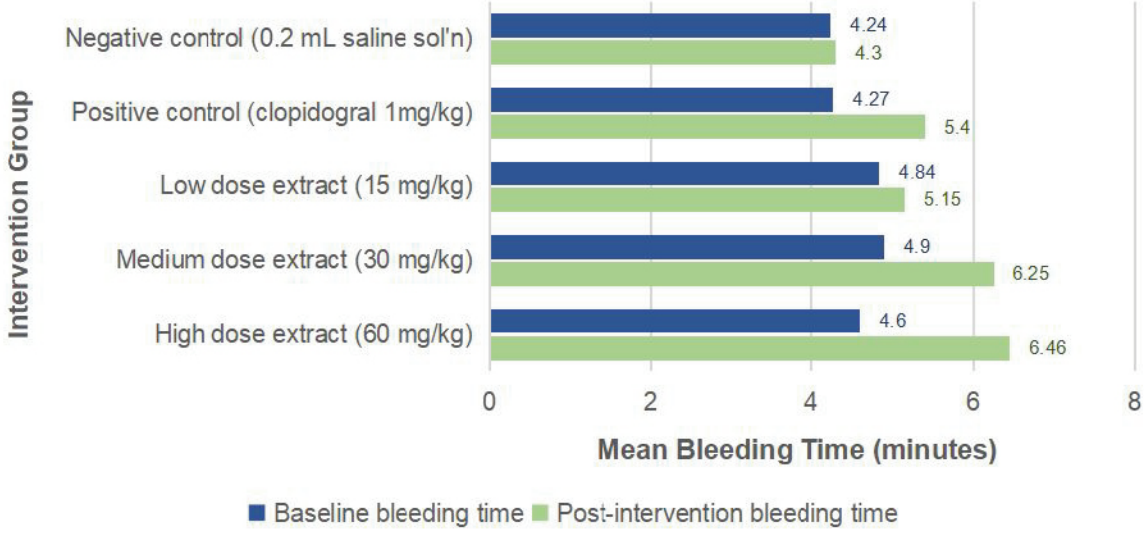

Figure 1. *Mean baseline and post-intervention rat bleeding time in minutes*

Figure 2. *Mean baseline and post-intervention rat clotting time in minutes*

values respectively. The baseline mean bleeding time values (MANOVA, p = 0.115), as well as the mean baseline clotting values (MANOVA, p = 0.579) do not vary greatly from each other. There was a dose dependent prolongation of mean clotting and bleeding time in post-intervention among the treatment groups. Effect was more pronounced as the dose was increased.

Baseline and post-intervention mean differences in bleeding and clotting time were compared among the different groups using MANOVA as shown in Table 1. MANOVA shows a p-value less than 0.05 denoting a significant difference in both the bleeding and clotting time.

A post hoc analysis using Duncan's Multiple Range Test (DMRT) showed that the medium dose extract ranked equally with Clopidogrel. This implies that the magnitude of prolongation of bleeding and clotting time by the medium dose extract approximates that of Clopidogrel. On the other hand, high dose pomelo peel extract ranked first among the intervention groups similar to medium dose in the bleeding time and similar to Clopidogrel in the clotting time. These imply that the higher the dose of the extract, the more prolongation of bleeding and clotting time can be expected.

DISCUSSION

The statistical data connotes that medium and high doses of pomelo peel extract can prolong bleeding and clotting time. The prolonged bleeding and clotting time of the rats may be attributed to the flavonoid components present in pomelo peel particularly naringin and hesperidin.[18-21] A study by Xi et al. found that one of the most abundant and predominant flavonoids in pomelo was naringin, which was confirmed through NMR spectroscopy.[19-21] Pomelo is widely cultivated and available throughout the Philippines. The flesh is consumed, and the peels are readily discarded. The flavonoid contents of *Citrus grandis* peel aqueous methanol extract were reported to be 149.05mg/L and 158.09mg/L.[20] In a study by Caengprasath et al., the methanolic extraction of pomelo peel yielded naringin (11.90 ±0.21 mg/g dried extract) and hesperidin (12.04 ±0.12 mg/g dried extract).[23]

Naringin and Hesperidin are known to possess antiplatelet effects.[9, 22, 24, 25] However, these were not measured because of equipment limitation. Platelet aggregation studies could have been a valuable tool to assess platelet function, but such test was not available in our setting. This study was only able to measure bleeding and clotting time which are not reliable measures of antiplatelet activity. But a dose-dependent prolongation in the bleeding and clotting times seen in this study could probably support a potential antiplatelet activity which can be further explored in future studies.

In summary, pomelo peel methanolic extract was not toxic at an oral single dose of 5000mg/kg.

Table 1. *Comparison of the mean differences between baseline and post-intervention bleeding and clotting times among the different groups using MANOVA and DMRT.*

Bleeding Time Mean Differences				Clotting Time Mean Differences			
Intervention	Homogeneous Groups			Intervention	Homogeneous Groups		
	1	2	3		1	2	3
High Dose	1.897			High Dose	2.467		
Medium Dose	1.355	1.355		Medium Dose	1.972	1.972	
Clopidogrel		1.153		Clopidogrel		1.660	
Low Dose			0.308	Low Dose			0.477
Negative			0.023	Negative			-
p-value	<0.001*			p-value	<0.001*		

Note: MANOVA: *significant p-value at 0.05; DMRT: Interventions in the same column are not statistically different.

The study shows significantly prolonged bleeding and clotting time of rats in a dose-dependent manner. Further studies involving isolation of the active components of the extract including assays such as platelet aggregation tests are essential to validate the findings of this study and to further demonstrate pomelo peel's potential as an antiplatelet agent.

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Pulmonary Function of Laundry Workers Chronically Exposed to Chlorine

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RESEARCH ARTICLE

Abstract

Chlorine is widely used as a bleaching agent in industry and society. In the hospital setting, it is used in increased concentration to remove bloodstains and dirt in bed linens. With its increasing use, this study aimed to evaluate the potential effects of chronic inhalation of chlorine in the respiratory function of laundry workers. The participants in the study were laundry workers from tertiary hospitals. The participants in the control group were comparable with the individual respondents in terms of demographic profile and past medical history. The pulmonary function was determined using a spirometer and parameters evaluated include Forced Expiratory Volume in 1 second (FEV_1), Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second/Forced Vital Capacity (FEV_1/FVC) ratio. The spirometry testing was conducted in San Pedro College, Davao City. A two-sample *t*-test statistical technique was employed on the data gathered. The laundry workers exhibited a decrease in FEV_1, FVC, and FEV_1/FVC ratio compared to the control group but there was no significant difference between the laundry workers chronically exposed to chlorine and the control group who are not exposed to chlorine in terms of FEV_1, FVC and FEV_1/FVC ratio. The results of this investigation suggest that although chronic chlorine inhalation produced a decrease in the pulmonary function parameters mentioned above, it was not enough to suggest an obstruction or restriction in the pulmonary passageway of the laundry workers. Hence the difference between the exposed and unexposed groups was not statistically significant.

Keywords: *Chlorine inhalation, Laundry workers, pulmonary function tests*

INTRODUCTION

Chlorine is known to be corrosive when it comes in contact with the respiratory epithelium due to its halogen characteristics which are very reactive and toxic to the human body.[1] Occupational exposures constitute the highest risk for serious toxicity from high concentration of chlorine.[2] The health hazard rating of the foregoing chemical is four, which is considered severe.[3]

Humans may come into contact with chlorine through its popular use as a bleaching agent. Higher concentration of chlorine powder is used by laundry workers to remove bloodstains from hospital bed linens. It is currently the most commonly used substance in the market due to its strong antiseptic effect and its stain removal properties. Many chemicals in work environments have not been studied for their potential to damaging effects on the workers' health. With the lack of information, many laundry workers may not know that problems related to their occupational exposure to chlorine may arise. Chronic exposures to chlorine among these laundry workers may pose a risk to their health.

With the increasing use of chlorine as a means of removing stains in household or hospital settings, this study intended to evaluate the occupational hazard of inhalation of chlorine present in bleach with lung function. With proper knowledge on the use of personal protective equipment (PPE), handling, storage and preparation following the

standards of mixing with emphasis on the time limit of exposure, one may lessen any deleterious effects associated with chlorine use.

This led the researchers to study the pulmonary function of laundry workers chronically exposed to chlorine. Specifically, it aimed to describe and compare the demographic data of the laundry workers and the control group in terms of age, height, weight and sex. Also, it sought to determine if there were significant differences between the laundry workers and the control group in terms of Mean Forced Expiratory volume in 1 second (FEV_1), Mean Forced Vital Capacity (FVC) and Mean Forced Expiratory volume in 1 second/Forced Vital Capacity (FEV_1/FVC) ratio.

METHODOLOGY

Research Design

The research utilized a cross-sectional analytical study design wherein exposed and unexposed participants were subjected to pulmonary function tests. FEV_1, FVC and FEV_1/FVC ratio were determined by the use of a spirometer.

Research Locale

Spirometry testing was conducted at San Pedro College, Davao City.

Participants

Participants included in the study were laundry workers from tertiary hospitals in Davao City chronically exposed to chlorine for at least three months. The study employed a control (unexposed group) whose characteristics were comparable with the individual laundry workers in terms of age, sex, height, weight, diseases present, lifestyle, family history of illnesses and presence or absence of allergies. The control (unexposed group) differed only from the participants (exposed group) on the basis of exposure to chlorine.

Variables and Measures

Spirometry testing was performed on the exposed and unexposed participants to determine their individual FEV_1, FVC, and FEV_1/FVC ratio. The spirometer utilized was an MIR Spirolab III provided by the San Pedro College Respiratory Therapy Department. Spirometry testing of the participants was done under the supervision of professional and trained respiratory therapists of San Pedro College.

Exclusion Criteria

The exculsion criteria were the following: laundry workers not exposed to chlorine for at least three months, any significant medical condition that would interfere with the test or evaluation of the results (e.g., asthma, pulmonary tuberculosis and/or any pulmonary diseases), current usage of drugs, history of pulmonary disease, and those who were currently participating in another trial.

Sampling Technique

Purposive sampling was used to determine the population who became the respondents of the study.

Procedure

The study first sought out the approval from the Institutional Ethics Research Committee. Approval from the tertiary hospital was done prior to the conduct of the study. A purposive sampling of participants was conducted. Survey and gathering of participants were made. Informed consent was secured from each potential participant. Exclusion parameters were thoroughly evaluated among all possible participants. Interview of the laundry workers followed and data pertaining to age, sex, height, weight, diseases present, lifestyle, family history of illnesses, and presence of allergies was gathered.

The control group was chosen based on the demographic data taken from the 12 laundry workers. The demographic data collected included information such as age, gender, weight, height and recent medical history. Based on the data, the researchers were able to select a control group that had a similar demographic data with the laundry workers being studied.

In performing the Spirometry test, an MIR Spirolab III was used in order to obtain the data needed for the study. Spirolab is a new generation spirometer that can facilitate a total evaluation of lung function. The product, designed for use by a specialist, requires a simple, compact device which is capable of calculating more than thirty spirometric parameters.

Spirolab assesses FVC, VC, IVC, MVV, and breathing pattern and calculates an index of test acceptability (test quality control) and a measure of reproducibility. It also gives a functional interpretation with eleven possible levels following the American Thoracic Society classification. The main spirometric parameters are measured and displayed and all data with flow/volume and time/volume curves can be printed out in seconds by the built-in thermal printer. The flow/volume

curve is shown in real time.

The test protocol and maneuver of the test was explained to the participants by the respiratory therapist. A time was given to the participants for the orientation and for them to get accustomed to the instrument and the procedure was enumerated before the actual test. The tests were conducted while the subjects sat comfortably. Each test was repeated three times, with an interval of five minutes between the tests. The lung function parameters such as the FEV_1, FVC, and FEV_1/FVC were determined.

No special preparations were needed prior to the test procedure. No discomfort was felt during the procedure. It took about 15 to 20 minutes per patient. The procedure was conducted inside a well-ventilated classroom of San Pedro College. A pulmonologist analyzed the data that was obtained from the results of the pulmonary function test.

Statistical Treatment

Data were reported using means, counts and percentages. The Wilcoxon signed rank test was used to determine if there is a significant difference in the demographic data of the laundry workers and control group. Two-sample *t*-test was utilized to compare the mean results of the pulmonary function tests between the two groups.

Ethical Consideration

The participants were carefully screened and limited to the exclusion criteria to minimize the possible risks that may arise. A registered respiratory therapist performed the pulmonary function test. Prior to the procedure, the participants were informed as to the nature and purpose of the research, the process used, the expected benefits, and the potentiality of foreseeable risk, stresses, and discomforts of participating in the research. Participants were given an informed consent form approved by the Davao Medical School Foundation, Inc. (DMSFI) Institute of Graduate Studies and Research (IGSR) prior to the conduct of the study. The participants' profile and test results gathered were treated with confidentiality and anonymity. The right to refuse further treatment and the right to withdraw from the study during its course were emphasized to the participants. The researchers declared that there was no conflict of interest. The proponents had no personal, financial and other connection with the respiratory therapist or the laboratory. The results gathered after the spirometry testing and interpretation of the pulmonologist were disclosed to the individual participants.

RESULTS

Wilcoxon signed rank test was used to compare the demographic data of the laundry workers and control group to see if there is a significant difference between the two as shown in Table 1. It showed that the two groups were indeed comparable in terms of age, height and weight. On the other hand, the laundry workers and control group had the same number of male and female participants. There were more males than females who participated in the study.

Table 1. *Descriptive statistics and test for significant Difference of Control Group and Laundry Workers' Demographics*

Profile	Laundry workers (Mean ± SD)	Control group (Mean ± SD)	*p*-value	Interpretation
Age (years)	36.25 ± 10.11	36.25 ± 10.62	1.000	No significant difference
Height (cm)	151.64 ± 12.35	152.25 ± 14.92	0.912	No significant difference
Weight (kg)	61.67 ± 15.57	61.27 ± 9.82	0.877	No significant difference
Sex count (%)				
Male	9 (75%)	9 (75%)		
Female	3 (25%)	3 (25%)		

Note: Significant at *p*-value ≤ 0.05

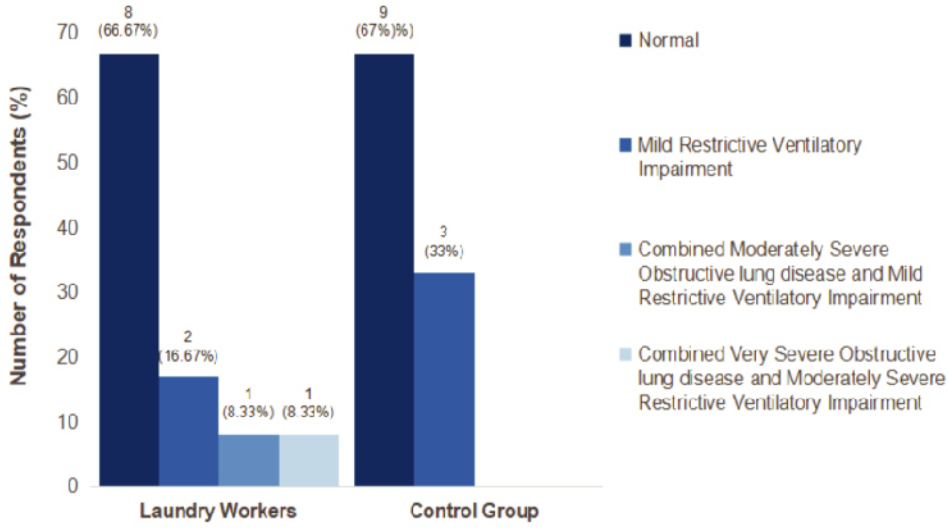

Figure 1. *Pulmonary Function Test Results of Laundry Workers and the Control Group*

Depicted in Figure 1 are the pulmonary function test results of the laundry workers and the control group as interpreted by a pulmonologist. The pulmonary function test results of the twelve laundry workers showed that two had mild restrictive ventilatory impairment; one had combined moderately severe obstructive lung disease and mild restrictive ventilatory impairment; one had combined very severe obstructive lung disease and moderately severe restrictive ventilatory impairment; and eight had normal results. On the other hand, pulmonary function test results of the control group showed that three out of the twelve respondents had mild restrictive ventilatory impairment and the rest had normal results.

Table 2 shows the results of the pulmonary function test of the laundry workers and control group in terms of FEV_1, FVC, and FEV_1/FVC ratio. The mean of the percent (%) predicted FEV_1 value of the laundry workers is lower than the control group. Same results were observed for the FVC and FEV_1/FVC ratio. Upon comparison, the mean level of the FEV_1 of the exposed (laundry workers) group (control group). Same results were observed for the FVC and FEV_1/FVC ratio.

DISCUSSION

Determination of demographic profile of the participants is important because it affects their pulmonary function test results. The lungs mature at age 20 to 25 years old and thereafter, aging is associated with progressive decline in lung function[4]. This is characterized by increased chances of small airways to collapse due to deterioration of collagen and elastin that supports lung structures. Such collapse will lead to certain problems such as the "Ventilation - Perfusion Mismatch".[5] In this study, the mean age of the participants were 36.25 ± 10.11 and 36.25 ± 10.62 for laundry workers and control groups respectively passing even beyond lung maturirty age range. Height also has an important effect on vital capacity. Taller people regardless of their age or gender usually have a higher lung capacity than shorter people. This might be due to the increased surface area of the lungs in relation with increasing height[6]. In terms of weight, obesity causes deleterious effects on lung volume and

Table 2. *Comparison of the results of the pulmonary function tests between laundry workers and the control group*

Pulmonary Function Test	Laundry workers (Mean ± SD)	Control group (Mean ± SD)	*p*-value	Interpretation
FEV_1	93.64 ± 31.10	96.13 ± 21.04	0.823	No significant difference
FVC	98.33 ± 29.89	103.22 ± 28.29	0.684	No significant difference
FEV_1/FVC ratio	98.37 ± 12.31	100.28 ± 13.51	0.754	No significant difference
Note: Significant at *p*-value ≤ 0.05				

capacity in children and adolescents, mainly by reducing functional residual capacity, expiratory reserve volume and residual volume. The increase in adipose tissue in the chest and abdomen area causes an increase in the intra-abdominal pressure, with consequent reduction in lung compliance and chest wall mobility.[7]

As shown in Figure 1, four out of twelve participants (33.3%) showed abnormal pulmonary function test ranging from mild to severe restrictive ventilator impairment as compared to the control group where three out of twelve participants (25%) showed only mild restriction. The abnormality in the test result could be supported by a study of Medina-Ramon[12] where symptoms of obstructive lung disease in domestic cleaners have been linked to the use of diluted bleach and other irritant cleaning products such as degreasing sprays/atomizers and air fresheners.

The decrease in the percent (%) predicted values of FEV_1, FVC, and FEV_1/FVC ratio of the laundry workers could be attributed to the chronic inhalation of chlorine. Chlorine has very corrosive properties when it comes in contact with the respiratory epithelium. Due to chlorine's halogen characteristics, it is often reduced when it interacts with moist tissues. This occurs when hydrogen separates from water in the moist tissues, thereby causing damage to the tissue as hydrochloric or hypochlorous acid is formed.[8] Upon hydration of chlorine, reactive oxygen species are generated. These reactive species can contribute to further airways injury, edema, and inflammation, immediate airway constriction, and persistent airways reactivity.[9]

Although chlorine exposure is already detected at low levels by feelings of mild irritation and discomfort, it is only at ≥30 part per million (ppm) that respiratory epithelium is altered and obstructive defect is seen.[10] Moreover, exposure to low concentrations of chlorine causes preferential damage to the large airways. Alveolar damage requires exposure to high concentrations of gas. This damage to large airways is minimal and cannot cause significant reduction in spirometry parameters like FEV_1, FVC, and FEV_1/FVC ratio.[11] This may explain the absence of significant statistical difference when pulmonary function tests between laundry workers and control were compared.

Moreover, the small number of participants in this study may not be enough to show a statistically significant result (see Table 2). However, the higher trend of mild to severe ventilator impairment among laundry workers should alert us to the possibility of significant work hazards among them. Health authorities should be vigilant of the possible dangers there laundry workers may be exposed to so that measures can be done to avoid or minimize such hazards.

In summary, the results showed that the mean age of the laundry workers was 31 years old. Their mean height was 142.998 cm and their mean weight was 69.667 kg. There were more male than female laundry workers who participated in the study. Two laundry workers (16.67%) had mild restrictive ventilatory impairment, one laundry worker (8.3%) had combined moderately severe obstructive lung disease and mild restrictive ventilatory impairment, and another one laundry worker (8.3%) had combined very severe obstructive lung disease and moderately severe restrictive ventilatory impairment which could be attributed to chronic inhalation of chlorine. The remaining eight had normal test results. Three of the control group had mild restrictive ventilatory impairment and the rest had normal results. When testing for the significant difference between the laundry workers and the control group, it showed that there was no significant difference in the FEV_1, FVC and FEV_1/FVC ratio between the laundry workers and the control group.

Future studies may include chest radiographs and its correlation with the spirometry parameters used in this study. Extensive history taking should be done to better exclude all other factors which may affect the pulmonary function of the respondents. Measurement of chlorine levels in the air in terms of parts per million (ppm) as stipulated in the occupational hazard guideline published by OSHA and description of the working area of the respondents as to surface area, height of the ceiling and presence of windows would also be valuable.

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Lymphoma in Kawasaki Disease: A case report of a rare association

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CASE STUDY

Abstract

Kawasaki Disease or mucocutaneous lymph node syndrome is an acute febrile vasculitic disorder of childhood. We present a case of a 2-year-old male who initially presented with cervical lymphadenopathy. Symptomatology and diagnostic evaluation led to the diagnosis of Classic Kawasaki Disease. The patient was initially managed with intravenous immunoglobulin and subsequently with Methylprednisolone and Infliximab due to persistence of fever despite changes in management. Cervical enlargement prompted biopsy and revealed Hodgkin's Lymphoma categorized as T-cell Anaplastic Large Cell Lymphoma. Chemotherapy was initiated and improved the patient's condition.

Keywords: *kawasaki disease, mucocutaneous lymph node syndrome, lymphoma, association*

CASE PROTOCOL

A 2-year-old Filipino boy from Matina, Davao City was brought in for fever. About two weeks prior to admission, an enlarged lymph node was noted on the left lateral neck. After a week, there was persistence of the lymph node swelling which was later associated with fever. Consult was made with a pediatrician and was managed as a case of bacterial lymphadenitis. He was given Azithromycin 200mg/5ml, 3.0 mL once daily or at 10 mg/kg once daily for five days with no improvement. Four days prior to admission, aside from the persistent enlarged lymph node and remittent fever, there was an onset of maculopapular rash and non-purulent conjunctivitis. Later, the condition was associated with the appearance of swelling of hands on the day of admission.

There was no history of cough, colds, hematuria, vomiting, diarrhea, abdominal pain, joint pain, or other associated signs and symptoms noted. There was no previous history of hospitalization. He completed immunization up to 1 year old at the local health center. He was breastfed exclusively up to 6 months old. There was no history of exposure to tuberculosis.

At the emergency room, the patient was irritable, febrile (temperature of 38.8^{0}C), tachycardic (cardiac rate of 150 beats per minute), with a respiratory rate of 45 cycles per minute, and O_2 saturation of 99%. He weighed 12.2kg, measured 92cm for height, with a body mass index of 14.4 and normal *z* scores. There were erythematous maculopapular rashes seen on the trunk and extremities. Examination of the eyes revealed bilateral nonsuppurative conjunctivitis. His lips were erythematous and cracked and the tongue appeared strawberry-like. A non-fluctuant, nontender left cervical lymphadenopathy measuring 2 cm was noted (see Figure 1). Pulmonary findings were unremarkable. Cardiac rhythm was regular, and no murmur was appreciated. Abdomen was nondistended and soft with no masses nor hepatosplenomegaly. There was mild nonpitting edema of both hands. Neurologic examination was unremarkable.

On admission, the 8th day of illness, he was managed as a case of Classic Kawasaki Disease (KD). Diagnostic investigation revealed anemia of 97 g/L, platelet count of 359 x 10^9 cells/L, leukocytosis of 17.9 x 10 9 cells/L with neutrophilia of 78%, a markedly elevated Erythrocyte Sedimentation Rate (ESR) of 144 mm/hr, and C-Reactive Protein (CRP) of 200 of mg/dl. Dilated coronary arteries were noted in the 2D-echocardiogram.

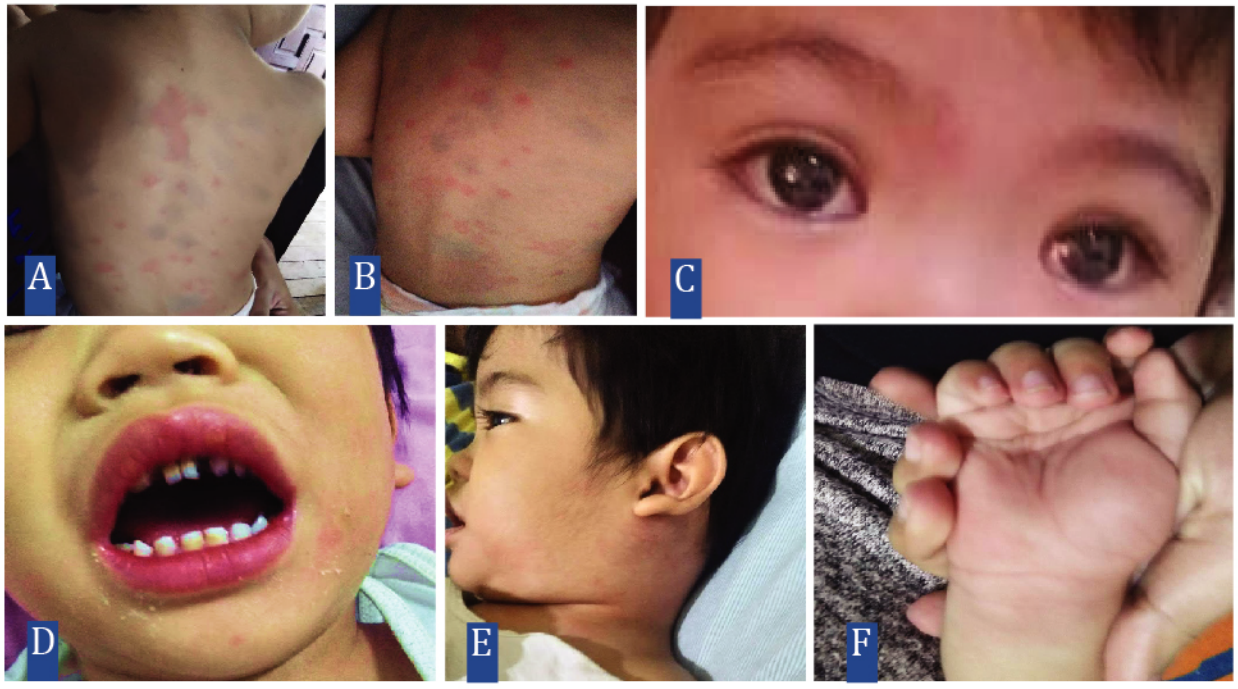

Figure 1: *Physical Features of the patient. (A) and (B) Skin showed erythematous maculopapular rashes. (C) Eyes manifested bilateral nonsuppurative conjunctivitis. (D) Erythematous and cracked lips. (E) Left cervical lymphadenopathy measuring 2cm, non-fluctuant, nontender. (F) Right hand showing nonpitting edema. He was started on anti-inflammatory dose of Aspirin 100mg/tablet, 3 tablets per orem every 6 hours.*

On the 9th day of illness, high dose intravenous immunoglobulin (IVIG) of 2g/kg body weight or 24 grams IV infusion to run over 12 hours was given. Patient was closely monitored and resolution of fever was noted within seven hours of completing IVIG infusion. Thereafter, fever recurred and was observed to persist until the 36th hour. At this point, other reasons for fever were investigated.

During the 13th day of illness, onset of cough was observed, with noted bilateral fine crackles. Rash, conjunctivitis, cracked lips, strawberry tongue, and lymphadenopathy persisted. On the 14th day of illness, periungual desquamation was noted (see Figure 2). Chest X-ray revealed Pneumonia and blood culture was negative. Cefepime 610mg IV every eight hours or at 50 mg/kg body weight per dose was given which resulted to improved cough and breath sounds. However, patient still had fever. A repeat 2D-echocardiogram revealed persistent dilatation of coronary arteries. Referral to an Infectious Disease Specialist and a Pediatric Cardiologist ruled in an IVIG-resistant KD.

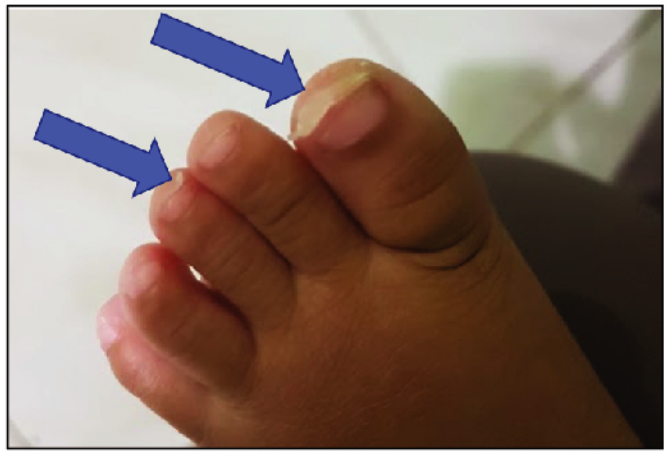

Figure 2: *Toes showing periungual desquamation.*

On the 19th day of illness, a second dose of IVIG 24 grams IV infusion to run over 12 hours (2g/kg) was given, after which the rash, conjunctivitis, cracked lips, strawberry tongue, and lymphadenopathy lessened. The fever lysed for five hours but recurred again. There was also an increase in the size of the left cervical lymphadenopathy from 2cm to 3cm.

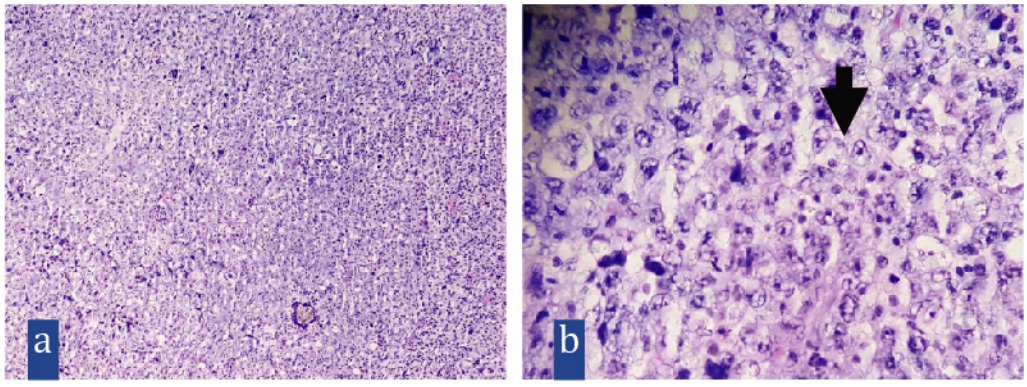

Figure 3: *Histopathology slides of patient. (a)LPO view: proliferation of round atypical cells with diffused pattern of growth; (b)HPO view: the individual cells have uniform large, vesicular nuclei with prominent nucleoli*

With the patient going on his 3rd week of illness, there was thrombocytosis of 1100 x 10^{12} cells/L and a comparative Chest X-ray revealed moderate clearing of infiltrates. A repeat 2D-echocardiogram continued to show persistent dilated coronary arteries. Ultrasound of the neck showed multiple lobulated, enlarged lymph nodes noted in left cervical, submandibular, posterior auricular and supraclavicular areas with maximum diameter of 3.4 cm x 2.1 cm. On consult with an ENT Specialist, the possibility of a cervical abscess was ruled out. The Infectious Disease Specialist added Clindamycin 122mg IV or at 10mg/kg/dose given every eight hours to the treatment regimen and referral to a Pediatric Rheumatologist was made. With the consideration of an IVIG-resistant Kawasaki Disease, Methylprednisolone pulse therapy at 366mg IV (30mg/kg/dose) once a day for three days was given which resulted to resolution of fever and improvement of patient's well-being for a few days. However, fever recurred and persisted. Further work-ups revealed leukocytosis of 23.54 x 10^9/L, thrombocytosis of 789 x 10^9/L, elevated CRP of 98.9 mg/L, negative rheumatoid factor and negative urine and stool KOH. Hence, working impression at this time still was IVIG-Resistant KD.

On the 4th week of illness, Infliximab infusion of 61mg IV at 5mg/kg body weight was given. Fever persisted and the left cervical lymphadenopathy rapidly enlarged in size to 5cm. Upon further examination, an enlarged, 2cm, non-fluctuant, nontender lymph node was also noted on the left axillary area. On referral to the Hematology-Oncology service, a bone marrow aspiration (BMA) was done which revealed a reactive marrow. ENT service performed incision biopsy of the left cervical mass which on gram stain revealed no organism and no acid-fast bacilli. However, on histopathology (see Figure 3), Non-Hodgkin's Lymphoma was considered. Slides were sent for Immunohistochemistry studies.

With the diagnosis of Non-Hodgkin's Lymphoma, Stage II, patient was started on chemotherapy with COP-Protocol comprising Vincristine, Cyclophosphamide and Prednisone. His condition improved significantly, fever lysed on the 30th day of illness and the lymphadenopathies gradually subsided. He was discharged improved.

Immunohistochemical staining revealed ALK-positive, CD3 (T cell marker)-positive and CD20 (B cell marker)-negative. Histopathology and immunohistochemistry categorized the patient as a T-Cell Anaplastic Large Cell Lymphoma. Further diagnostic investigation was performed, biopsy slides were sent to St. Jude Children's Research Hospital in Memphis, Tennessee. Paraffin immunohistochemistry showed that the tumor cells were positive for CD45, CD30, ALK1 (nuclear and cytoplasmic) while negative for CD15, CD20, PAX5. Epstein-Barr encoding region (EBER) in situ hybridization was negative. These evidences strongly support the diagnosis of T-Cell Anaplastic Large Cell Lymphoma.

Bone marrow and central nervous system (CNS) disease were absent in this case as evidenced by BMA and cerebrospinal fluid analysis.

Up to this date, he has received BFM-NHL-90 Protocol for Anaplastic Large Cell Lymphoma, and has completed the sixth cycle of Vincristine, Cyclophosphamide, Doxorubicin, Methotrexate and Prednisone.

DISCUSSION

The law of parsimony is a principle in which "the simplest explanation of an event or observation is the preferred explanation".[1] Thus, if one diagnosis can explain all the signs and symptoms, then it must be the correct diagnosis. However, as the patient's clinical picture evolves, one disease might no longer be enough to explain everything, and another diagnosis can also be considered.

A 2-year-old boy presented with lymphadenopathy which was later associated with fever, rash, bilateral nonsuppurative conjunctivitis, cracked lips and strawberry tongue, as well as edema of hands. These manifestations complete the clinical criteria to diagnose Classic Kawasaki Disease.

Kawasaki Disease, or mucocutaneous lymph node syndrome, is an acute febrile vasculitic disorder of childhood.[2,3] The incidence of Kawasaki disease is higher in Japan than in any other country, although the disease has been reported worldwide.[3] Based on the Philippine Pediatric Society, Inc. website, ICD-10 search for Kawasaki Disease (M30.3) revealed 6,653 cases from January 2000 to present.[4]

Kawasaki Disease almost always affects children. Most patients are under 5 years old. The average age for children affected with the syndrome is about two years. Boys develop the illness almost twice as often as girls.[3] In the last four decades of investigation, the cause of Kawasaki disease remains unknown.[5]

Studies have demonstrated generalized alterations in immune function in patients with Kawasaki disease, particularly involving T lymphocytes. However, while an increased risk of lymphoid malignancies may be observed in other acquired disorders of immunity, patients with Kawasaki disease who developed malignancies have not been extensively reported.[6]

Evaluation and treatment of lymphadenopathy is guided by the probable etiologic factor, as determined from the history and physical examination.[7]

Pertinent from the patient's history are the initial manifestations of lymphadenitis and fever, and he was initially managed as bacterial lymphadenitis. It is worth noting that he was already treated with antibacterial medications even as an outpatient, but still signs of infection still persisted. Blood culture was negative. Poor response to antibiotics during hospital stay also ruled this out.

Another plausible consideration in this patient is the presence of Koch's infection. Tuberculous lymphadenitis is the most common form of extrapulmonary tuberculosis (TB). The most common presentation is a unilateral single or multiple slowly growing nontender lymphadenopathy which can be present for up to 12 months before diagnosis.[8] In this patient, lymph node enlargement was noted two weeks prior to admission. However, he had no exposure to TB with no history of respiratory infections. Despite the fever and the cervical lymphadenopathy, the patient had other manifestations (oral changes, conjunctivitis, swelling of hands, rashes) which could not be attributed to TB alone. This was further ruled out by the absence of acid-fast bacilli on biopsy.

Malignancy as a cause of lymphadenopathy was also considered. Lymphoma may present with varied clinical pictures but may commonly present as lymphadenopathy, fever, weight loss and hepatomegaly.[9] Weight loss and hepatomegaly were absent in this case. Chest X-ray also showed no mediastinal mass. The presence of other signs and symptoms in the patient on admission also pointed to a classic case of Kawasaki Disease over any malignancy such as Lymphoma.

Presenting with a history of lymphadenitis and fever of more than five days as the first manifestations, along with five of the clinical features: erythema and cracking of the lips and erythematous tongue, bilateral bulbar conjunctival injection without exudate, maculopapular rash, edema of hands, and left cervical lymphadenopathy, the patient fulfilled the case definition of Classic Kawasaki Disease (see Table 1). Based on the American Heart Association Scientific Statement, diagnosis of Classic Kawasaki Disease is based on the presence of fever for five days or more and the presence of four or more of the five principal clinical features.[5]

Diagnostic evaluation further supports the diagnosis of Kawasaki Disease: anemia, leukocytosis, elevated acute phase reactants, ESR and CRP, and later, thrombocytosis was noted. According to American Heart Association (AHA) Scientific Statement, anemia occur commonly; leukocytosis is typical during the acute stage of illness; elevation of ESR and CRP is nearly universal; thrombocytosis peaks on third week of illness.[5] Although these

Table 1: *Diagnosis of Classic Kawasaki Disease taken from American Heart Association Scientific Statement (Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals from the American Heart Association, 2017)*[5]

Classic Kawasaki Disease is diagnosed in the presence of at least five days (the day of fever onset is taken to be the first day of fever) together with at least four of the five following principal clinical features. In the presence of less than four principal clinical features, particularly when redness and swelling of the hands and feet are present, the diagnosis of KD can be made with four days of fever, although experienced clinicians who have treated many patients with KD may establish the diagnosis with three days of fever in rare cases:
1. Erythema and cracking of lips, strawberry tongue, and/or erythema of oral and pharyngeal mucosa
2. Bilateral bulbar conjunctival injection with exudate
3. Rash: maculopapular, diffuse erythroderma, or erythema multiforme-like
4. Erythema and edema of the hands and feet in acute phase and/or periungual desquamation in subacute phase
5. Cervical lymphadenopathy (less than 1.5 cm diameter), usually unilateral

laboratory results could also point to a possible Lymphoma, 2D-echocardiogram revealed dilated coronary arteries. The presence of coronary abnormalities is considered a specific criterion supportive of KD, thus a possible Lymphoma, which was one of the initial considerations for fever and cervical lymphadenopathy, was not pursued.

Imaging study of the persistent left cervical lymphadenopathy was performed. In Kawasaki Disease, multiple lymph nodes are enlarged, and retropharyngeal edema or phlegmon is common.[5] This was apparent in the patient's ultrasonography result which revealed multiple lobulated, enlarged lymph nodes on the left cervical, submandibular, posterior auricular and supraclavicular areas. This would rule out bacterial lymphadenitis, which is most frequently associated with a single node with a hypoechoic core.

Primary medical treatment for Kawasaki Disease comprising of intravenous immunoglobulin and aspirin were administered to the patient. Approximately 10 - 20% of patients with Kawasaki Disease who develop persistent fever at least 36 hours after the end of their IVIG infusion are considered IVIG Resistant.[5] Based on recent guidelines released by AHA as shown in Table 2, IVIG retreatment, corticosteroids and infliximab are the most frequently administered medications to IVIG Resistant KD. However, there are no robust data from clinical trials to guide the clinician in the choice of therapeutic agents.[5]

Kawasaki Disease, in this case, remained as the diagnosis because as the illness progressed, further signs and diagnostic tests continue to support it. KD can be divided into three clinical phases. The acute febrile phase is characterized by fever and the other acute signs of illness which usually last for one to two weeks. The patient came in our institution during the first phase. But as the disease progressed, periungual desquamation and thrombocytosis were both noted. These signs belong to the second phase of KD, which is termed the subacute phase. The subacute phase is associated with desquamation, thrombocytosis, and the development of coronary artery aneurysm, and this phase generally lasts three weeks, further supporting the diagnosis of Kawasaki Disease in this patient. It is also of utmost significance that during the subacute phase, the highest risk of sudden death occurs in patients in whom aneurysms have developed. Coronary artery aneurysm was not evident in the patient, but persistent coronary artery dilatation seen in a series of 2D-echocardiogram studies has posed fear for the worse. Hence, the risk of sudden death has always occurred in the minds of the attending physicians, making them more vigilant while the patient was under their care. The third stage is the convalescent phase. This phase begins when all clinical signs of illness have disappeared and continues until the erythrocyte sedimentation rate (ESR) returns to normal, typically about six to eight weeks after the onset of illness.[7,10]

Table 2: *Treatment Options for IVIG-Resistant Kawasaki Disease taken from American Heart Association Scientific Statement (Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals from the American Heart Association, 2017)*[5]

Agent	Description	Dose
Most frequently administered		
IVIG: Second Infusion	Pooled polyclonal IG	2g/kg IV
IVIG + Prednisolone	IVIG + Steroid	IVIG: 2g/kg IV + prednisolone 2mg/kg/d IV divided every eight hours until afebrile, then prednisone orally until CRP normalized, then taper over two to three weeks
Infliximab	Monoclonal antibody against TNF-α	Single infusion: 5mg/kg IV given over two hours
Alternative treatments		
Cyclosporine	Inhibitor of calcineurin-NFAT pathway	IV: 3mg/kg/d divided every 12 h PO: 4–8 mg/kg/d divided every 12 h Adjust dose to achieve trough 50–150 ng/mL; 2-h peak level 300–600 ng/mL
Anakinra	Recombinant IL-1β receptor antagonist	2–6 mg/kg/d given by subcutaneous
Cyclophosphamide	Alkylating agent blocks DNA replication	2mg/kg/d IV
Plasma exchange	Replaces plasma with albumin	Not applicable

Kawasaki Disease was clearly established in this case. History, physical exam, laboratory tests and disease course strongly supported the diagnosis. But why did the patient end up developing a Lymphoma? Did Kawasaki Disease cause the Lymphoma or is it the other way around? Very limited data is available to answer these questions, thus making this case indeed rare and very interesting.

Numerous analyses have demonstrated generalized alterations in immune function in patients with Kawasaki Disease, particularly involving T lymphocytes.[6] Recent studies favored that activation of innate immune system occurs as an early event in Kawasaki Disease, with high numbers of activated, circulating neutrophils and evidence for activation of interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF) signaling pathways. Study of the adaptive immune response demonstrated that both proinflammatory and regulatory T cells can be found in the circulation in the first week after fever onset.[5]

In an article by J. Murray, *et al*, in 1995, two case reports of children diagnosed and treated with Kawasaki Disease were consequently diagnosed with lymphoid malignancies. Activation of T cells in Kawasaki Disease could lead to malignant lymphocyte dysregulation.[6]

A study disclosed key mechanisms of peripheral T cell transformation explaining the pathogenesis of Lymphomas, particularly T cell lymphomas. One of which is deregulation of signaling pathways controlling T cell development, differentiation, and maturation. Two other mechanisms are the following: the remodeling of the peritumor microenvironment and the virus-mediated rewiring of T cell biology.[11] Furthermore, Non-Hodgkin's Lymphoma results from alteration in T cell regulation with consequent clonal expression of a select lymphoid subpopulation.

This suggests that there may be a relationship between the immunopathy accompanying Kawasaki disease and the development of malignancy. Marked activation of T lymphocytes has been documented in Kawasaki Disease. The polyclonal activation of T cells, in Kawasaki Disease, leads to the speculation that there may be the potential for further lymphocyte dysregulation resulting in the selection of a subpopulation of cells capable of expanding in a monoclonal or malignant fashion.[6]

Non-Hodgkin's lymphoma (NHL) accounts for approximately 60% of lymphomas in children. Although most children and adolescents with NHL present with de novo disease, a small number of patients have NHL secondary to specific etiologies, including inherited or acquired immune deficiencies such as severe combined immunodeficiency syndrome, Wiskott-Aldrich syndrome, viruses such as HIV and EBV, and as part of genetic syndromes such as ataxia-telangiectasia, and Bloom syndrome.[7,9] The four major pathologic subtypes of childhood and adolescent NHL are lymphoblastic lymphoma, Burkitt lymphoma, diffuse large B-cel lymphoma, and anaplastic large cell lymphoma.[7]

The immunohistochemistry report in this patient revealed T-cell Anaplastic Large Cell Lymphoma (ALCL). ALCL manifests either as a primary cutaneous manifestation (10%) or as a systemic disease (90%) with dissemination to liver, spleen, lung, or mediastinum. Bone marrow or CNS disease is rare in ALCL. In this case, site-specific manifestations of NHL occurred as presented by a rapidly enlarging lymph node. Other manifestations include cough or dyspnea with thoracic involvement; superior mediastinal syndrome; ascites, increased abdominal girth or intestinal obstruction with an abdominal mass; nasal congestion, earache, hearing loss, or tonsil enlargement with Waldeyer ring involvement; and localized bone pain.[7] The rest were absent in the patient.

The morphology of ALCL consists of large lymphoid cells with pleomorphic or multiple prominent nuclei and abundant cytoplasm. Tumor cells grow in a cohesive pattern, and there is often sinusoidal spread in the lymph nodes.[12,13] This was evident in the patient's histopathology slides. The biopsy slides that were sent to St. Jude Children's Research Hospital in Memphis, Tennessee showed that the tumor cells are positive for CD45, CD30, ALK1 (nuclear and cytoplasmic) markers. CD45 is a lymphocyte common antigen. It has been established that the expression of CD45 is essential for the activation of T cells via the T-cell receptor.[14] CD30 is a member of the tumor necrosis factor receptor superfamily. It is characteristically expressed in certain hematopoietic malignancies, including anaplastic large cell lymphoma [15]

The same diagnostic study revealed that the tumor cells are negative for CD15, CD20, PAX5. CD15 is a marker for the Reed-Sternberg cells of classic Hodgkin disease. It is negative in most non-Hodgkin's lymphomas, with the exception of some primary cutaneous Anaplastic large-cell lymphomas and other peripheral T-cell lymphomas.[16] It is strongly positive on approximately half of lymphoblastic lymphoma-leukemias, almost all mature B-cell lymphomas (except plasma cell lesions), Reed- Sternberg cells in roughly one quarter of the cases of classic Hodgkin disease and almost no T-cell lymphomas.[16] PAX5 is an immunomarker of B-cell origin and useful in the diagnosis of lymphoma. PAX5 gene, located on chromosome 9p13, expresses transcription factor PAX5 that is also known as B-cell Lineage Specific Activator Protein (BSAP), which is involved in the development of B-lymphocytes.[17]

Epstein-Barr encoding region (EBER) in situ hybridization, which was also performed, is negative. It is a useful test to detect Epstein-Barr virus (EBV) infection in various types of lymphomas, including Hodgkin where EBV infection is more common.

The results of the histopathology and immunohistochemistry studies performed all support the diagnosis of T-cell Anaplastic large-cell lymphoma.

Anaplastic large-cell lymphoma (ALCL) mainly affects lymph nodes, although extranodal sites may be involved. ALCL may be divided based on the expression of the tyrosine kinase anaplastic lymphoma kinase (ALK). Immunohistochemistry studies in this case showed ALK-positive result. The determination of ALK positivity is important because it denotes a significant favorable prognosis, with a reported 5-year overall survival rates of 79% in contrast to 46% for patients with ALK-negative ALCL. [12]

The treatment for ALCL would be based on the extent of the disease, whether it is localized or advanced. The clinical staging system proposed at the St. Jude Children's Research Hospital has been widely accepted. The goal of staging studies should

be to rapidly assess the extent of disease, to determine prognosis and to assign appropriate therapy.[19]

Based on the St. Jude Staging System for Childhood NHL, this case was classified as Stage II with the presence of two affected lymph nodes. Multiagent chemotherapy is required. Various chemotherapy regimens have been studied with similar outcomes and survival rates ranging from 70-79%. CNS prophylaxis consists of intrathecal chemotherapy. CNS disease, although rare, can be seen and is treated with intrathecal chemotherapy and cranial radiation.[7] In this case, he was diagnosed with NHL, T-cell ALCL Stage II. Systemic chemotherapy based on ALCL-BFM-NHL-90 Protocol was given to the patient, which included the following agents given in specific number of days and cycles: Vincristine, Cyclophosphamide, Doxorubicin, Methotrexate and Prednisone.

The association of Kawasaki Disease and malignancies, specifically Non-Hodgkin's Lymphoma, has been such rare case that only three (3) journals were retrieved documenting lymphoid malignancies following Kawasaki Disease. A sole journal documented concurrent Adrenal Neuroblastoma and Kawasaki Disease while another journal linked Kawasaki Disease to Mycoplasma pneumoniae and ALCL.[6,20,21,22,23] Development of Lymphoma from Kawasaki Disease is documented in an article published in the Journal of Mazandaran University of Medical Science in 2005. A 10-year-old boy who presented with signs of Kawasaki Disease, received two high doses of IVIG. However, his cervical lymph node continued to enlarge. Lymph node biopsy revealed T-cell lymphoma.[20]

A study from Taiwan confirmed autoimmunity associated with site-specific and hematological malignancies and provided clinical evidence of an association between inflammation and subsequent site-specific cancer development. In this study done in 2016, Kawasaki Disease showed the least cancer incidence rate, still concluding its rarity. These findings supported the importance of inflammation in site-specific organ system carcinogenesis. However, there are still no guidelines in medical literature with surveillance for cancer after diagnosis and treatment of Kawasaki Disease, or any other Autoimmune Disorder.[24] In the Philippines, there have been no journal reports on this rare association.

CONCLUSION/LESSONS LEARNED

This case documented the association of Kawasaki Disease and Non-Hodgkin's Lymphoma. Kawasaki Disease was clearly established but had a rare turn out with the presence of Non-Hodgkins Lymphoma. The rarity of the disease and unconventional progression of symptoms were a dilemma to the medical team. A multidisciplinary approach led to favorable outcomes for the patient. Looking forward with hope, the family continues to pray for healing and recovery, and sustenance of their child's medical treatment.

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Knowledge Gaps in the Transmission Dynamics of Chikungunya in the Davao Region

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LETTER TO EDITOR

Chikungunya (CHIK), like Dengue Fever, is characterized by abrupt onset of high fever, rashes, and polyarthralgia. The causal microorganism, Chikungunya virus (CHIKV), is a mosquito-borne Alphavirus with *Aedes aegypti* and *Aedes albopictus* as vectors.[1] CHIK is one of the important endemic arboviral diseases in the Philippines[2] and considered as a nationally notifiable disease.[3] A total of 8,920 cases were reported in the county from January 2016 to March 2018.[4,5,6]

Chikungunya virus (CHIKV) is transmitted through two cycles. The enzootic or sylvatic cycle involving forest mosquitoes, non-human primates (NHPs) and an urban epidemic cycle or the human-mosquito-human transmission.[7] Both cycles have been reported in the Philippines[8,9] and Malaysia.[10] The transmission of Chikungunya in the Philippines is episodic[11] with no new diseases in between outbreaks.

In this paper, we identify the knowledge gaps in the CHIKV transmission dynamics in the Davao Region by reviewing the available recent scientific literature and existing preliminary data.

Is there underreporting of CHIK in the Philippines?

CHIK is commonly self-limiting and rarely fatal. Around 43% to 75% of patients infected with Chikungunya experience continued morbidity, mostly persistent arthralgia.[12] Painful and debilitating, Chikungunya-induced arthralgia may persist for one week up to two years[1] and delay in the management of acute symptoms increases the risk. Thus, it is imperative to establish the true burden of disease in the country.

As a notifiable disease, health care facilities and establishments are required by law (RA 3573) to report suspected CHIK cases, based on signs and symptoms, through the respective regional Department of Health, Epidemiology and Surveillance Units.[3] Laboratory confirmation of CHIK is not routinely performed in most hospitals, [13] and blood samples for confirmatory testing have to be sent to the Virology Department of the Research Institute for Tropical Medicine. The 2018 Chikungunya Surveillance Report documented 8,155 suspected CHIK cases. Among these, only 1,804 (22%) had blood samples for confirmation. Using the standard serologic test, CHIKV IgM, only 45% were confirmed CHIK cases.[3,6] Serological analyses in humans have proven that Chikungunya infection can be mistaken for Dengue fever based on clinical presentation alone.[14]

Subclinical CHIK may be more common, as shown in a prospective cohort study in Cebu in 2012 to 2013, wherein 10.03 per 100 person-years seroconverted but did not experience clinical CHIK.[15] Hence, accurate reporting of CHIK cases is required to estimate the burden of CHIK infections in Filipinos.

What are the CHIKV reservoir species?

Recent studies in Senegal proved that monkeys are only amplification hosts of CHIKV, and that non-primatophilic mosquitoes play an important role in maintaining sylvatic CHIKV.[16] Two Philippine studies on long-tailed macaques have provided serological evidence that Philippine macaques are natural hosts of CHIKV.[8,9] A report by Inoue *et al* stated that juvenile captive-bred and adult wild macaques from Zamboanga carried CHIKV IgM (14.8%) and IgG (59.3%). A study done by Otero found that 26% of captive and wild macaques from Davao del Norte and Davao City have CHIKV IgM. This proves that both human epidemic and sylvatic transmission cycles are happening in the Philippines.

In Malaysia, CHIKV isolated from wild monkey serum through viral culture was found to be genetically related to human CHIKV that caused the 2006 Bagan Panchor and the 1998 Klang CHIK outbreaks.[10] However, there is limited Philippine data to support that there is spillover of sylvatic CHIK to humans (or vice versa), and more studies are needed to establish the role of macaques in CHIKV transmission in Southeast Asia.

Other animals that are potential CHIKV reservoirs have been studied. Anti-CHIKV antibodies were detected in the wild Norway rat *(Rattus norvegicus)*, brown lemur *(Eulemur fulvus)* and the crab-eating macaques *(Macaca fascicularis)* after the 2006 Chikungunya outbreak in the Indian Ocean Islands[17] suggesting natural exposure to CHIKV. Pigs[18], palm squirrels, bats[19], buffalos and elephants[20] were also found to harbor anti-CHIKV antibodies. However, there is still no report of successful viral isolation or CHIKV RNA detection from vertebrates other than NHPs. Thus, it is speculative that non-NHP vertebrates are dead-end hosts of CHIKV.[17]

What are the other mosquito species that can transmit CHIKV?

In between CHIK outbreaks, CHIKV is said to be maintained through the sylvatic transmissions cycle that involve mosquito species other than *Aedes aegypti* and *Aedes albopictus.* In Africa, *Ae. africanus, Ae. furcifer, Ae. leteocephalus,* and *Ae. taylori* are known sylvatic CHIKV vectors.[19] CHIKV was also detected in *Culex* spp., *Mansonia* spp., and *Anopheles* spp., but their vector competence are unestablished.[21]

Preliminary entomologic profiling in a wildlife rescue center in Davao City revealed that there are 10 species of zoophilic mosquitoes (including *Ae. atropalpus, Ae. mediovittatus, Ae. taylori*) coexisting with the medically important anthropophilic mosquitoes *Aedes aegypti, Aedes albopictus,* and *Culex quinquefasciatus.*[22] Very few studies have focused on zoophilic mosquitoes in the Philippines[23,24] and none have detected CHIKV from these types of mosquitoes. The results of the mosquito profiling are ominous, and it underlines the importance of studying reservoir and amplification hosts in the prevention and control of arboviral zoonotic diseases, like CHIK.

What other prevention and control measures should be devised?

Figure 1 shows the putative transmission dynamics of CHIKV in the Davao Region, and the proposed prevention and control strategies based on previous studies.

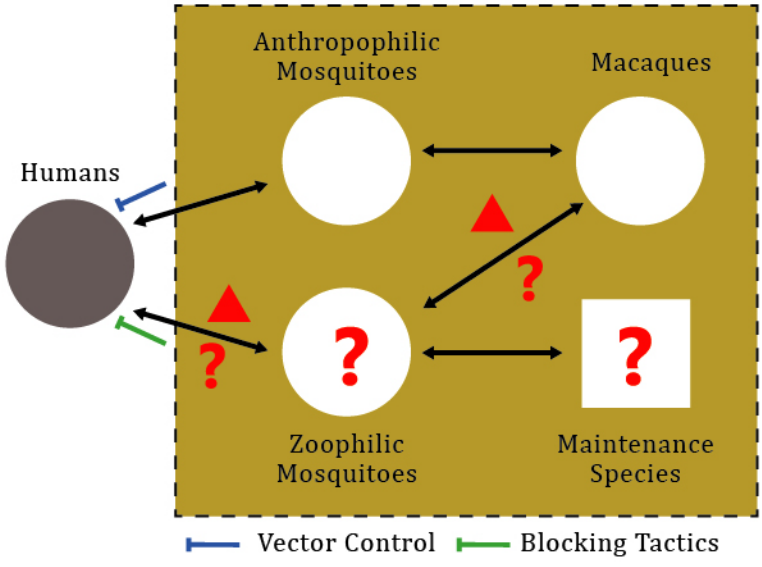

Figure 1: *Proposed transmission dynamics, prevention and control measures for Chikungunya in the Davao Region. The brown box represents the reservoir system of CHIKV. The reservoirs in circle are the non-maintenance population. The black arrows show the direction of CHIKV transmission. The red triangles represent the spillover of CHIKV to humans or macaques. The question marks denote the knowledge gaps that need to be studied.*

The framework of Haydon[25] was adopted on infection reservoirs where the target populations are the humans and the potential reservoir are all other susceptible hosts that are connected epidemiologically, whether directly or indirectly, to the target population. The maintenance population, which must be above the still-undetermined Critical Community Size for CHIKV infection to persist indefinitely, may be the Philippine macaques, or other non-human primates (NHPs). The Philippine macaque, or other non-primate animals, such as rodents, reptiles, birds, and domestic animals, may also comprise the non-maintenance population.

CHIKV from the urban epidemic can spillover to animals, in the same way that sylvatic CHIKV can spillover to humans.[1] Thus, vector control alone may not be sufficient as other animals like the Philippine macaque, can contract the infection and be possible vessels for transmission. In the absence of readily available CHIKV vaccines, strategies that block transmission, such as the use of mosquito repellants or mosquito nets when in the forest, may aide in prevention and control of CHIKV infections. Massive Information and Education Campaigns about sylvatic transmission of CHIKV targeted to high-risk persons (e.g. field biologists) can also help in raising functional knowledge about prevention of CHIKV transmission.

Conclusion

These are the knowledge gaps in the transmission dynamics of CHIKV in the Davao Region: 1) the spillover of sylvatic CHIKV from macaques to human, 2) the spillover of human CHIKV to macaques, 3) the zoophilic mosquito vectors of CHIKV, 4) the species of animals where sylvatic CHIKV is maintained. With active surveillance in humans, mosquitoes, and macaques in high CHIK incidence areas, the chances of picking up spillover events will increase. Vector control alone is not sufficient, and these knowledge gaps require a shift of perspective for public health professionals when dealing with arboviral diseases with zoonotic potential. When not addressed, these gaps can impact the implementation and success of prevention and control strategies against Chikungunya.

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AUTHOR GUIDELINES

Journal Submission

Only online submission of unpublished manuscript to Health Sciences Mindanao (HSM) are accepted through email at *healthsciencesmindanao@email.dmsf.edu.ph* with subject HSM | Paper Submission. Attachments should include the cover letter, manuscripts (with file formats Microsoft Office, Open Office or RTF document) and supporting files (text, figures, supplementary information, video, certificates).

Types of Peer-Reviewed Manuscripts

1. *Research articles* are detailed unpublished original study. The main text should have maximum of 3,000 words excluding tables, figures, and references. Maximum of 30 references are allowed.
2. *Reviews* are balanced and critical analysis of recent developments in a research field with maximum of 3,000 words excluding tables, figures, and references. Maximum of 100 references are allowed. Authors are encouraged to have short annotations of selecting key contributions.
3. *Case studies* reports real patient cases' details from medical practice with maximum of 1,200 words excluding tables, figures and references. Maximum of 20 references are allowed.
4. *Letters* are important research results that must begin with an introductory paragraph (not abstract) of approximately 150 words. The main text should have maximum of 1,200 words excluding tables, figures, and references. Maximum of 20 references are allowed.
5. *Conference summaries and announcements* should have maximum of 500 words.

PREPARATION OF MANUSCRIPT

General Guidelines

1. Manuscript has not been previously published, nor is it submitted in another journal for consideration (or an explanation has been provided in Cover Letter).
2. **Text format:** Submitted manuscript should be in A4 paper format, double-spaced, left justified, one-inch margin each side, use 12 point Arial and with line numbers.
3. **Cover letter:** It explains the impact and salient features of the article addressed to the Editor-In-Chief claiming that the study has not been previously published, nor submitted in another journal. Manuscripts submission without this will not be accepted.
4. **Content Order:** Main article file should be in this order:
    a. Title page
    b. Abstract with keywords
    c. Report Body
    d. References
    e. Tables
    f. Figures
    g. Appendices (if relevant)
5. **Style Points:** The following formats must be observed:
    a. For abbreviations, spell out meaning when first used, followed by the abbreviation in parenthesis. Henceforth, use the abbreviation only.
    b. Use only SI or SI-derived units.
    c. Numbers less than 10 are spelled out, except for measurement, age, and lists with other number.
    d. Do not use trade names.
    e. Authors must abide by the relevant standards:
        i. CONSORT for reporting randomized controlled trials,
        ii. PRISMA for systematic reviews and meta-analyses,
        iii. STROBE for observational studies,
        iv. COREQ for qualitative studies,
        v. STARD for reporting diagnostic tests,
        vi. EQUATOR Network, or
        vii. ARRIVE guidelines.

6. Ethical Consent: Manuscripts with human and/or animal studies will be accepted if study has complied with institutional policies of ethical standards for experimental studies. These documents must be attached in the Appendices. All studies involving:
    a. Humans should include an Ethics approval letter identifying the Ethics Committee that approved the protocol,
    b. Animals should include Animal Care and Use approval letter, identifying the IACUC that approved the protocol,
    c. Microorganisms or viruses should include Biosecurity approval letter identifying the Biosafety Committee that approved the protocol, and
    d. Case reports should include a consent declaration from the person/s involved with strict anonymity observed.

Title Page

The title page must contain a short running title (less than 40 characters) without abbreviations, full names (First-Middle Initial-Surname-Extensions), institutional affiliations and work contribution to the study of ALL authors. The corresponding author must be clearly indicated with email and telephone numbers.

Abstract

Abstract must be 300 words or less. Original research articles must have an extended structured abstract with background, objectives, methods, results and conclusion. After this, a list of at least five keywords useful for indexing must be included. For keywords, please refer to NLM Medical Subject Heading (MeSH), or established indexing systems (e.g. PubMed).

Report Body

The body must be organized with proper numbering as follows:

1.0 **Introduction** explains the importance of the research by describing the problem for conducting the study, current situation in the field, and study gap. Primarily, it states the research's objectives, scope, significance and hypothesis (if relevant). Authors are encouraged to avoid subheadings.

2.0 **Methodology** reveals how the study is conducted. It provides a detailed information about population, sample, materials, procedures, and data analysis. This part typically has subheadings.

3.0 **Results** presents the findings of the study. This may be presented based on research framework or objectives. Tables and figures from statistical analysis are presented in this section.

4.0 **Discussion** summarizes, evaluates and interprets the findings of the study. It revisits the related literature and studies for newfound knowledge. This may include limitations of the study if these are factors in the results that have been gathered. This also includes recommendation that discusses the limitations of the study to suggest ideas for future studies. The Conclusion and Recommendation will be given at the end of the Discussion section.

5.0 **Acknowledgements/Funding** pertains to individuals, groups, institutions or agencies that supported the research study. This must be after Conclusion or Discussion.

6.0 **Conflict of Interest Statement** must be observed for authors to identify and declare clearly any conflict to avoid any future investigations by HSM.

Subheadings should be numbered (e.g. 2.1 Research Location, 2.2. Research Instrument which are subsections of 2.0 Methodology).

References

References should follow the Vancouver format which is commonly used in medicine and science. These are its general guidelines.

1. References are cited in sequence using Arabic numerals enclosed in a square bracket right after the sentence' last word and before any punctuation mark. If two or more references are cited, it should be separated by a comma.
2. References are listed in numerical order and in the same order in which they are cited in text.
3. Only a maximum of six authors per article must be cited. If more than this, only the first three authors will be mentioned followed by "*et al.*"
4. The following are examples of listing of references.

Journal article – one author

Snowdon J. Severe depression in old age. Med Today. 2002 Dec;3(12):40-47.

Journal article – two authors

McInnes D, Bollen J. Learning on the job: metaphors of choreography and the practice of sex in sex-on-premises venues. Venereology. 2000;13(1):27-36.

Journal article – three to six authors

Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, Paul M. Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. Br Med J (Clin Res Ed). 2008 Mar 29;336(7646):701-4

Journal article – more than six authors

Hanna JN, McBride WJ, Brookes DL, et al. Hendra virus infection in veterinarian. Med J Aust. 2006 Nov 20;185(10):562-64.

Journal article – no author

New accreditation product approved for systems under the ambulatory and home care programs. Comm Perspect. 2005 May;25(5):8.

Journal article – in press

Rourke E, Hussain R, Buscombe JR, Hilson AJ. Overlying urostomy bag simulating urinary leak in a postrenal transplant MAG3 study. Clin Nucl Med. Forthcoming 2006

Journal article – electronic with DOI

Puri S, O'Brian MR. The hmuQ and hmuD genes from Bradyrhizobium japonicum encode heme-degrading enzymes. J Bacteriol [Internet]. 2006 Sep [cited 2012 Aug 2];188(18):6476-82. doi:10.1016/j.psychsport.2009.03.009

Journal article – electronic without DOI

Lemanek K. Adherence issues in the medical management of asthma. J Pediatr Psychol [Internet]. 1990 [cited 2010 Apr 22];15(4):437-58.

Online resource

U.S. Department of Health and Human Services. Managing asthma: A guide for schools. 2003

Book

Beck CAJ, Sales BD. Family mediation: Facts, myths, and future prospects. Washington, DC: American Psychological Association. 2001

Tables

The following are guidelines for table formatting:

1. The main table heading, which appears above the table, should include a table number (Arabic) and a title. Legend and explanatory notes must appear below the table.
2. Tables should be single-spaced and in 10 point Arial.
3. Tables, which are meant to supplement information in the text, must be self-explanatory.
4. Manuscript should contain no more than 3 tables.
5. Tables must be submitted as a separated file.

Figures

The following are guidelines for figure formatting:

1. Figures or graphs should be identified by Arabic Numbers with titles and explanations underneath. The numbers should correspond to the order in which the figures/graph occur in the text. It is recommended that figures/graphs also be submitted as image files (preferably as jpeg, tiff, or png files) of 300 dpi.
2. All identifying data of the subject/s or patient/s under study, such as name, case numbers, etc., particularly in case reports, should be removed.
3. Manuscript should contain no more than 3 figures.
4. Figures must be submitted as a separate file.

Appendices

An appendix contains supplementary information not essential in the report body but is helpful in further comprehensive understanding of the research. This must be submitted in a separate file. These may include the following:

1. Supporting evidence (e.g. raw data)
2. Contributory facts or specialized data
3. Sample size calculations
4. Technical figures, graphs, tables, statistics
5. Detailed description of research instruments
6. Maps, charts, photographs, drawings
7. Letters, emails and other copies of correspondence
8. Questionnaire/survey instruments
9. Complete transcripts of interviews
10. Complete field notes from observations
11. Specification or data sheets
12. Certificates

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