

# **Journal of US-China Medical Science**

Volume 11, Number 2, Apr.-Jun. 2014 (Serial Number 94)



David Publishing Company  
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**Publication Information:**

*Journal of US-China Medical Science* (ISSN 1548-6648) is published quarterly in print and online by David Publishing Company located at 240 Nagle Avenue #15C, New York, NY 10034, USA

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# Journal of US-China Medical Science

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# ***In vitro* Study of Apically Extruded Debris and Irrigant Following the Use of Conventional and Rotary Instrumentation Techniques**

Elka Radeva and Vassileva Radosveta

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**Abstract:** The complete preparation of the root canal space is one of the most important stages in endodontic treatment. While the root canal space is being prepared, debris, irrigant, necrotic tissue or bacteria may be extruded into the periapical region, leading to periapical inflammation and postoperative flare-ups. The aim of this study is to compare *in vitro* the amount of debris and irrigant extruded apically after a root canal preparation, using two instrumentation techniques—the conventional hand “step back” technique with SS K-files and the engine-driven “crown down” technique with Ni-Ti K3 files. Two groups of 20 extracted teeth with single canals were used. In group 1 (10 teeth), the root canals were instrumented using a “step back” technique and stainless steel K-files. In group 2 (10 teeth), the root canals were instrumented using a “crown-down” technique and nickel-titanium K3 rotary instruments. Debris and irrigant extruded from the apical foramen during instrumentation were collected into vials and the amounts were measured. The debris extruded through the apical foramen in group 1 (K-files) was 0.400 mg and in group 2 (K3 files)—0.225 mg. The volume of the extruded irrigant was 0.443 mL in group 1 and 0.247 mL in group 2. The time taken for instrumentation was 13 min for the step back technique and 8.7 min for the crown-down technique. There is a significant difference in the amount of debris and irrigant produced between the two groups. During biomechanical preparation of the root canal space, debris and irrigant were extruded through the apical foramen by both instrumentation techniques.

**Key words:** Apical extrusion, step back technique, crown down technique.

## **1. Introduction**

Apical extrusion of debris and irrigant during the cleaning and shaping of the root canal is one of the common problems encountered by the endodontist. The debris, which contains necrotic pulp and bacteria, initiate post-instrumentation inflammatory processes in the periapical region. Thus, it is necessary to look for a proper way to decrease the extrusion of debris via the periapical area in order to reduce post-treatment problems.

Various studies have attempted to quantify the amount of debris, irrigant and bacteria extruded beyond the apical foramen [1-5].

Moreover, many authors' researches support the

statement that applying all techniques leads to production of debris extrusion into the periapical area, but to varying degree [6-9].

The aim of this study is to establish and compare *in vitro* the amount of debris and irrigant extruded apically, using two root canal instrumentation techniques—the conventional “step back” technique with SS K-files and the engine-driven “crown down” technique with Ni-Ti K3 files.

## **2. Materials and Methods**

Twenty extracted teeth with single canals were used. All teeth had similar root curvatures of 0-10 degrees. The external surface of all teeth was cleaned with a periodontal curette. All teeth were controlled with digital radiographs in buccal and proximal directions to check for a single canal and one apical foramen. The

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teeth were stored in physiological saline solution.

The teeth crowns were cut at the cement-enamel junction and their length were standardised to 15 mm. The working length was determined by means of electronic apex-locating device Raypex-5. All teeth were instrumented by a single practitioner.

The teeth were divided into two experimental groups of 10 teeth.

Group 1: Step back hand conventional technique (stainless steel K-files-Dentsply Maillefer, Switzerland)

The root canals in this group were instrumented using the “step back” technique to a size 35 file to the apical construction and a size 60 in coronal part of the teeth. Each file was used in push and pull motion until it was loose in the canal before the next size file was used. The apical preparation was enlarged to size 35 and then the larger files were used 1 mm shorter than the previous file. The file used to prepare the apical stop was applied to recapitulate. Distilled water was used as irrigant solution. Each canal was irrigated with 10 mL distilled water in total applying 27G 3/4 (0.4 × 19 mm) needle.

Group 2: Crown down engine-driven technique (nickel-titanium K3 rotary instruments, Sybron Endo, USA)

The root canals in this group were instrumented using “crown down” technique and nickel-titanium K3 rotary instruments: Taper 0.08—in the coronal part; Taper 0.06/25—1/2 of working length; Taper 0.06/20—between 1/2 and 2/3 of working length; Taper 0.04/20, 0.04/25, 0.04/30, 0.04/35—full working length.

After each applied instrument the root canal was irrigated with distilled water. Each canal was irrigated with 10 mL distilled water in total applying 27G 3/4 (0.4 × 19 mm) needle.

Control group—five vials of distilled water were used as a control measure. They were dried in the same way and weighed.

Time taken for each instrumentation technique was

also determined.

Debris and irrigant extruded from the apical foramen during instrumentation were collected into vials (using the Myers and Montgomery technique) and the amounts were measured (Fig. 1).

The experimental model consisted of two glass vials, one of them larger, the other smaller (the smaller one was inside the larger one—but they were separate vials). The large vial was closed with a rubber plug, which was pierced, the opening corresponding to the root diameter of the tooth. The root of the tooth was placed in this opening and the surface was sealed with plastic.

During the processing of the root canals irrigant, debris passed through the apical foramen are collected in a small glass vial. This vial contained distilled water, and the apical part of the root was immersed in it. All vials were numbered and measured by the quantity of distilled water in them prior to the root canal processing.

Immediately after the canal instrumentation, the smaller vials were removed from the larger ones and measured. Then they were placed in an electric oven at a temperature of 50-60 °C for 12-24 h until the liquid had evaporated. All the tubes with vaporized liquid were placed in a desiccator containing CaCl<sub>2</sub> in order to prevent absorption and measured three times with an analytical balance. The average of the measured values has been taken into consideration for the final result.



**Fig. 1** Experimental model for collecting debris and irrigant during root canal preparation.

The measurements were done with an analytical balance with an accuracy of five decimal places.

The data was input and processed using the statistical software package SPSS 17.0.1. The level of significance for rejecting the null hypothesis was fixed at  $P < 0.05$ .

The following methods were applied:

- (1) Analysis of variance—for calculating the estimates for the central tendency and dispersion;
- (2) Graphical analysis—for visualizing the obtained results;
- (3) Shapiro-Wilk test—for checking the normality of distribution;
- (4) Student’s parametric test—for checking hypotheses of difference between two independent samples;
- (5) Mann-Whitney nonparametric test—for checking hypotheses of difference between two independent samples.

### 3. Results

The data regarding the volume of irrigant and weight of debris extruded are presented in Table 1 and Figs. 2-4, respectively.

The conventional “step back” technique showed a greater extrusion of debris and irrigant than cleaning and shaping by the engine-driven technique with K3 Ni-Ti files.

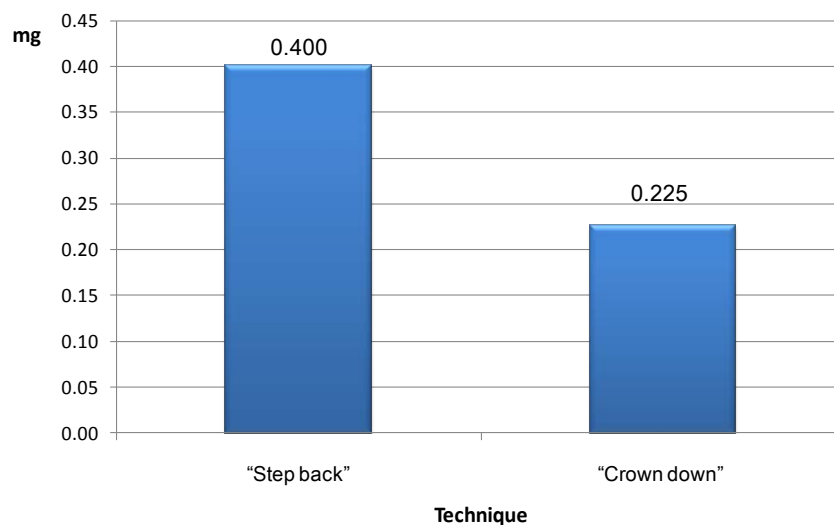
The data revealed there showed a significant difference between the average of extruded debris and irrigant in hand and rotary techniques.

The amount of debris extruded through apical foramen by “step back” technique with stainless steel K-files was 0.400 mg. The volume of extruded irrigant was 0.433 mL.

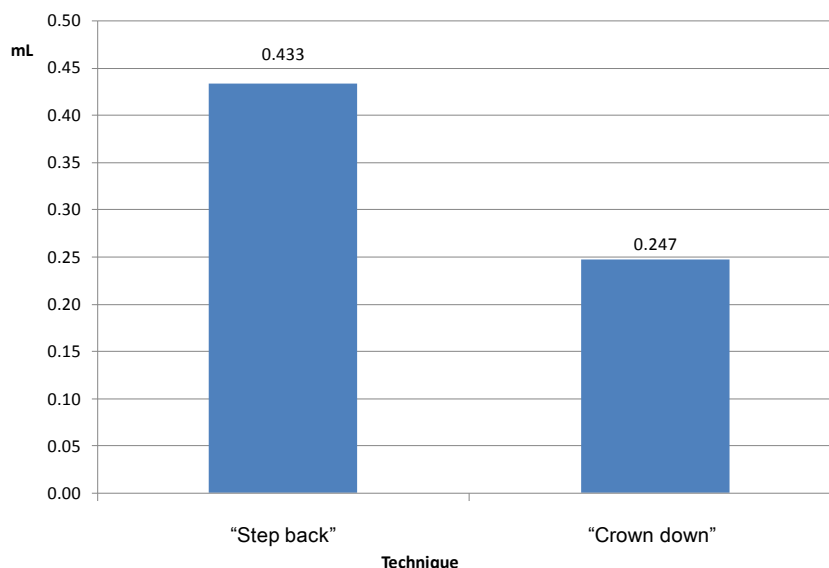
The amount of debris extruded through apical foramen by “crown down” technique with nickel-titanium K3 was 0.225 mg. The volume of extruded irrigant was 0.247 mL.

**Table 1 The weight of dry debris and irrigant extruded apically during cleaning and shaping by means of each technique.**

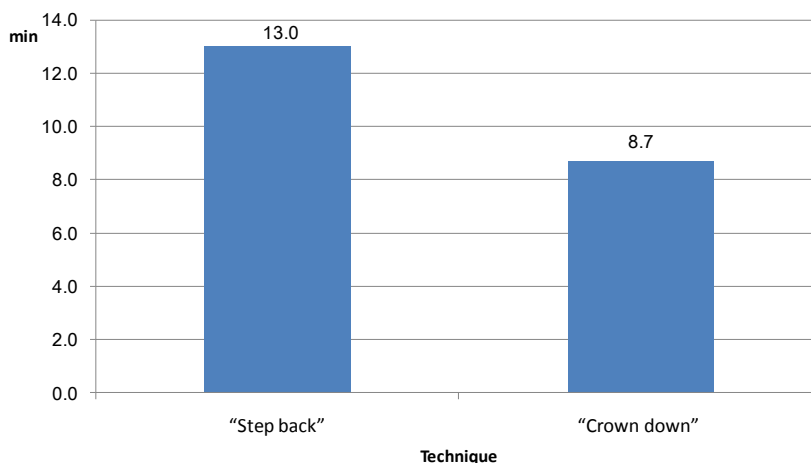
Index	Technique				P
	“Step back” (n = 10)		“Crown down” (n = 10)		
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	
Weight of dry debris (mg)	0.400	0.193	0.225	0.079	0.021
Weight of extruded irrigant (mL)	0.433	0.154	0.247	0.098	0.005
Time taken for instrumentation (min)	13.000	0.816	8.700	0.675	< 0.001



**Fig. 2 Average extruded debris in “step back” and “crown down” techniques.**



**Fig. 3** Average extruded irrigant in “step back” and “crown down” techniques.



**Fig. 4** Average time taken in the two techniques of canal instrumentation.

The results show that both techniques have produced extruded debris and irrigant through apical foramen. (Table 1 and Figs. 2-4). Significant differences can be observed in the amount of debris and irrigant produced between two groups.

The processing time for a root canal engine driven techniques is significantly less (8.7 min during treatment with Ni-Ti K3-file) in comparison with conventional methods (13 min during step back hand technique).

## 5. Discussion

The main objective of the present study was to assess

the apical extrusion of the debris and irrigant as a result of canal shaping by means of different preparation techniques and instruments.

The results presented here are consistent with those of other investigators and confirm the conclusion that linear filing motion extrudes more debris and irrigant. Some previous studies have shown that the initial coronal flaring and crown down techniques produce less apical extrusion [8-13]. Bidar et al. [10] confirmed that using a rotary technique is beneficial in decreasing the amount of debris extrusion to the periapical area, since, in the case of the rotary technique, debris is blocked in file flutes and moves to the coronal portion.



In group 1, where a step back technique is applied, the probable reason for more apical extrusion of debris is that the file acting in the apical one third as a piston which tends to push the debris through the foramen and less space is available to flush it out coronally.

In group 2, a crown-down technique is used by the K3 files. Despite being a 0.04 taper instrument, the extrusion of debris is due to its typical instrument design.

The rotation during instrumentation tended to pack the dentinal debris into the flutes of the files and directed them toward the orifice.

Kustarci et al. [2] compared three engine-driven techniques (RaCe, K3, FlexMaster) with each other and with the conventional step back technique. The obtained results have shown that the amount of extruded material in the step back technique is the largest and that it is the least when Ni-Ti RaCe rotary instruments are applied.

The application of engine-driven instruments and crown down technique for root canals leads to overcoming the disadvantages of the step back technique to a significant degree; namely an increased possibility of extrusion of the canal contents into the periapical area, inability to complete irrigation—solutions cannot reach the apical part—high probability of formation of ledges and plugs and a reduction of the working length [14].

The engine-driven technique has been shown to prepare the root canal more rapidly than applying hand technique. In *in vitro* studies, the tooth is suspended in air or vacuum, but *in vivo* it is surrounded by periapical tissues. One of the aims of the canal preparation should be to minimize the apical extrusion in order to prevent unwanted pain and inflammation. Therefore, it is logical to use a technique which minimizes this occurrence.

## 6. Conclusions

Measurable apical extrusion of debris and irrigant is produced during biomechanical preparation of the root

canal space by conventional and engine-driven techniques.

A significant amount of extruded debris and irrigant is produced by the conventional step back technique.

A significant reduction in the amount of extruded debris and irrigant is obtained in the case of the crown down technique.

The engine-driven technique has been shown to be more rapid for preparing the root canal than the hand technique.

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# Minimally Invasive Approach to Visceral Microsurgical Autoflaps

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**Abstract:** Organ-preserving and function-sparing treatment, which provides reduction of the duration of treatment and rehabilitation periods, is a promising direction in clinical oncology. Such approach requires application of the most modern, effective and high-tech method of reconstructive and plastic surgery, which is the microsurgical autotransplantation of tissues and organs. The method of minimally invasive abdominal approach to visceral autoflaps in cancer patients was developed. Thirty one male and twenty two female patients with malignant tumors of craniofacial (27) and oropharyngeal (26) localization underwent reconstructive surgery after tumor resection. Visceral autoflaps were recovered using minilaparotomy. The minilaparotomy was successful in 50 cases. Twenty six omental flaps, seven gastro-omental flaps, fifteen colonic-omental flaps and five small intestinal flaps were formed and prepared for transplantation. No intra- or postoperative complications associated with abdominal organs were observed. The use of minimally invasive techniques to form visceral autotransplants for reconstruction of head and neck organs, provides significant reduction of surgical trauma and shortening of treatment duration. This method should be indicated for impaired cancer patients or young patients willing to avoid an additional scar in the donor area.

**Key words:** Head and neck cancer, head and neck reconstruction, visceral flaps, microsurgery, autoflaps, video assistance, minimally invasive abdominal approach.

## 1. Introduction

Surgery for tumors of digestive tract, head, neck, etc., may be very traumatic and disabling for a patient, resulting in isolation from family, social networks and participation in everyday life [1, 2]. The possibility of having oro-, pharyngo-, esophago- and tracheostomas, salivation, tube feeding, etc., causes irreparable psychological damage to patient's personality, and in some cases, forces to refuse the required radical treatment [3].

Currently, one of the promising directions of oncology is performing reconstructive interventions on the stages of combined and/or complex treatment of patients with malignant tumors. Regarding this, the time of treatment is considerably reduced and the

quality of patient's rehabilitation is improved, and additionally the degree of disability in the patients who underwent surgery, is also decreased.

Obviously, the most promising direction in clinical oncology is organ-preserving and function-sparing treatment, which provides reduction of the treatment duration and rehabilitation periods. Such kind of approach requires application of the most modern, effective and high-tech method of reconstructive and plastic surgery, which is the microsurgical autotransplantation of tissues and organs [4].

Another promising direction in modern oncology is minimally invasive diagnostics and surgery of tumors of different localization and morphological structure. The method of minimally invasive abdominal approach for formation of visceral autoflaps in cancer patients was developed in the P.A. Herten Moscow Cancer Research Institute.

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**Corresponding author:** Igor Reshetov, M.D., professor, research fields: oncology, head and neck tumors, reconstructive surgery, microsurgery and tissue engineering. E-mail: reshetoviv@mail.ru.

## 2. Materials and Methods

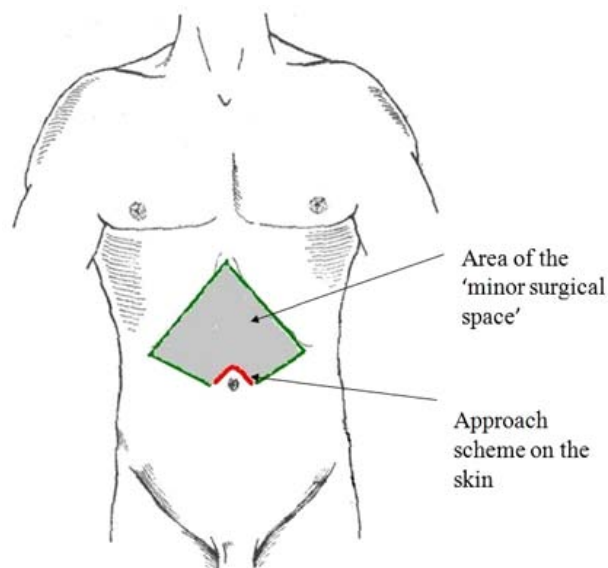
The presented report is based on the clinical experience of treatment of 53 cancer patients in the age from 16 to 63 years (Table 1). Thirty one male and twenty two female patients (Table 1) had malignant tumors of craniofacial (27) and oropharyngeal (26) localization. Seventeen patients had tumors of the scalp, 5—tumors of the maxillary area, 5—tumors of the ethmoidal labyrinth, 4—cancer of the oral cavity floor, 5—tongue cancer, 3—oropharyngeal cancer, 8—laryngopharyngeal cancer, 1—soft tissue sarcoma of the face, 1—sarcoma of mandible, and 4—parotid salivary gland cancer.

The chosen periomphalic 2/3 circle incision, provided an adequate mono-approach to the abdominal cavity, and was associated with minimal external trauma of the anterior abdominal wall (Fig. 1).

Then the dissection of the aponeurosis in the midline, laparotomy and revision of the abdominal cavity was performed with the video-assistant support (video

**Table 1** Distribution of patients by age and sex.

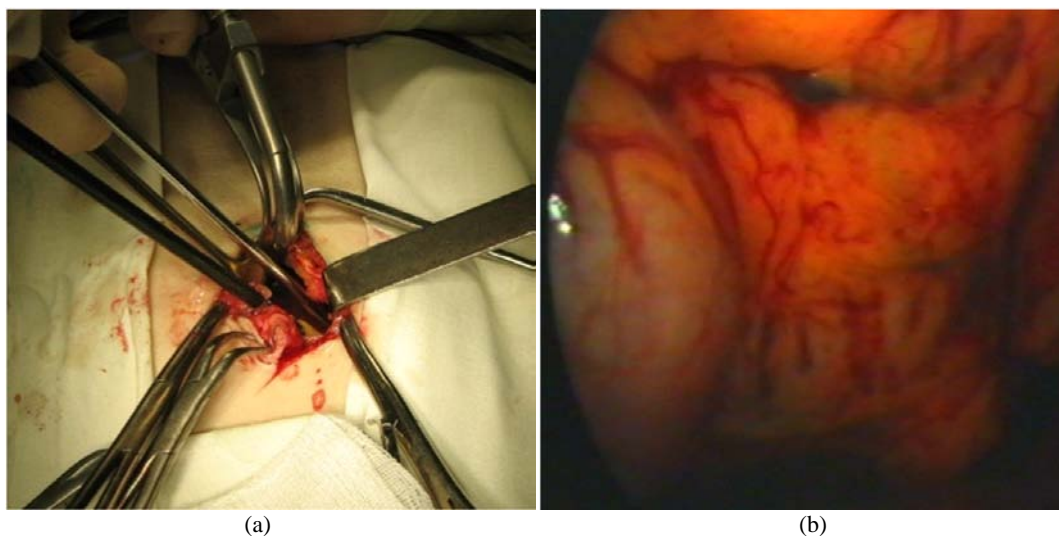
Age	Sex	
	Male	Female
< 40	18	11
40-60	13	9
> 60	0	2
Total	31	22



**Fig. 1** Marking of the periomphalic approach.

endoscopic rank and tools) (Fig. 2). It was essential to abandon gas-laparoscopy in order to create the “minor surgical space”, using retractors with illumination. This permits to carry out the abdominal revision and accurate mobilization of the potential autoflap through the minimal mono-approach.

Further, the dissection of the adhesions in the abdominal cavity was performed, if necessary. Donor organs (omentum, greater curvature of the stomach, transverse colon, and small intestine) were recovered through the minilaparotomy opening on the anterior abdominal wall. The mobilization of the vascular pedicle



**Fig. 2** Laparotomy and revision of the abdominal cavity. (a) Minilaparotomy; (b) Abdominal revision with the assistance of endoscopic equipment.

(right gastroepiploic vessels, medial colonic vessels, and jejunal vessels) and forming of the visceral graft were performed afterwards. After forming and dissection of the graft, organ anastomoses were made extracorporeally.

Unlike the method of routine resections of abdominal organs, an important feature of the autoflaps' forming is the requirement of adequate perfusion of transplanted tissue flap, which has an artery and a vein within its vascular pedicle, while preserving blood circulation and function of the donor organs.

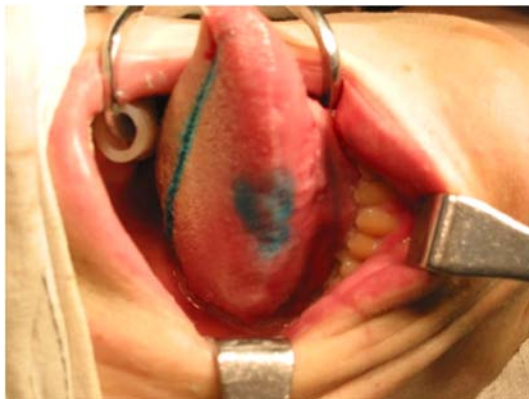
Postoperative care of patients with transplanted visceral flaps was similar to cases with casual abdominal surgery.

Case report (Fig. 3):

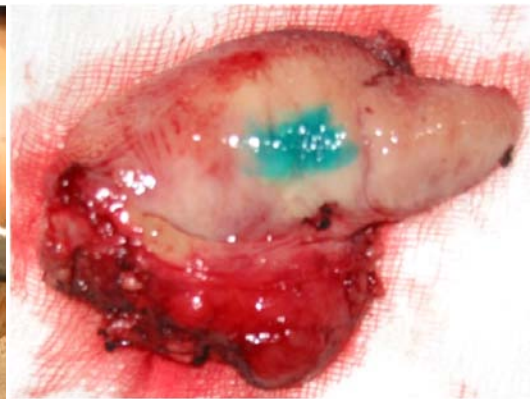
Patient X., 29 years old, case record number OA-3600 with diagnosis: stage II (T2N0M0) tongue cancer, condition after combined treatment in 2004. Histology study #918/04 showed invasive highly

differentiated squamous cell cancer.

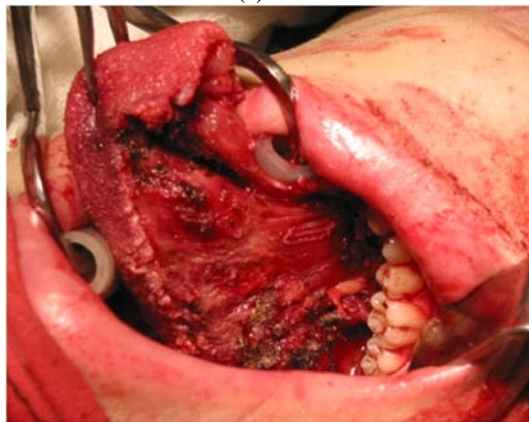
Anamnesis: The disease started in Feb. 2004, when the patient first started to experience pain and ulceration in the right part of the tongue. The patient attended the P.A. Herten Moscow Cancer Research Institute. Ulcerated tumor of 2 cm in diameter in the middle third of the lateral part of the tongue was revealed on the examination. Histology study #918/04 showed invasive highly differentiated squamous cell cancer. Regional lymphatic nodes were not involved. No data confirming regional or distant metastases were found during further complex examination. This case was discussed at the consultation with surgeons, radiologists and chemotherapists. Based on localization, histological structure and extent of tumor, the combined treatment plan was approved, with chemo radiotherapy to be applied during the 1st stage of the treatment. From Aug. 17, 2004 to Sep. 28, 2004 the patient underwent hospital treatment in the Radiation



(a)



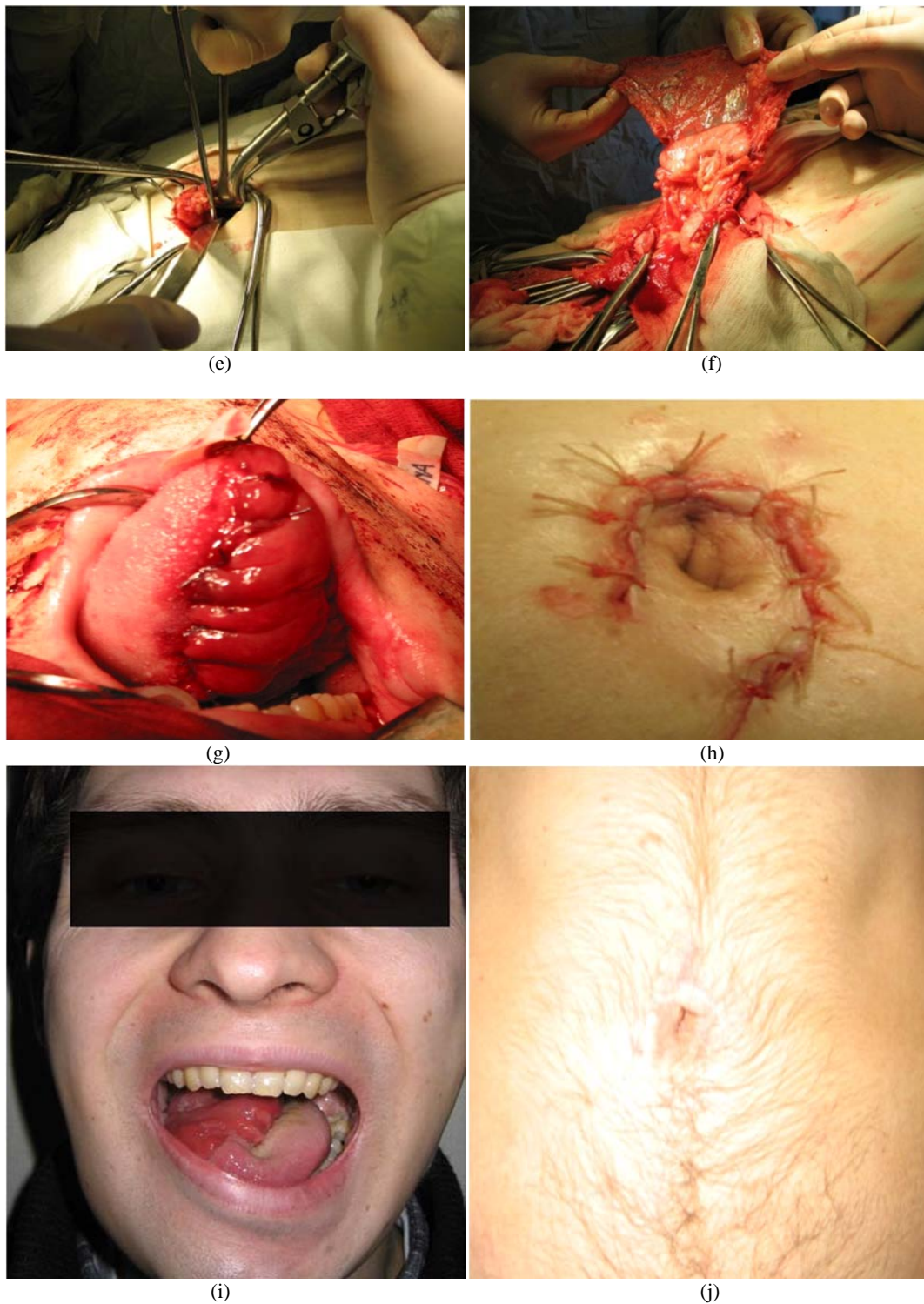
(b)



(c)



(d)



**Fig. 3** Clinical example of microsurgical tongue plastic with colonic-omental flap, performed through a minilaparatomic approach. (a) Tongue resection borders; (b) Removed specimen; (c) Tongue after resection; (d) Marking of the periomphalic incision; (e) Revision of the abdominal cavity through a mini-laparatomic approach; (f) Extracorporeal forming of colonic-omental flap; (g) Colonic part of the flap is fixed in a position of resected right half of the tongue; (h) Final view of a minilaparatomic wound; (i) View of colonic mucosa in the oral cavity 6 months after surgery; (j) Scar on the anterior abdominal wall 6 months after surgery.

therapy department, where he received with 5-FU and platinum, with the total boost dose of 22 Gy (finished on Sep. 3, 2004). Treatment was terminated at this dose because of severe radiation reactions. After reactions healing, at the 2nd stage of the combined treatment, a right hemiglossectomy with submandibular lymph node dissection on the right, microsurgical reconstruction using colono-omentalis free flap and tracheostomy were performed on Sep. 29, 2004.

Lower tracheostomy was performed on the IV semiring level under local anesthesia. The Portex No. 8 tracheostomy tube was inserted. Inhalation anesthesia with artificial lung ventilation was started. Through a transoral approach, resection of the right half of the tongue and mucosa of the floor of the mouth was performed using electrosurgical equipment. Skin incision was performed in right submandibular area. Submandibular salivary gland with surrounding fat tissue was mobilized and removed. Right facial artery and vein were mobilized and prepared for further anastomosis. After that, a periomphalic incision was made. Minilaparotomy was performed under video-assisted control by means of endoscopic equipment. Examination of abdominal cavity revealed no pathologic changes. The transverse colon and greater omentum were exteriorized through the minilaparotomy opening on the anterior abdominal wall. Visceral peritoneum was dissected over the site of middle colonic vessels. Vascular pedicle of the flap, containing middle colonic arteria and vein, was mobilized. Vascular clip was placed on the pedicle. After making sure that the collateral circulation in the transverse colon is adequate, the clip was removed. Considering the location of the ramus of collaterals going to the greater omentum from colonic vessels, 10 cm part of the transverse colon was cut using automatic suturing device, additionally a middle part of the greater omentum was included in the flap. After cutting the supplying vessels of the flap, the flap was placed on the facial defect. The vascular pedicle and the omental part of the flap were introduced through the

transmuscular tunnel in the floor of mouth to submandibular area, where recipient vessels are located. The flap was revascularized by creating the end-to-end type anastomosis between the middle colic artery and vein and the right facial artery and vein, under optical magnification. After restarting blood circulation, the viability of the flap was restored. The colonic part of the flap was dissected along the antimesenteric margin and fixed around the borders of the tongue defect and floor of the oral cavity on the right. A vascular anastomosis zone in submandibular area was covered with the omental part of the flap.

The end-to-end type anastomosis with two rows of sutures between parts of the transversal colon was created simultaneously. The abdominal cavity is drained and sutured tightly.

No complications were observed during the postoperative period. Wounds healed by primary intention. The flap is alive, adapted, without any signs of circulation disturbances. In the early postoperative period, patient was fed via naso-gastral feeding tube. Per oral feeding was started on day 14. Tracheal cannula was removed on day 16. The patient was discharged from the hospital in a satisfactory condition. No signs of recurrence were found during control examination after 6 years. The patient is eating by mouth to the full extent, has no dyspeptic syndrome and gained body weight. Currently, the patient is under dynamic monitoring.

### 3. Results

In 50 cases, the minilaparotomy was successful (3 of these patients had a history of abdominal surgery). Twenty six omental flaps, seven gastro-omental flaps, fifteen colonic-omental flaps and five small intestinal flaps were formed and prepared for transplantation.

In three patients, standard upper-medial laparotomy was performed after revision of abdominal cavity organs. The reason for an extension of the surgical approach was the presence of severe peritoneal adhesions revealed during an attempt to form 1 gastro-

**Table 2 Type of the transplant and different complications.**

Autotransplant	Total	Donor wound	Recipient wound
Omental	26	-	Flap necrosis—1 (0.2%) Flap margin necrosis—1 (0.2%)
Gasto-omental	7	-	-
Colono-omental	15	-	Marginal necrosis of the omental part of the flap—1 (0.2%)
Small intestinal	5	-	-
Total	53	0	3 (0.1%)

omental flap, 1 omental flap and 1 colono-omental flap. The surgery was also successful in these cases, as the flaps were formed and transplanted on recipient wounds.

No intra- or postoperative complications associated with abdominal organs were observed. Three patients in the postoperative period were observed in the transplanted ischemic flap. One patient developed complete necrosis of the flap, two segmental necrosis (Table 2).

#### 4. Discussion

Our own experience in creating visceral autotransplants using video-assisted equipment for their subsequent microsurgical revascularization with the purpose of reconstruction of organs and tissues, shows that the method can be successfully used in clinical oncology. According to the case analysis (Table 1), the minimal invasive approach was mostly indicated in young patients willing to avoid an additional scar.

The same opinion we met in other publications about privileges of miniinvasive access for removal of inflamed organs of abdominal cavity as gall bladder, appendicitis etc. [5, 6].

The discussion about readaptation of microsurgically transferred tissues at recipient site is still continuing [7]. Clinical and morphological comparisons did not demonstrate any reliable signs of morphological and functional changes in the mucosa of gastric and colonic fragments after their one-year

persistence in the oral cavity or pharynx.

#### 5. Conclusions

The use of minimally invasive techniques to form visceral autotransplants for reconstruction of head and neck organs, provides significant reduction of surgical trauma and shortening of treatment duration. This method should be indicated in young patients' willing to avoid an additional scar in the donor area.

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# Efficiency of Immunological Methods in the Diagnosis of Active Tuberculosis in Children

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**Abstract:** Nowadays, no clear diagnostic criteria for tuberculosis in children exist as absolute majority of tuberculosis cases in childhood have no bacterial excretion, unlike adults where bacterial excretion is observed not rarely. This fact leads to necessity to implement immunological methods in diagnostic complex in children. Tuberculin test is a routine one which is used for diagnosis of tuberculosis. However, there are a number of factors which interfere with the diagnostic value of tuberculin test. This study helped to ascertain the superiority of DST (Diaskintest) and QFT-G (QuantiFERON®-TB Gold IT) with respect to the wealth of information they provide when compared with the TST (tuberculin screening test) in determining the activity of tuberculosis infection in children. The evaluation parameters of diagnostic value (DSS (diagnostic sensitivity), the DSC (diagnostic specificity), PPV (positive predictive value) and NPV (negative predictive value), and DE (diagnostic efficiency)) with DST, QFT and TST (tuberculin skin test) were: DSS—84.3%, DSC—59.1%, PPV—61.9%, NPV—82.7%, DE—71.7%) with QFT (DSS—78.4%, DSC—68.2%, PPV—57.7%, NPV—85.1%, DE—73.3%) were significantly higher than with TST (DSS—91.5%, DSC—15.7%, PPV—74.4%, NPV—45.8%, DE—53.6%). New immunologic methods DST and QFT-G have higher specificity and diagnostic value in comparison with TST that makes their implementation in the diagnosis of TB in children essential.

**Key words:** Tuberculosis, children, Diaskintest, QuantiFERON-TB test, immunology.

## 1. Introduction

In Russian Federation, TILN (tuberculosis of intrathoracic lymph nodes) prevails in children, commonly found in 74% of all cases [1, 2]. Nowadays, no clear diagnostic criteria for tuberculosis in children exist as absolute majority of tuberculosis cases in childhood have no bacterial excretion unlike adults where bacterial excretion is observed in 36% cases of the disease [3, 4]. This fact leads to necessity to implement immunological methods in diagnostic complex in children.

Tuberculosis is diagnosed by a data set: clinical symptoms, positive TST (tuberculin skin test) and radiologic data. However, the frequent absence of the intoxication symptoms of tuberculosis in children

along with the increasing comorbidity in modern scenarios significantly impedes the assessment of the intoxication symptoms and nature of sensitivity to tuberculin, which has a numeric response in 60% of the patients with tuberculosis disease [5, 6]. Often TST gives an incorrect result, because many factors affect diagnostic value of tuberculin test. BCG (Bacillus Calmette-Guerin) vaccination, growth of allergic disorders and concomitant diseases in children lead to false positive or sometimes to false negative results of the test [7, 8]. Due to this, new immunologic methods and criteria are required for diagnosis of tuberculosis infection in children. This indicates late detection of the disease; therefore, there is an urgent need for the introduction of new immunological tests for early detection and determination of the activity of the tuberculosis infection.

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The aim of presenting research was to identify immunologic features in children with tuberculosis of intrathoracic lymph nodes.

## 2. Material and Methods

Between 2010 and 2013, a prospective study of the information by employing in a complex diagnosis of tuberculosis in children was conducted in the Department of Pediatric Phthisiopulmonology. We examined 213 children from 3 to 14 years old with a positive tuberculin test, of whom 102 (47.8%) were between 3 to 6 years old and 111 (52.2%) were between 7 to 14 years old. The patients were assessed by clinical symptoms and results of CT (computer tomography). Assessment of intrathoracic LNs (lymph nodes) in children using X-ray methods was performed in line with the recommendations, according to which the transverse dimension of the lymph nodes in children (from 3 to 14 years old) should not exceed 10 cm depending on the group and the age of the child; however, that did not exclude the presence of pathological changes, including specific changes, in the smaller nodes [9]. According to Lazoreva et al. [10], Gegeeva [11], and Dauletova [12], all LNs between 5 mm to 10 mm should be treated as manifestations of tuberculosis in intrathoracic LN, although, the standard CT imaging studies can be the limit for visualization of the LNs according to the criteria of “imaging”.

According to the results, they were split in two groups. The first group includes 70 children who were infected by MBT (*Mycobacterium tuberculosis*), but have no tuberculosis disease—it was taken in the research as a control group; the second group includes 143 children with tuberculosis of intrathoracic lymph nodes. Both groups were tested by DST using recombinant tuberculosis allergen based on MBT specific proteins: ESAT-6 (early secreted antigenic target 6 kD protein) and CFP-10 (culture filtrate protein 10), and by its nature it represents an immunologic skin-test. This test is designed in Russia and used for diagnosis of tuberculosis since 2009. An analysis of the

results of the tuberculin skin test (administration of the purified tuberculin in standard dilution (ready form)) and DST (tests with recombinant TB allergens in standard dilution) was conducted [13, 14].

In addition QuantiFERON test (“Tubiniferon” test system) has been used. Before performing these tests, a venous blood sample was drawn for QFT (QuantiFERON-TB test), which is the reference method and allows a qualitative assessment of the information contained. The QuantiFERON®-TB Gold In-Tube is a diagnostic tool designed for the diagnosis of tuberculosis *in vitro*. This method is based on using a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7 (p4) proteins to stimulate the cells in heparinized whole blood. Determination of the quantification of IFN- $\gamma$  in anda-TB (BioChimMak) as performed to identify *in vitro* the cellular response to the stimulation of these peptide antigens associated with *Mycobacterium tuberculosis* infection. Since tuberculosis in literature described as the disease with immunosuppressive pattern, the following immunologic parameters were chosen for assessment: leucocytes’ subsets identification (CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD4<sup>+</sup>/CD3<sup>+</sup>, CD8<sup>+</sup>, CD16<sup>+</sup>, CD20<sup>+</sup>, CD25<sup>+</sup>, CD95<sup>+</sup> and HLAI), induced cytokines levels (TNF- $\alpha$ , IL-2, IL-4 and INF- $\gamma$ ), and assessment of antibodies IgA, IgG and IgM (anda-tb ELISA).

Apart from these X-ray method diagnosis ((MSCT (multislice spiral computed tomography) and MSCT-AG (MSCT angiography)) and X-ray examination were done using a spiral CT scanner with a multirow detector (multislice) “Aquilion-32” (Toshiba Medical Systems Corporation, Japan).

In clinical example 1 (Fig. 1), child P.S., 5 years old, was vaccinated by BCG. Tuberculin test in 2007—p 13 mm, 2008—p 15mm, 2009—p 16mm and 2010—p 15mm. In anamnesis, she has allergy. In examination, CD16<sup>+</sup> is low, DST-negative, QFT-negative, IgG is low, and no symptoms of intoxication. In CT, in lungs and intrathoracic lymph nodes tuberculosis changes are not identified. In this



(a) Tuberculin test is positive



(b) Diaskintest® is negative

**Fig. 1** Child P.S., 5 years old.

example, high result of TST is influenced by allergy growth.

In clinical example 2 (Fig. 2), child P.M. was 3 years old. She has a contact with her father, who is ill since 2008 with lung tuberculosis MBT (+) with has MDR (multidrug resistance). BCG vaccination, preventive course of therapy in 2007 and 2008. Tuberculin test: 2009—p 6mm, 2010—p 18mm; CD16<sup>+</sup> is high, DST—p 20mm, QFT—positive, IgG is high, symptoms of intoxication. CT and CT-Ag—lymph nodes > 1.0 cm paratracheal, bifurcation, bronchopulmonal groups. In this example, child who had tuberculosis of intrathoracic lymph nodes and course of therapy has been administered.

All the data was processed employing the variation statistics methods using the software Microsoft Office Word Excel 2007, Statistica 8. The difference was considered reliable when  $P < 0.05$ . The Mann-Whitney (U Test) was used to compare the differences between

two independent groups (for nonparametric data). The mean ( $M$ ) and standard error of the mean ( $M$ ) were deduced. Pearson's Correlation Coefficient ( $r$ ) was used to determine the strength of the relationship between two continuous variables.  $P$  value less than 0.05 was considered significant. Spearman's rank correlation coefficient was also used. The diagnostic accuracy of the tests employed was analyzed as well as the method used to calculate the operating characteristics: DSS, DSC, PPV, NPV and DE.

### 3. Results and Discussion

No clinical manifestations of the intoxication syndrome were significantly higher in the I group (78.6% vs. 13.3%,  $\chi^2 = 74.9$ ;  $P < 0.001$ ) in comparison with the II group and manifestation intoxication syndrome in the II group were significantly higher in comparison with the I group (61.5% vs. 4.3%,  $\chi^2 = 53.1$ ;  $P < 0.001$ ) (Table 1).



(a) Tuberculin test—p18 mm



(b) Diaskintest®—p20 mm

**Fig. 2** Child P.M., 3 years old.

**Table 1** Clinical manifestations of the intoxication syndrome in the groups.

Groups	No intoxication syndrome (%, n)	Moderate intoxication syndrome	Manifestation intoxication syndrome
I group (n = 70)	78.6% (55)*	17.1% (12)	4.3% (3)
II group (n = 143)	13.3% (19)	25.2% (36)	61.5% (88)*

\* $P < 0.001$ .

In Fig. 3, in the II group high sensitivity to TST was observed in 42% cases, which was significantly higher in comparison with the control group (42.0% vs. 20.0%,  $\chi^2 = 9.99$ ,  $P < 0.01$ ). Results of DST and QFT tests were significantly higher in the II group compared to I group. Results of DST and results of QFT were comparable in 95% cases.

Positive DST was marked in 84.4% in the II group, which was significantly higher compared to the I group (41.5%,  $\chi^2 = 40.36$ ,  $P < 0.001$ ). At the same time, negative DST was detected significantly more frequently among healthy children in the group I (50.7% vs. 12.7%,  $\chi^2 = 52.16$ ,  $P < 0.001$ ).

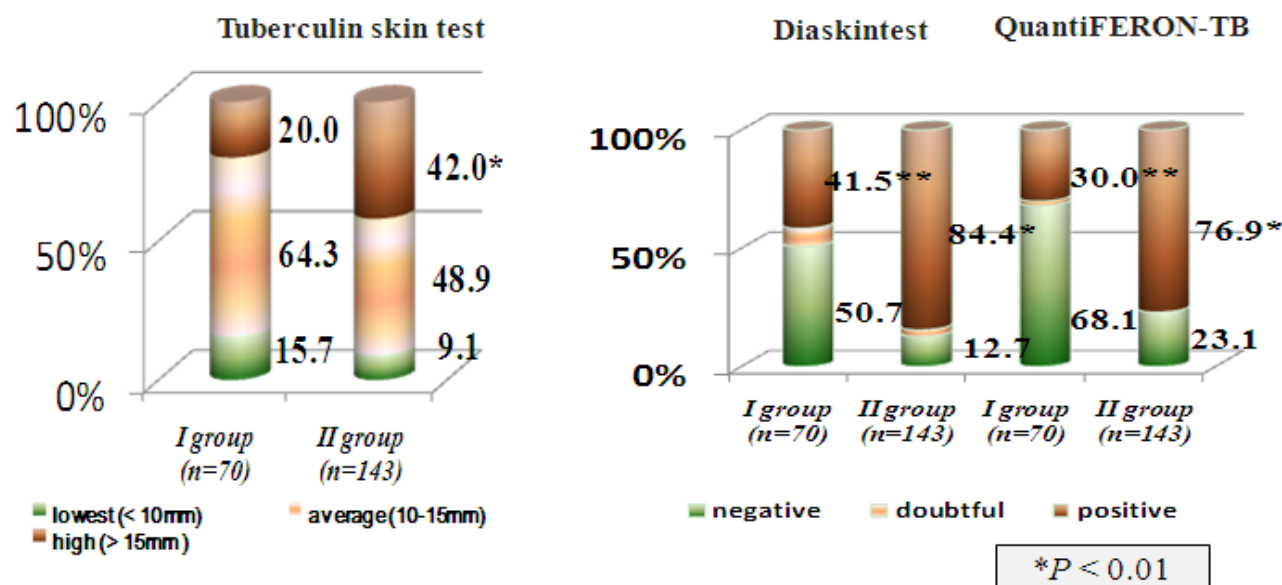
Negative QFT test result in the group I (68.1%) was significantly higher negative results in the II group (23.1%), as well positive results of QFT test have significant difference between groups: (30% in the I group) vs. (76.9% in the II group).

Analysis of leukocyte subsets being compared to normal ranges revealed significant decrease in  $CD4^+$

(57.1 vs. 37.7,  $\chi^2 = 3.99$ ,  $P < 0.05$ ) and twice lower the level of  $CD25^+$  (11.9 vs. 26.4,  $\chi^2 = 4.68$ ,  $P < 0.05$ ) in children infected by MBT may be considered as a sign of immunosuppression (Fig. 4). In the II group (in children with tuberculosis of intrathoracic lymph nodes), the increased level of  $CD4^+$  (14.2 vs. 4.8,  $\chi^2 = 38.74$ ,  $P < 0.01$ ) and  $CD16^+$  (58.5 vs. 2.4,  $\chi^2 = 38.74$ ,  $P < 0.001$ ) may be assumed as a sign of activation. In both groups, elevation of  $CD25^+$  and  $CD95^+$  levels were observed with the same frequency of occurrence.

No significant differences were identified between groups in the levels of induced cytokines, nevertheless the tendency for elevation of  $TNF-\alpha$  and IL-4 in children with TB of intrathoracic lymph nodes was marked (II group) (Fig. 5).

Comparison of the levels of specific immunoglobulins between groups revealed significant elevation of IgM (45.8% vs. 25.6%,  $\chi^2 = 4.07$ ,  $P < 0.05$ ) and a tendency with high level of IgG in children with TB of intrathoracic lymph nodes (Fig. 6).

**Fig. 3** Results of TST, DST and QFT.

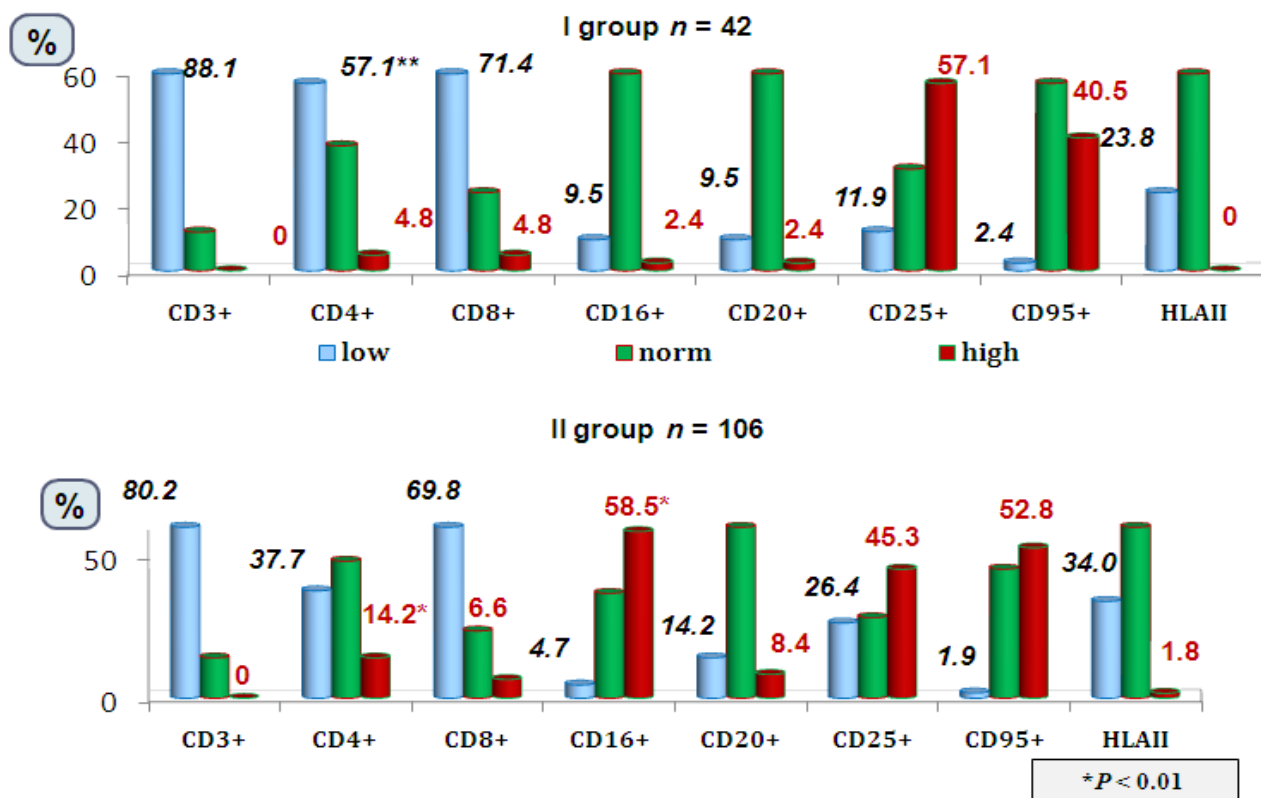


Fig. 4 Leucocytes' subsets in the groups.

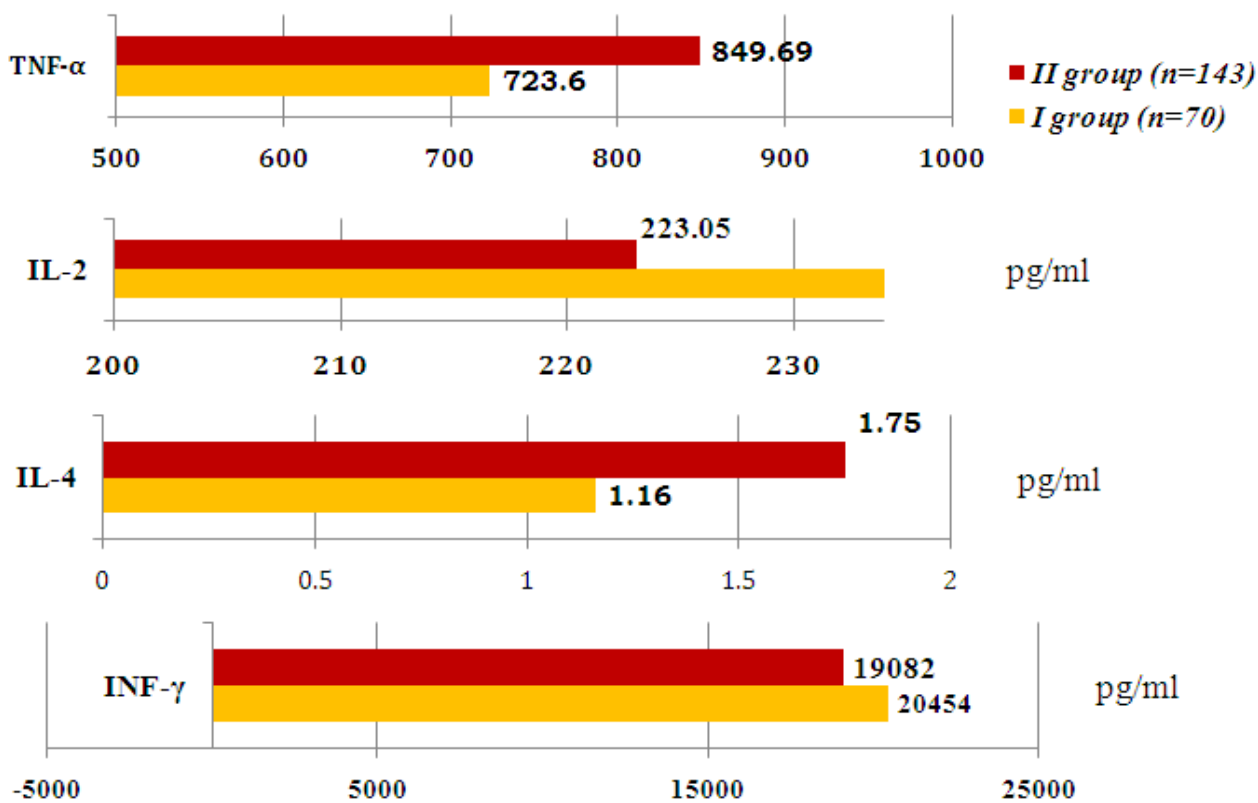
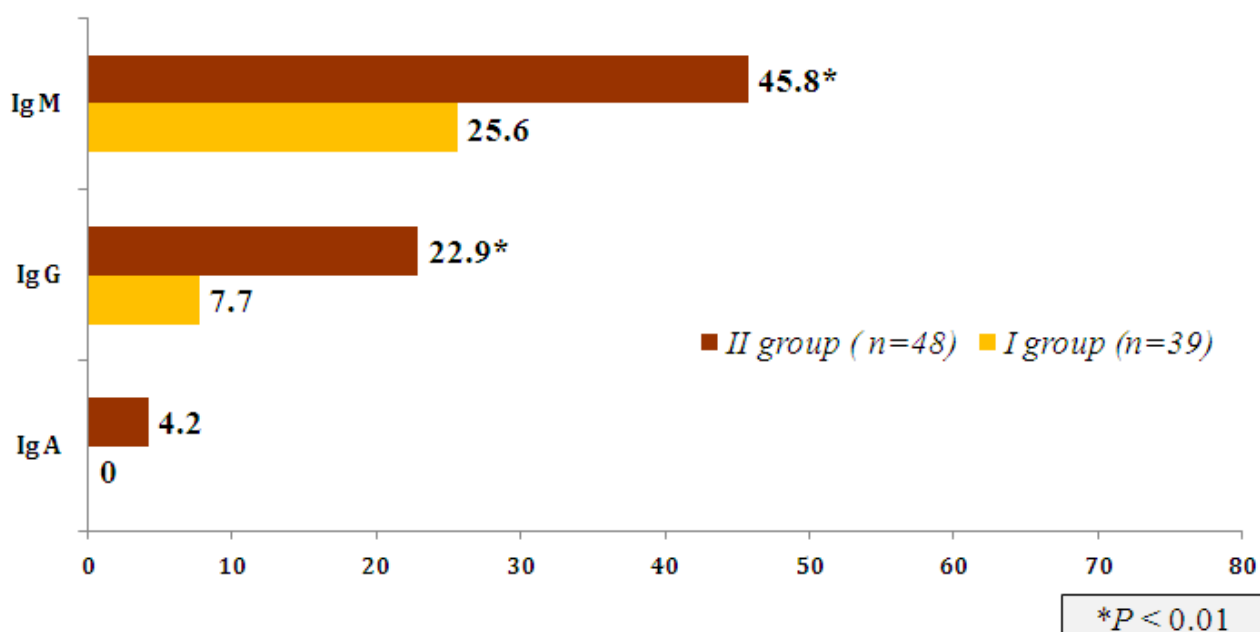


Fig. 5 Levels of cytokine-induced.



**Fig. 6** Levels of specific antibodies in immunological reaction.

Collected in the research data allowed us to figure out diagnostic values of the most relevant immunologic methods. The calculated data on the diagnostic value of TTS (DSS—91.5%, DSC—15.7%, PPV—74.4%, NPV—45.8%, and DE—53.6%) gives evidence of its low degree of information and the pressing need for the introduction of new methods to determine tuberculosis activity. New immunologic methods have higher specificity and diagnostic value in comparison with TT that makes their implementation in the diagnosis of TB in children essential. The data of the diagnostic value of DST (DSS—84.3%, DSC—59.1%, PPV—61.9%, NPV—82.7%, DE—71.7%) and the QFT (DSS—78.4%, DSC—68.2%, PPV—57.7%, NPV—85.1%, DE—73.3%) do not exhibit significant differences among themselves. However, the degree of information provided by DST is twice as high as the data from TTS, which are confirmed during QFT.

Thus, TTS does not provide sufficient information to determine the activity of tuberculosis infection in children infected with MBT, which leads to a late diagnosis of the disease and identification of the specific process involved in the phase of reverse development.

#### 4. Conclusions

In children with tuberculosis, no significant immunologic differences vs. normal ranges were identified, except elevated rate of CD16<sup>+</sup>. The most informative signs of active tuberculosis in children are positive QFT-G, DST and higher levels of specific IgM. QFT-G and DST are very informative tests in diagnosis of tuberculosis in children and these tests are comparable.

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# The Optimal Dose of *Euphorbia milii* Extracts in NKp46 Expression against Mice Infected with *Mycobacterium tuberculosis*

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**Abstract:** Objective: To compare the effect of EEM (*Euphorbia milii*) extract in various dose for NKp46 expression in mice infected with MTB (*Mycobacterium tuberculosis*). Methods: An experimental study with 24 mice Balbc were divided into 8 groups of treatment which is observed at weeks I and II. All groups were infected with *Mycobacterium tuberculosis* strain H37Rv then each successive group was given sterile distilled water (P0.1 and P0.2), extract of EEM 5 mg/20 grbw (gram body weight) (P1.1 and P1.2), 10 mg/20 grbw (P2.1 and P2.2), and 15 mg/20 grbw (P3.1 and P3.2). Then, termination in each group at weeks I (P0.1, P1.1, P2.1 and P3.1) and II (P0.2, P1.2, P2.2 and P3.2). Pulmonary organ harvesting conducted for immunohistochemistry of NKp46 expression. Result: Quantitative results of NKp46 expression from successive groups (1 to 8) are 48.33, 25.33, 58.67, 46.67, 35.67, 58.67, 61.33 and 40.67, respectively. One Way ANOVA showed significant difference between groups ( $P < 0.05$ ). Conclusions: EEM with doses of 5 mg/20 grbw and 15 mg/20 grbw are able to increase the expression of NKp46 in the second week, whereas a dose of 10 mg/20 grbw is able to increase expression of NKp46 on the first and second week. It is make an opinion if EEM with 10 mg/20 grbw is the optimal dose to increase the expression of NKp46 in mice infected with *Mycobacterium tuberculosis*.

**Key word:** Lung tuberculosis, natural killer cells, *Euphorbia milii*, NKp46.

## 1. Introduction

According to WHO data in 2010, Indonesia ranked fifth in the country with the highest tuberculosis burden in the world. The mechanism of pulmonary parenchymal tissue damage which is manifested as pulmonary tuberculosis is based on immunopathogenesis. Characteristic lesions of pulmonary tuberculosis is granulomas, due process based on the reaction of cell interactions including macrophages, lymphocytes, epithelioid cells, NK cells (natural killer cells), and neutrophils against

*Mycobacterium tuberculosis* [1, 2]. NK cells are effector in the innate immune response to patrolling against pathogens and tumor invasion. The main biological function of NK cell activity include natural cytotoxic and cytokine secretion that are directly or indirectly control infections and tumors as well as regulate the immune system. Through the dual role of NK cells in the secretion of IL-22 which activate macrophages infected with *Mycobacterium tuberculosis* and NKp46 expression which lyse macrophages infected with *Mycobacterium tuberculosis*. NKp46 is a natural cytotoxic receptors [3-5]. Active NK cells are expected to work optimally in eliminating MTB in macrophages infected either by

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increasing the activation of macrophages and lyse infected macrophages so that tuberculosis can be controlled. *Euphorbia milii* is a species of “euphorbiaceae” contains flavonoids (phenols and phenolic glycosides), saponins (terpenoids and steroids), and tannins (carbohydrat). Several studies have shown antibacterial effects, inhibitors against aspergillus, mollusidal, as well as non-teratogenic [6-8]. This study aimed to investigate the effect of EEM (*Euphorbia milii* extract) increased expression of NKp46 of the lungs of mice infected with *Mycobacterium tuberculosis*.

## 2. Material and Methods

### 2.1 Preparing Suspension of *Mycobacterium tuberculosis* Strain H37Rv

*Mycobacterium tuberculosis* strain H37Rv (ATCC27294) were grown in liquid medium Middlebrook 7H9 for 2 weeks, then stored at -70 °C and is ready to used for research. Before use bacterial suspension was thawing at 37 °C and sonification for 10 s to dissolve the clot of bacteria became homogeneous and separate one cell to another. To infected the mice we inoculated intranasally 60 µL suspension contain  $10^5$  bacteria per mL [9].

### 2.2 Preparing Ethanol Ekstrak of *Euphorbia milii* (EEM)

*Euphorbia milii* flowers are taken from Ketewel (region of Gianyar, Bali). These flowers are taken in a fresh state, colored pink with the whole look, subsequently collected and aerated to dry. Once dried, they are crushed with a blender, and then weighed 100 g. *E. milii* flower powder was added 300 mL of methanol and stirred with a magnetic stirrer for 1 h at room temperature. Then, filtered with Whatman paper No. 42 in order to obtain the filtrate 1. Obtained residue was re-extracted filtrate thus obtained 2. Filtrates 1 and 2 mixed filtrate was then evaporated with a rotary evaporator.

### 2.3 Mice Infection with *Mycobacterium tuberculosis* Strain H37Rv

Mice were infected with  $10^5$  bacteria per mL intranasally, initially created as suspension equivalent to  $10^7$  McFarland 1, then diluted until a concentration of  $10^5$  bacteria, as many as 60 µL of suspension in the nostrils of mice inoculated with a micropipette. Then, the mice were placed in the cabinet for further observations [9].

### 2.4 Immunohistochemistry NKp46 Expression in Mice Lung Tissue

Right lobe of the lung tissues were fixed in 10% formaldehyde solution in PBS for 2 days and then soaked in paraffin and cutting is done along the widest area of each lobe. This is done for all the examined lung tissue so that all the lung tissue of mice examined in the same area. Lung tissue with 5 µm thickness separated 100 µm each other then process with immunohistochemical staining technique based on rabbit anti-mouse NKp46 (Bioss, Bs-2417R) then using secondary antibody from Dako (LSAB + systems-HRP). Then see below the light microscope CX3100 with 100× magnification. Numbers of positive NKp46 cells expression were counted in 5 fields then calculated the average per 100 cells.

### 2.5 Data Analysis

Data quantity of NKp46 expression were tabulated then analyzed with SPSS 14.0 programme. The homogeneity by Lavene test with a confidence level  $\alpha = 5\%$  followed by normality test with the Kolmogorov-Smirnov, finally tested with One Way ANOVA ( $P < 0.05$ ) to analyze the average difference in the treatment group and examine the interaction between each of the effects continue with Scheffe test to know which groups differed significantly.

### 3. Results

#### 3.1 The Effect of EEM against NKp46 Expression in Lung Tissue of Mice Infected with *Mycobacterium tuberculosis*

The experiment was conducted in June 2013 at Department of Histology, Faculty of Medicine and Department of Pathology, Faculty of Veterinary, University of Udayana. Primary antibody kit of anti-mouse NKp46 from Bioss (Bs-2417R) and secondary antibody of anti-rabbit from Dako were used. Immunohistochemistry results of anti NKp46 in samples which were terminated on day 8 and 15 consist of 8 groups: P0.1 is the control 1, terminated at day 8; P1.1 is the treatment 1, with 5 mg/20 grbw EEM, terminated at day 8; P2.1 is the treatment 2, with 10/ 20 grbw EEM, terminated at day 8; P3.1 is the treatment 3, with 15 mg/20 grbw EEM, terminated at day 8; P0.2 is the control 2, terminated at day 15; Group P1.2 is treatment 4, with 5 mg/20 grbw EEM, terminated at day 15; P2.2 is the treatment 5, with 10 mg/20 grbw EEM, terminated at day 15; P3.2 is treatment 6, with 15 mg/20 grbw EEM, terminated at day 15. The example of NKp46 immunostaining in lung mice was showed in Fig. 1.

#### 3.2 Histopathological Picture of the NKp46 Expression in the Study Group

The immunohistochemistry staining of NKp46 expression in the above 8 groups are presented.

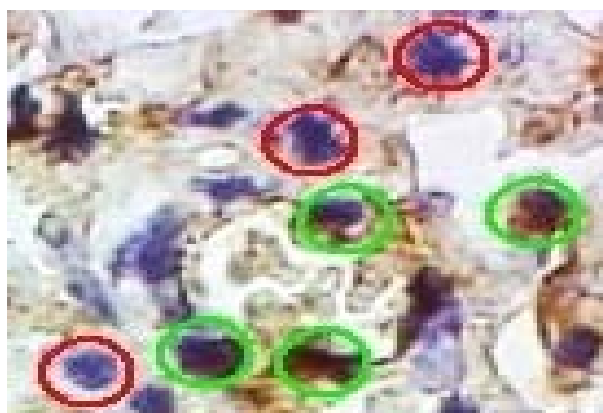
The fourth picture in Fig. 2 is the result of NKp46 immunostaining in mice lung tissue which were terminated on day 8. The fourth picture in Fig. 3 shows the result which terminated on day 15. Positive cells expressing NKp46 is the cell with the cell membrane brown with or without brown nuclei. Whereas, cells that do not express NKp46 are mononuclear cells with blue nuclei, cytoplasm and cell membrane. In Fig. 1, positive cells showed brown nuclei is marked with a green circle while negative cells showed blue nuclei which is marked with a red circle. The number of

positive cells in Figs. 2 and 3 were counted in five fields of view using Optilab camera cell count programme. Each group contained three replications. Then the mean of NKp46-positive cells per 100 cells of lung tissue were calculated.

Fig. 4 showed the mean and standard deviation of NKp46-positive cells per 100 cells of lung tissue, with its significantly different.

### 4. Discussion

Based on the results, there are different expressions of NKp46 between eight groups. In the first week, NKp46 was expressed successively from the lowest group to the groups as high as P1.1, P3.1, P0.1 and P2.1. When compared with the control group (P0.1), the expression of NKp46 on lower P1.1 group is probably due to EEM at a dose of 5 mg/20 grbw within 1 week which is not optimal for increasing the expression of NKp46. A dose of 15 mg/20grbw may cause resistance to the receptors so that the expression is not increased. However, at a dose of 10 mg/20 grbw (P2.1), the expression is increased compared to the control group although the Scheffe test showed no significant increase ( $P = 0.82$ ), which suggested the possibility of an optimal dosage for increased activity NK cells in NKp46 expression .



**Fig. 1** The example of immunohistochemistry staining in lung mice with anti-mouse NKp46 (Bioss) and secondary antibody (Dako). Green circled is positive cells and red circle is negative (CX3100 Olympus Microscope, magnification 100 $\times$ , Optilab doc, Histology Department, Medical Faculty of Udayana University).

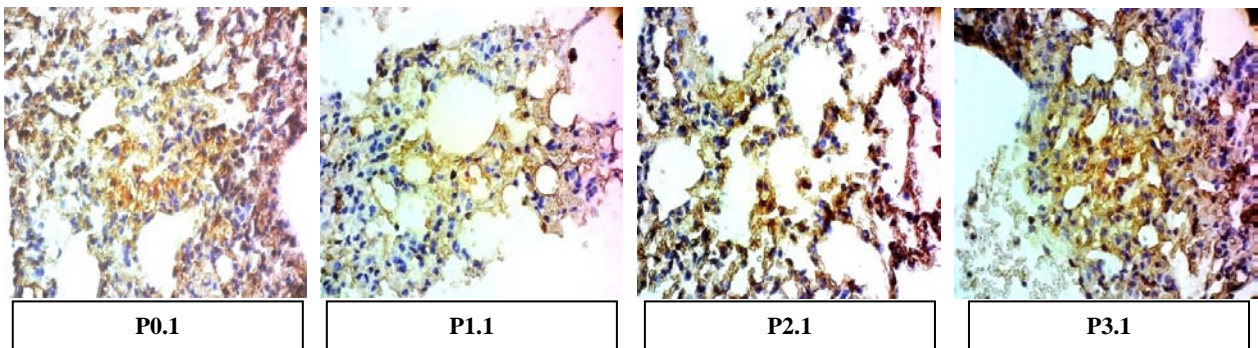


Fig. 2 The NKp46 immunostaining (Bioss) and secondary antibody (Daco) of mice lung from 4 treated group in the first week. (CX3100 Olympus Microscope, magnification 100×, Optilab doc, Histology Department, Medical Faculty of Udayana University).

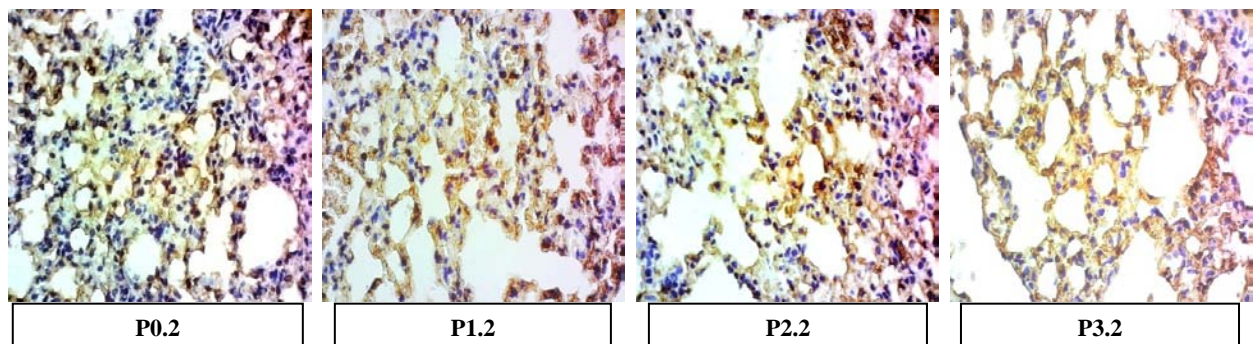


Fig. 3 The NKp46 immunostaining (Bioss) and secondary antibody (Daco) of mice lung from 4 treated group in the second week. (CX3100 Olympus Microscope, magnification 100×, Optilab doc, Histology Department, Medical Faculty of Udayana University).

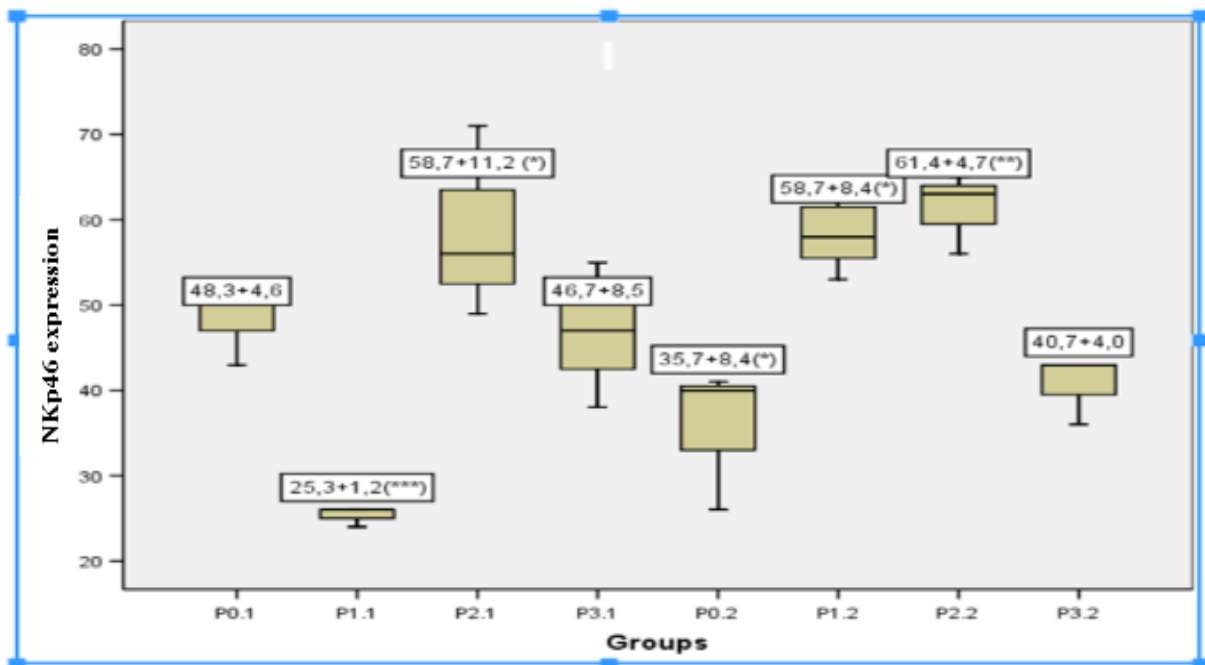


Fig. 4 The mean (with standard deviation) of NKp46 expression in each groups of study. (\*): Significant different with another group. P2.1 is significantly different with P1.1, and P1.2 is also significantly different with P1.1. (\*\*): Significant different with the other two groups. P2.2 is significantly different with P0.2 and also P1.1. (\*\*\*): Significant different with the other three groups. P1.1 is significantly different with the other three group such as P2.1, P1.2 and P2.2.

In the second week, the lowest expression of NKp46 is in the control group (P0.2), while the highest is in P2.2 (dose 10 mg/ 20 grbw). It shows EEM flower extract in different doses at 2-week administration was able to increase the expression of NKp46 compared to the control group. Overall, it shows the content of the active substance in the EEM on duration of 2 weeks were able to increase the expression of NKp46 at all doses (Fig. 4). Although when it is analyzed by Scheffe test, it showed a significant increase ( $P = 0.03$ ) only at doses 10 mg/20 grbw (P2.2) when compared with the control group (P0.2). NKp46 expression was detected in all groups, because the infection with *Mycobacterium tuberculosis* is a potent inducer for NK cells that are activated to express NKp46. It is in accordance with the experiments performed by Vankalayapati et al. [10] who found an increase in the expression of NKp46 in NK cells and macrophages co-cultures which are infected with *Mycobacterium tuberculosis*, then causing lysis of macrophages infected with *Mycobacterium tuberculosis*. In addition to infection with *Mycobacterium tuberculosis*, Culley [3] also found that NKp46 receptor plays an important role in infection control against influenza virus hemagglutinin which binds to NKp46 expressed in cells infected with influenza virus and causes lysis of the infected cells. Increased expression of NKp46 in the group with the dose of EEM 10 mg/20 grbw in both first and second week suggests the possibility of optimal dose is 10 mg/20 grbw. There was an increase in the second week of NKp46 expression at all doses when compared to the control group EEM. It is because of the active substance content in the EEM, containing a variety of active substances such as flavonoids, saponins, triterpenoids, phenols, and alkaloids.

Flavonoid is one of the compounds contained in *Euphorbia milii*. Flavonoid compounds are said to have immunomodulatory effects on several immune cells. In NK cells, it was found to have immunostimulatory effects such as IFN- $\gamma$  synthesis and inhibitory action on the cytotoxic activity. These different effects can be

caused by the concentrations or conditions of different flavonoids from natural materials [11].

Increased expression of NKp46 is an inducer for NK cells to produce IFN- $\gamma$ . Flavonoids increase the activity of NK cells through the high expression of NKp46, which can increase the secretion of IFN- $\gamma$ . The content of flavonoids in *Euphorbia milii* flowers gives the possibility to act as an immunomodulator for NK cells. Increased expression of NKp46 of NK cell would increase IFN- $\gamma$  levels many times higher after cytokine stimulated [12].

## 6. Conclusions

Ethanol extract of flowers of *Euphorbia milii* (EEM) at a dose of 5 mg/20 grbw; 10 mg/20 grbw, and 15 mg/20 grbw are able to increase the expression of NKp46 in lung tissue of mice infected with *Mycobacterium tuberculosis* in the second week. EEM at dose of 10 mg/20 grbw is an optimal dose because it increase the expression of NKp46 in lung tissue of mice infected with *Mycobacterium tuberculosis* in the first and second week.

## Acknowledgment

Thank to the Higher Education Grant Programme through Decentralization Competitive Grant 2013 for funding this research, and the research centre in Udayana University.

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# Evaluation of Immune Response in Mouse Blood Post Injection with Gamma Ray-Attenuated Sporozoites of *Plasmodium berghei*

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**Abstract:** Ionizing radiation has been successfully used to attenuate parasites for malaria vaccine development. To get a deeper insight in the immune response post injection of irradiated pre-erythrocytic stage of malaria parasites (sporozoites), we used *Plasmodium berghei* as a model. *Anopheles sp.* with infective parasites in their salivary glands was irradiated with 0-225 Gy of gamma rays. The isolated sporozoites were intravenously injected into groups of mouse followed by a booster and challenge in interval time of 2-4 weeks. The detection of parasite in the infected mosquito was done with nested-PCR (polymerase chain reaction). Immune response in mouse blood was examined with ELISA (enzyme-linked immunosorbent assay) and protein profile of irradiated sporozoites was studied with SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis). By using *Anopheles farauti* as the more susceptible mosquito to parasite infection, based on molecular detection and parasites observation in mouse blood, there was low or no effect of irradiated sporozoites on the immune responses in mouse serum. Some factors affecting these results are discussed. There was a slight alteration of protein profile of sporozoite infected salivary glands post gamma ray irradiation except for its band intensity, indicating the low effectiveness of gamma irradiation at optimal dose. It can be concluded that irradiated sporozoite as vaccine materials was failed in eliciting immune response.

**Key words:** Gamma rays, malaria, *P. berghei*, sporozoites, vaccine materials.

## 1. Introduction

Malaria is still a major health problem in the world, causing high mortality mostly among children under the age of five years living in sub-Saharan Africa. More than 500 million people are infected by malaria and over one million of them die each year, accounts for 2.23% of deaths worldwide [1, 2]. In Indonesia, almost 45% of populations are having risk for malaria infection [3]. Indonesian Ministry of Health reported that the number of malaria cases dropped to

over 1.1 million in 2009 from about 3 million per year in the past due to appropriate techniques in combating the deadly disease.

However, there are serious issues around the effective use of antimalarial drugs that include increasing multidrug resistance to *P. falciparum* and *P. vivax*. Consequently, a malaria vaccine that protects against infection and severe disease is urgently needed [4]. Therefore, research and development of a cost-effective deployable vaccine will be needed to facilitate elimination of malaria [5].

Research showed that the irradiated sporozoite remains the “gold standard” for the development of

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malaria vaccines that target the pre-erythrocytic stages of parasites [6]. Early studies demonstrated that experimental rodent hosts and human volunteers immunized with irradiated sporozoites developed antibodies that neutralized sporozoite infectivity [6-8]. These antibodies inhibit sporozoite motility, migration to the liver, and invasion of host hepatic cells [9, 10]. Many preliminary vaccines have been based on the *P. falciparum* circumsporozoite surface antigen, circumsporozoite protein, or its epitopes because immune response of the body generally targets this protein when mounting an attack on the parasite [11]. The most promising being the demonstration that non immune volunteers repeatedly challenged and cured with pre-erythrocytic-stage parasites developed immunity to subsequent challenge as well as the demonstration of the efficacy of the first liver-stage vaccine tested in a malaria endemic area to reduce parasite density in children [12, 13].

Thus, the irradiated sporozoite vaccine is an excellent model for malaria vaccine development, because irradiated sporozoites enter hepatocytes and only partially develop within these cells. Such approaches have been repeated in mice using otherwise lethal infections of *P. berghei* [14, 15] and *Plasmodium yoelii* [16]. From these, a number of sporozoite surface antigens have already been identified and considered as vaccine candidates, but it is still in limited success. Here, we attempt to analyze the immunity in malaria by using *P. berghei* as a model. Due to protective immunity to parasitosis requires repeated, a booster was also done. Since vaccines rely strongly on stimulation of antibody responses, understanding the effects of infection on humoral immunity is an important step in effective vaccine development.

The objective of this study was to obtain the optimum irradiation dose delivered to parasites to attenuate and characterize all parameters required to obtain reliably malaria vaccine materials. We showed that irradiated parasite as vaccine material was not fully effective in eliciting immune response.

## 2. Materials and Methods

### 2.1 Parasite and Mouse

*P. berghei* ANKA (Antwerpen-Kasapa) strain infected mouse bloods were obtained from Eijkman Institute for Molecular Biology, Indonesian Ministry of Research and Technology. Male Swiss-Webster mice (6-8 weeks old) were purchased from Tropical Medicine Laboratory, National Institute of Health Research and Development, Indonesian Ministry of Health and were housed at the Biomedical Laboratory of The Center for Technology of Radiation Safety and Metrology, National Nuclear Energy Agency of Indonesia (BATAN) animal facility and handled according to institutional guidelines. All procedures were reviewed and approved by the Animal Care and Use National Commission, Institute of Health Research and Development, the Indonesian Ministry of Health.

### 2.2 *Anopheles* sp. Rearing

*Anopheles maculates* and *An. farauti* were reared at a temperature of around 26 °C and 70% relative humidity in the insectarium of the Center for Application of Isotope and Radiation, BATAN. For the selection experiment, 3-8 days old female mosquitoes were first separated out into groups of 100-200 individuals per cage. Experiments comprised of mosquitoes which were 1-3 days apart in age. Mosquitoes were maintained on distilled water for 12-15 hours prior to feeding on a restrained guinea for up to 45 minutes.

### 2.3 Infection of Mouse

Mice were IP (intraperitoneally) injected with parasitized mouse blood containing about  $10^6$  *P. berghei*/mL. Parasitaemia was monitored started in the third day after infection by Giemsa-stained blood smears using light microscopy and was repeated every two days. When the parasitaemia was about 1%-5% (on day 5-7 of infection or gametocyte formation),

mice were used for mosquito infection.

#### 2.4 Infection of Mosquito

Standard protocols were used for infecting mosquitoes. It was done by feeding upon gametocyte-carrying 6-8 week old mice. Mosquitoes that had been starved overnight were placed into plastic containers (bugdorm). Hair of *P. berghei* infected mouse was removed from around 4 cm<sup>2</sup> area of the back. Mosquitoes were allowed to feed on mice for 1-2 hours. After feeding, only fully engorged mosquitoes were kept up to 14-15 days securely in about 1/5 liter cartoon cages, a cotton impregnated with a solution of sugar water was put on it and covered with a piece of net and placed in clipped transparent plastic completed with 50 mL centrifuge tube containing distilled water for humidity. Mosquitoes were maintained on glucose water soaked sterile cotton balls and changed every three days until dissection. The parasite infection status within mosquito was monitored at 8-9 days by examining 3-5 mosquito midguts for oocysts.

#### 2.5 Extraction of Mosquito DNA

Head and thorax of mosquitoes were ground with teflon pestles in 1.5 mL Eppendorf microtubes containing 25 µL of grinding buffer (0.1 M NaCl, 0.2 M sucrose, 0.1 M Tris HCl, 0.05 M EDTA, 0.5% sodium dodecyl sulfate; pH adjusted to 9.2). Genomic nucleic acid was isolated from mosquito by the method as described in Ref. [17].

#### 2.6 Detection of Sporozoite in Mosquito with Nested-PCR

The presence of sporozoite was assessed on days 14-15 by using PCR method. Oligonucleotide primers for PCR assay were designed based on the *Plasmodium* small subunit ribosomal RNA genes. Each 25 µL reaction mixture for nest 1 amplifications contained 5 µL of DNA template, 250 nM of each primer (rPLU 1 and rPLU 5), 4 mM MgCl<sub>2</sub>, PCR buffer (50 mM KCl, 10 mM Tris-HCl), 200 mM of each deoxynucleoside

triphosphate, and 1.25 units of *Taq* DNA polymerase. Nested 1 amplification conditions were: initial denaturation at 94 °C for 5 min, followed by 35 cycles that consists of denaturation at 94 °C for 30 sec; annealing at 55 °C for 1 min; extension at 72 °C for 1 min; final elongation at 72 °C for 5 min. Two microliters of the nested 1 amplification product served as the DNA template for each of the 20-µL nested 2 amplifications. The concentration of the nested 2 primers and other constituents were identical to nested 1 amplifications except that 0.5 units of *Taq* DNA polymerase were used. Nested 2 amplification conditions were identical to those of nest 1 except that the extension at 72 °C was for 1 min and 30 seconds of 30 cyclus for the genus-specific primers (rPLU 3 and rPLU 4). The PCR products of nested 2 amplifications were analyzed by gel electrophoresis and stained with ethidium bromide.

#### 2.7 In vivo Irradiation and Isolation of Sporozoites

Fourteen days after infective blood meal mosquitoes with the presence of sporozoites in their salivary glands were irradiated *in vivo* with doses of 0, 100, 125, 150, 175 and 200 Gy at a dose rate of 380.2 Gy/hour. Irradiation was done with gamma source of Cobalt-60 machine located at the Center for Application of Isotope and Radiation, National Nuclear Energy Agency. Dose rate of irradiation was determined by Fricke dosimeter. Sporozoites for immunization were isolated by anesthetized mosquitoes on ice after the legs and wings were removed. Infected salivary glands were dissected out and triturated in saline solution (0.9% NaCl), after which freed sporozoites were intravenously injected to 2-4 healthy mice. We used 10 pairs of salivary glands for each of mouse.

#### 2.8 First Injection, Booster and Challenge for Sporozoites

For immunization, mouse received an initial injection of irradiated sporozoites. Intravenously injections into the tail of mouse were given in a volume



of 50-100  $\mu\text{L}$  of saline solution containing salivary glands per mouse. This process was repeated for the booster immunization two weeks after the first injection. Some mice that were not treated for a booster acted as control. Mice were challenged in the same way by injection of non irradiated parasites with lower concentration (about half of immunization and booster) 2-4 weeks after booster. Some mice were not treated for a challenge. Thin blood smears were prepared from drops of tail blood on a glass slide starting on day 4 up to 14 days after challenge. Slides were air dried, fixed with 100% methanol and stained with 10% Giemsa for 10 minutes. The percentage of parasitized red blood cells was determined microscopically. The mice were sacrificed 2-4 weeks post challenge and blood was collected from the heart. Serum was isolated by centrifugation, and antibody levels were determined using ELISA (Enzyme linked immunosorbent assay) kit.

### 2.9 ELISA

Procedure for the determination of immune response in mouse serum with ELISA method was done according to standard protocol [18]. After coated with 50  $\mu\text{L}$  of antigens (Pv (*Plasmodium vivax*) 247, Pv210 and Pf (*P. falciparum*)) at 10 mg/mL in coating buffer, the plate was kept at 4  $^{\circ}\text{C}$  overnight. Plate was blocked with 100  $\mu\text{L}$  of 0.5% BSA in coating buffer for 3-4 h at 37  $^{\circ}\text{C}$  and washed four times with washing buffer. Fifty microliter of serum samples diluted 1:100 (v/v) in blocking buffer was added and leaved them for 1 h at 37  $^{\circ}\text{C}$ . After four times with washed buffer, 50  $\mu\text{L}$  of ALP-conjugated or biotinylated anti-Ig of appropriate specificity at the recommended concentration in Tween-buffer was added and leaved for 1 h at 37  $^{\circ}\text{C}$ . The sample was washed four times with 0.9% NaCl plus 0.05% Tween. Fifty microliter of streptavidin—ALP diluted 1:2,000 in Tween-buffer was added and leaved the sample for 1 h at 37  $^{\circ}\text{C}$ . The sample was washed four times with 0.9% NaCl plus 0.05% Tween. The sample was developed with 50  $\mu\text{L}$

of NPP (1 tablet/5 mL of substrate buffer) and read at OD (optical density) 405 nm. Positive signal cut-off was defined as two times the mean OD value from normal serum. Antibody titer was determined as the highest sample dilution which produced an OD value greater than or equal to the cut-off.

### 2.10 Protein Profile Post Irradiation

The procedure was done according to standard protocol [19]. Samples of mosquito salivary glands that were irradiated with 0, 150, 175 and 200 Gy of gamma rays were added with acetone and sonicated for 15 minutes, added with Laemli buffer solution, warmed in boiled water for five minutes, centrifuged at 8,000 rpm for five minutes, and electrophoreted onto 12% SDS-polyacrylamide gel. A total volume of 15  $\mu\text{L}$  protein extract solution was loaded into each well and electrophoresis was carried out at 200 V until the bromophenol blue dye reaches 1 cm above the bottom of the gel. After stained with Commassie R-250 for 1 h, gel was destained for 24 hours, and the appeared protein bands on gel were analyzed. As a molecular weight marker, SeeBluePlus (Invitrogen) was used.

## 3. Results

In the current study, we investigate the effectiveness of radiation-attenuated pre-erythrocytic parasites for protection against parasitemia and severe disease in experimental model of malaria. We used the highly virulent murine malaria parasite *P. berghei* ANKA which produces two distinct yet uniformly fatal pathologies and because its ability to sequester within the microcirculation which is the characteristic of severe malaria [7].

Our experiment was dependent on the sporozoites existence in salivary glands of mosquito. Mass production of sporozoites requires intensive production of mosquitoes and experimental infection of vector that is determined by the existence of gametocytes in mouse blood. Some factors interfering with breeding and experimental infection of this anopheline species. They

are including insectarium temperature, pattern and rhythm of blood feeding, and parasite, host and mosquito parameters which interfere with the yield of experimental infections. The increase of temperature promotes growth of parasite, but it also enhances bacterial and mycosal pollution so that it reduces survival of mosquitoes [20]. A condition that should be addressed in sporozoite production is also the existence of oocyst in midgut of mosquito after infection. One experiment found the oocyst in midgut of *An. farauti* at day 8 after *P. berghei* infected blood meal (Fig. 1), indicating that *An. farauti* was the most susceptible mosquito to get sporozoites.

Several factors related to genetic and environmental affecting sporozoite production in anopheles mosquitoes mainly the genome numbers per oocyst such as the size of the mosquito and the number of oocysts on the midgut. Moreover, the potential of the mosquito to serve as a vector depends on the ability to support sporogony, mosquito abundance, and contact with humans, which are all influenced by climatic and ecological factors. The ability to support sporogony is largely dependent upon species in that not all species of *Anopheles* are susceptible to *Plasmodium* infection. Temperature and mosquito longevity are other key factors affecting the parasite's interaction with the vector. Temperature also affects the time of

development in that the duration of sporogony is substantially shorter at higher temperatures. A shorter duration of sporogony increases the chances that the mosquito will transmit the infection within its lifespan [21].

It was found that vaccine materials created from irradiated sporozoite was not seen its full effectivity in eliciting immunity after injected into the mouse. Several factors may affect this finding, one of them was predicted that sporozoite suspensions used for immunization may heavily contaminated with mosquito components. There is an alternate approach by allowed irradiated mosquitoes to directly inoculate attenuated sporozoites into hosts, the mosquitoes thereby acting as vehicles of immunization. Beside that, it is known that a large number of irradiated sporozoites are required to elicit protection in host. Another reason is that there were very low parasites in blood of mouse after bitten by infected mosquitoes. This is mainly due to, in our opinion, low capacity of all species of mosquito used as malaria vector in this experiment (*An. maculates* and *An. farauti*). There was no infection found in sporozoite injected healthy mouse at days of observation. This might be due to low number of sporozoite or no sporogoni processes taken place in the mosquitoes that may cause no transmission of infection from mouse-sporozoite-mouse cycle.

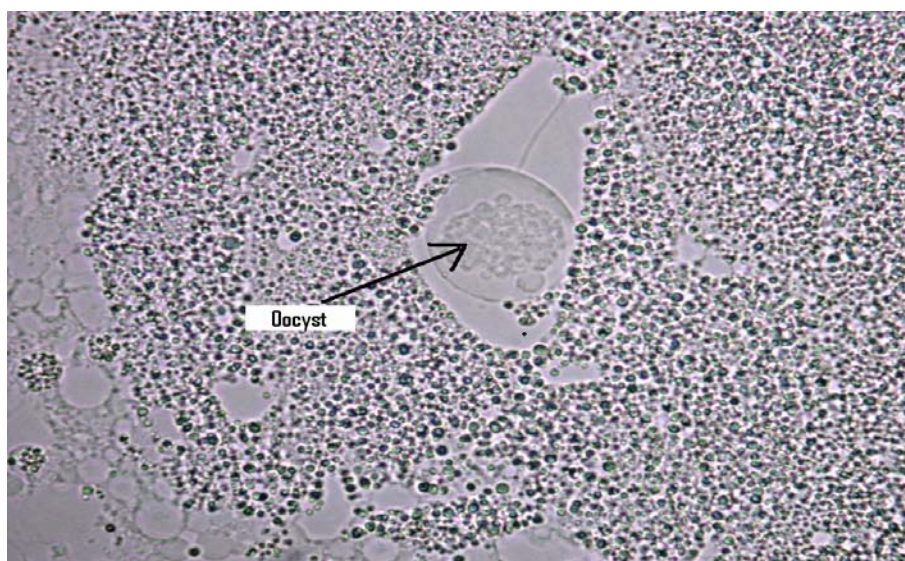


Fig. 1 Microscopic view of oocysts (arrow) in the midgut of *P. berghei* infected *An. farauti* at day 8 after infective blood meal.

The detection of parasite in the mosquito with nested PCR by using the primers designed for the parasites characterization showed that *An. farauti* was more susceptible to parasite infection and supports the complete life cycle of *P. berghei* compared to *An. maculates* (Fig. 2), even though the number of sporozoites was very low to induce an infection in mouse. The mosquito species that mostly used in such studies is *An. stephensi*, which is robust mosquito considered to be highly efficient for sporozoite production [7, 22].

The observation of parasites in thin mouse blood smear showed that there was no parasite found in blood at days post challenge.

ELISA results presented in Table 1 shows that there was seemingly low effect of the booster and challenge on the positivity immune responses. However, there was tendency in increasing of the number of antigenic positive in serum samples with the increasing of irradiation doses mainly for 175 Gy. For 0, all samples (100%) were positives in immune response, indicating that the immunity developed after one or two infections with infective parasites. For 100 Gy,

50%-75% samples were positives, whereas for 125 Gy it was 0-50%, and for 150, 175 and 200 Gy there were 0-100% positive to Pf antigen. This also showing that Pf antigen was more recognized by mouse serum compared to Pv247 and Pv210 antigens that may be

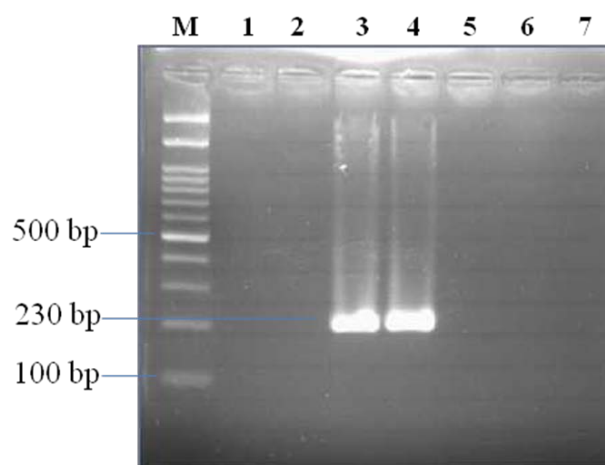


Fig. 2 The result of detection of parasite's DNA in the mosquito with nested PCR using the primers designed for the parasites characterization and 230 bp products. M, marker of 100 bp ladder; Lane 1, *Anopheles maculates* 1; lane 2, *Anopheles maculates* 2; lane 3, *Anopheles farauti* 1; lane 4, *Anopheles farauti* 2; lane 5, DNA of body of mosquitoes; lane 6, negative control for 1st PCR; lane 7, negative control for 2nd PCR.

Table 1 Results of ELISA on blood serum of mouse injected with irradiated sporozoite isolated from salivary glands of *An. farauti*, followed by booster and challenge.

Irradiation dose (Gy)	No. mouse (sex; F: female; M: male)	Booster (weeks post prime)	Challenge test (weeks post booster)	No. positive samples to antigens of ELISA/total		
				Pv247	Pv210	Pf
0	4, M	-	-	0	0	4/4
	2, F	2	-	0	0	2/2
	2, M	2	4	0	0	2/2
100	4, M	-	-	0	0	3/4
	2, M	2	2	0	0	1/2
125	4, M	-	-	0	0	1/4
	2, M	-	2	0	0	0/2
	2, M	2	4	1/2	0	1/2
	2, M	-	-	0	0	2/2
150	2, M	-	2	0	0	0/2
	2, M	2	-	0	1/2	0/2
	2, M	2	2	0	1/2	2/2
	2, M	2	4	0	0	2/2
	4, M	-	-	0	0	3/4
175	2, M	-	2	0	0	0/2
	2, F	2	2	0	0	1/2
	2, M	2	4	0	0	2/2
200	2, F	2	-	0	0	0/2
	4, M	2	2	0	0	0/4
	2, F	2	4	0	0	2/2

due to the similarity of Pf with *P. berghei*, but its molecular structure should be checked. It is in accordance with the fact that in *P. falciparum* malaria, the immunity induced by irradiated sporozoites is species-dependent yet it is cross-protective against different parasite strains [23]. Pv and Pf antigens were used due to unavailability of *P. berghei* antigen. This is done based on the finding that even though early human studies in one volunteer suggested species specificity, but recent studies with rodent models have showed cross-species protection [24, 25]. There were positive results in some serum to Pv247 and Pv210 antigens. There was low effect of booster to the challenge that were done 2-4 weeks post booster. From these, it was known that injection with irradiation-attenuated sporozoites of *P. berghei* conferred low or no protection against live sporozoite challenge, even though it was supported by booster injected two weeks after the first injection.

Free radicals induced by irradiation may cause breaks in the protein chains or changes in their secondary or tertiary structure, resulting in modifications of their physicochemical properties such as fragmentation, crosslinking and aggregation. However, the knowledge on the alteration of protein profile of parasites after irradiation is still limited and it is needed to study the vaccine efficacy. Result of protein profiling of gamma ray irradiated sporozoites of *P. berghei* showed that there was a slight difference in the profiles of protein except for CSP (circumsporozoite protein) with molecular weight of 62 kDa among doses of gamma rays of 0, 150, 175 and 200 Gy. However, the higher number and thicker bands (intensity) was seen in higher dose (200 Gy), showing the effectiveness of high dose of gamma rays in attenuating parasites (Fig. 3).

In this study with SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), it was known that protein profiling of *P. berghei* infected salivary glands of *Anopheles sp.* showed four main polypeptide bands with the sizes of about 32, 40, 58

and 80 kDa. Besides that, in this experiment there was no separation between lateral and distal parts of salivary glands which has various proteins with different function and profiles. The main proteins were seen at the molecular weights of 62 kDa and 110 kDa. It seems that protein of 110 kDa was only seen at irradiation dose of 200 Gy. These results provide basic information that would lead to further study on the role of sporozoite proteins in malaria vaccine development.

In another experiment, a band with molecular weight of 62 kDa that corresponds to circumsporozoites protein was prominent and detected only in 150 and 175 irradiated salivary glands, suggesting the effect of gamma irradiation to the profile of sporozoite protein that depending on the dose of irradiation (Fig. 4). Again,

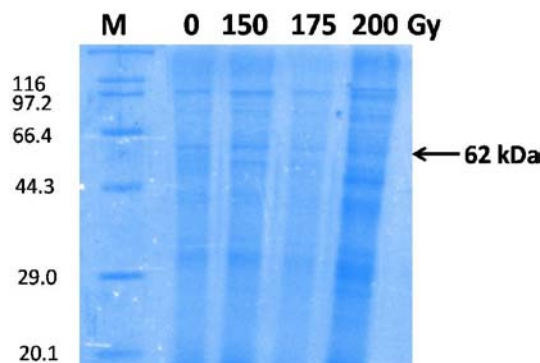


Fig. 3 Protein profile of irradiated sporozoites of *P. berghei* (molecular weight of 62 kDa) with SDS-PAGE.

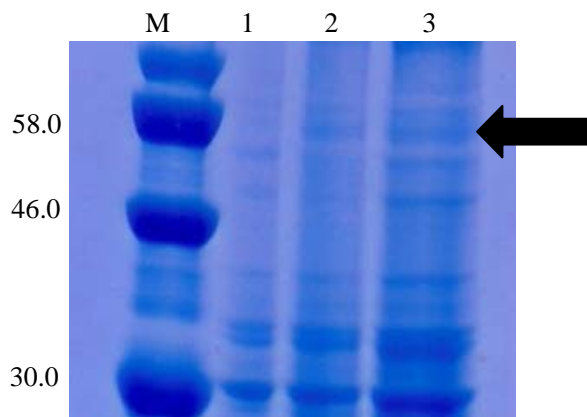


Fig. 4 Protein profile of irradiated sporozoites of *P. berghei* with SDS-PAGE where a protein with molecular weight of 62 kDa (arrow) was detected only in irradiated salivary glands. M, marker; lanes 1, 0; 2, 150 Gy; and 3, 175 Gy. No available data for 200 Gy.

the higher dose resulted in thicker bands where the intensity of several other subunit bands were increased as results of gamma radiation and reached maximum intensity at 175 Gy. There was no increase in bands associated with low molecular weight fragments (below 30 kDa) with the increase in the irradiation dose. Radiation may dissociate these protein fractions to small subunits and rearrangement to form a complex protein even high or small molecular weight compounds. Thus, the changes in protein profile were depended on radiation dose. To our opinion, this is the first report on the mosquito's salivary gland protein profiling after irradiation.

#### 4. Discussion

In this report, we present our simple but valuable results on immune response in mouse blood post gamma rays attenuated whole parasites of *P. berghei* injection for malaria vaccine development. As compared to developing vaccines against viruses and bacteria, developing a vaccine against malaria is complicated by the complexity of the parasite as well as the host's response to the parasite. Host genetic variations play a significant role in conferring predisposition to malaria infection and determine the outcome of interactions between host and pathogen. Genetic variation in malaria parasite is central to the pathogenesis of the organism, as allelic variability in different clones is thought to facilitate immune evasion [26]. Based on these facts, it urgently needed a malaria vaccine that is specific for Indonesian people. To realize this expectation, this preliminary study was conducted by using murine malaria models to understanding the nature of biological responses and the role in development of immunity to malaria.

Injection with radiation-attenuated sporozoites uniquely leads to long lasting sterile immunity against malaria infection. In this experiment, we obtained the similar results, even though only in limited data. Complete protection against malaria in humans and mice can be obtained by inoculation with irradiated

*Plasmodium* sporozoites which infect hepatocytes *in vivo* as normal sporozoites, but they do not progress further to blood-stage infections and, therefore, do not induce malaria-associated pathology [20, 27, 28].

The major target of pre-erythrocytic vaccine development is the high numbers of sporozoites obtained from mosquitoes. As discussed partly in sentences above, to do that some factors contributes such as the susceptibility of mosquito to parasites. Parasites must complete a complex life cycle in mosquitoes in order to be transmitted from human to human. Vectors must be able to support parasite development through several key stages over 8 days to 15 days. Temperature changes will also shorten the development time of parasites in mosquitoes. Thus, geographic distribution of malaria is confined within climates favoring its extrinsic cycle, provided that other conditions do not limit mosquito survival. In turn, duration of the sporogonic development of malaria parasites in mosquitoes (Fig. 2) is an important component of vectoral capacity that measures the potential rate of contact between infectious vectors and susceptible hosts [29].

Among the practical applications of radiobiological techniques that may be of considerable interest for public health is the use of ionizing radiation in the preparation of vaccines. Radiation attenuated vaccines have been demonstrated to be an effective means of controlling certain parasitic infections such as malaria. Irradiation is a technically simple process that retains structural features of the microbial pathogen without destroying the natural antigens or the intrinsic adjuvants. Therefore, a strong immune response is induced in the vaccinated host. Irradiation destroys nucleic acid, making the microorganism unable to replicate so it can not establish an infection, but some residual metabolic activity may survive, so the irradiated microorganism can still find its natural target in the host. The successful use of irradiated plasmodium as a vaccine depends on finding a radiation dose which will significantly reduce the

pathogenic effect of the larvae without seriously impairing their immunogenic power [30].

The dose of irradiation used to attenuate plasmodium is a most crucial factor. Therefore, to determine the dose of gamma-irradiation which will produce attenuated parasites without affecting the pre-erythrocytic stabilities is the ultimate goal of many studies on irradiation vaccine. It is thought that the amount of radiation required to render the parasites non-viable is about 150 Gy for an inoculum of certain number of parasites. There is also evidence that exoerythrocytic stages may be more susceptible to gamma-rays than are blood parasites. It means that a most important step is to determine the minimum dose of irradiation required to adequately attenuate each sporozoite. To ensure that the parasites are sufficiently weakened for the vaccine, yet remain alive, they must be exposed to a radiation dose of at least 150 Gy, but not much more. The researchers have revealed that to be safe for human trials, all mosquitoes must get their minimum radiation dose of 150 Gy. It is only at this dose that they can be sure that the parasites are weak enough for the vaccine, but are still alive [31]. Our experiment on blood (erythrocytic) stage parasites also revealed that this dose of irradiation was most effective dose for attenuating the parasites to obtain vaccine material [32].

In this experiment, as the main target, radiation exposure is intended to inactivate parasites. According to the category of biological agents of radiological protection, the irradiation used in this vaccine materials development is the deterministic effects (harmful tissue reactions) due to large part of the killing/malfunction of cells following high doses (more than 100 Gy) rather than stochastic effects. And the induction of tissue reactions is generally characterized by a threshold dose. The reason for the presence of this threshold dose is that radiation damage (serious malfunction or death) of a critical population of parasite cells. So that at high doses radiation exposures may cause deterministic effects (tissue reactions). The extent of damage

depends upon the absorbed dose and dose rate as well as radiation quality and the sensitivity of the target materials [31, 33].

Here, we sought the most effective dose of irradiation to attenuate parasites through protein profiling. Radiation is known to cause disintegration and aggregation of protein molecule and this is predicted occurred in the present study. Gamma radiation is generally attributed to direct breakdown in protein where the irradiation dose used considered very important factor. Radiation may also dissociate these protein fractions to small subunits and rearrangement to form a complex protein even high or small molecular weight compounds. The direct breakdown of the molecule by ionization capacity of the passing photon may be one possibility. In addition, radiation normally cause the formation of disulphide bridge between polypeptide chain which may be effect on the aggregation of the low molecular weight protein and effect on its conformation [34]. Thus, irradiation produces some chemical changes, which lead to the production of small amounts of radiolytic products that may cause breaks in the protein chains or changes in their secondary or tertiary structure, resulting in modifications of their physicochemical properties, namely, fragmentation, crosslinking and aggregation [35]. These changes were confirmed by SDS-PAGE.

## 5. Conclusions

The experiments showed that irradiated sporozoite as vaccine material had a low effectivity or did not seem in eliciting immune response upon booster and challenge. The low capacity of *An. farauti* as the most potential vector in our experiment affecting the number of sporozoites produced in the salivary glands. There was no alteration of protein profile of sporozoite infected salivary glands post gamma ray irradiation, indicating the effectiveness of irradiation.

## Acknowledgment

This research in part was conducted under Incentive

Research Project No. RD-2009-0047 from the Indonesian Ministry of Research and Technology.

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# Daily Life Experience of Institutionalized Elderly People

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**Abstract:** There is a continuous increase in the number of elderly people moving in to residential care facility in the Kingdom of Bahrain. Admission in to the residential facility perceived in Bahrain society as stigma, rejection for elders by their family members and a violation to Bahrain family values. Many adverse effects of institutionalizing elderly people were reported in literatures. This study aimed to explore the lived experience of institutionalized elderly people in order to generate appropriate interventions to smooth the transition in to the residential facility. A qualitative, phenomenological approach was used. The study was carried in one of the elderly residential health care institutions in the kingdom of Bahrain. The sample composed nine participants, were interviewed and audio recorded. The five themes emerged from the data analysis included: no choice, no freedom, killing routine, home of negligence, and elderly refuge. The conclusions involved implication for clinical practice, education and management. Future research in the same area was recommended.

**Key words:** Daily experience, institutionalization, residential care.

## 1. Introduction

Life expectancy increased dramatically all around the world. In Bahrain, although the percentage of elderly population remained static during the past ten years, the number of admission in to residential facilities dramatically increased. The family had traditionally been the primary health care provider for elderly people in Bahrain. Admission to residential care represent for elderly stigma and loss of value. In addition, many health adverse consequences had found to be associated with elderly people transition. This study assumed to be significant to nursing practice because the negative effects of elderly institutionalization could be preventable through soothing the transition process. The transition process considered a unique experience that can be understood by elders' stories and descriptions of this event, which can be achieved through this study. The results of this study is expected to help in developing a better health care planning for relocated elderly people in Bahrain. The most important objective of this study is to reinforce coping skills and help the older person find

new strategies in the new living situation.

## 2. Methodology

Naturalism was used as the most relevant paradigm of this study, and phenomenology was used as the most appropriate research approach. The setting of the study was in one of the elderly residential health care institutions in Bahrain. The purposive sample included nine participants, five males and four females. Inclusion criteria were all those residents for at least two months at the health care institution, and the elderly people who are able to describe their situation. Exclusive criteria were recently admitted elderly who lived less than two month at the residential institution, and elderly who are unable to describe their situation. The researcher followed strictly ethical approval by obtaining two institutional ethical reviews, assessing mental legibility for the participants, providing written/verbal information to the participants and obtaining informed consent from each participant to be interviewed and tape-record. It is believed that standards related to credibility, and authenticity was met. Data was collected by conducting a conversational, semi-structural, audio recorded and interviews. Each individual was interviewed

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individually between 30 min to 45 min.

### 3. Analysis

The analysis process allowed emerging themes to be identified. This included reading the entire transcripts, initial coding of interviews and identification of five themes. The process of themes identification was done by using Giorgi (1985) four steps of qualitative data analysis [1].

#### 3.1 Demographic and Personal Information

The sample represented nine participants, five male and four female, mean age 72.7 years old. All participants suffered from chronic diseases such as diabetes mellitus, ischemic heart disease, hypertension, arthritis, and bronchial asthma. The shortest residential duration was nine months and the longest duration was six years. Refer to Table 1 to view participant demographic data, diagnosis, physical dependency level, and residential duration.

#### 3.2 Emerging Themes

The categorization strategy was carried out to identify commonalities and differences in the data depending on the number of occurrence of the particular category in each transcript, which indicated

the importance of the category, as expressed by the participants. Elderly experiences of residential life became the basis for the theoretical ideas presented in the following themes: no choice, no freedom, killing routine, home of negligence, and elderly refuge. A description of each theme, supported by participants' statements will be elaborated in the discussion. Table 2 illustrates the categorization mechanism leading to the identification of five themes.

### 4. Discussion

#### 4.1 No Choice

All participants expressed that they had no choice in regards to their relocation in elderly care institution, but in a different ways. "I was forced to enter here and I hope I can go out one day and live my life again. Looking to go out again is just like prisoner who looks for rope to climb and go out", "My son brought me to elderly home. He said that the government provide home for cases like me. I didn't reject the idea because I don't have another choice".

Few elderly people actively plan to be in a care home. They find themselves living in one because of a crises in their health, or because somebody else persuade them [2-5]. Elderly people feared the residential care institutions and view them as a dumping place [6, 7].

**Table 1** Summery of participants' demographic and personal information.

Code	Age	Sex	Medical diagnosis	Residential duration	Admission decision maker	Reason for admission
T1	63	M	DM, HTN, Cataract Arthritis, Depression	One year & nine months	Client	Homeless
T2	84	M	Bronchial Asthma	One year & six months	Health professional	Homeless Lack of care providers
T3	87	M	Poor vision	Two years & two months	Son	Living alone Lack of care providers
T4	67	M	HTN, Poor vision	Two years & three months	Health professional	Homeless Lack of care providers
T5	64	M	Benign prostate, DM, HTN	Two years & two months	Health professional	Living alone Lack of care providers
T6	64	F	DM, HTN	Nine months	Nephew	Living alone Lack of care providers
T7	64	F	Sickle cell disease, DM, HTN , depression	Three years & seven months	Brother	Lack of care provider
T8	78	F	IHD, Osteoporosis	One year & five months	Brother	Lack of care provider
T9	84	F	DM, HTN, IHD	Six years	Client	Living alone

M: Male; F: Female; DM: Diabetes mellitus; HTN: Hypertension; IHD: Ischemic heart disease.

**Table 2 Summary of the categorization mechanism leading to the identification of five themes.**

Sub-themes	Emerging themes
Lack of care providers	No choice
Loss of health	
Loss of productivity	
Fear of outside world	
Saving dignity	
Nobody is listening	No freedom
Restricting role and regulation	
Humiliation	
Lack of privacy	
Lack of autonomy	
Routine	Killing routine
Limitation in activities	
Boredom	
Loneliness	
Nobody is listening	
Health worker carelessness	Home of negligence
Lack of touch	
Availability of accommodation and services	
Building new relationships	Elderly refuge

Participants expressed a hidden helplessness in term of their relocation. For instance, their long term illnesses, physical dependency, and lack of care providers were main reasons for their relocation and not their own wishes. "I entered here because I was sick", "The reason I came here was that I'm not walking anymore and I need someone always with me to attend my needs. My brother is telling here is better for me, and they will provide me with a better care. He told me that if I could walk again he would keep me at home". Some participants expressed their refusal to be burden on others, and deciding to relocate as a way to save their dignity. "Once I was in my nephew house, setting in their living room and hearing them inside the kitchen discussing something. His wife said to him I don't want your aunty to live here, and my nephew didn't say anything about it, and kept quite. I felt hurt because that was only a visit to my nephew house, and I didn't intend to live there, and the wife couldn't bear me! The worse was the response of my nephew, instead of clarifying to his wife my position, as the eldest aunty, and the next after his mother; he was just like agreeing with her. I don't want any mercy from these people. I prefer to save my dignity. God mercy is better.". In a similar view [8], the residents may feel that the best

they had left to offer loved ones was freedom from the burden of their care.

Some participants expressed fearing the outside world, where they were unable to live safely alone or trust anybody. "Actually I'm afraid to go out of here, because I don't have place to go and I don't have anyone to look after me." A similar result [9] found where participants expressed feeling very comfortable and safe when recalling their worries before the admission about having home accidents.

A group of researchers identify health deterioration to be predictors for elderly people relocation [2, 3, 10]. Residents may face the challenge of coping with the loss of independence, reduced physical abilities, and feelings of isolation, anxiety, depression, and helplessness, all of which may result in increase in their morbidity and mortality [11-13]. Anyhow, there is a view [14] that successful aging as a successful adaptation of the individual to changes during the aging process. Positive perception of elderly residents to themselves might be an important factor to adjustment the residential life, regardless their health status particularly in dealing with relocation distress, having self-worth and participating in group life [7]. These findings imply that elderly people may successfully age in residential facility if their adaptation capability was empowered. Positive perception of elderly residents to themselves might be an important factor to adjustment the residential life, regardless their health status particularly in dealing with relocation distress, having self-worth and participating in group life [7]. These findings imply that elderly people may successfully age in residential facility if their adaptation capability was empowered.

A group of researchers had studied the voluntary and involuntary relocation and their influence in elderly people adjustment to residential life [12, 15, 16]. In this study, despite the variations in reasons for admission, admission decision makers, and elderly involvement in the decision, the researcher came up with the fact that there was no real voluntary relocation for all

participants. Comments that were given by participants who made the decision revealed that it was taken in a situation where they perceived that there were no other alternatives. Relocating to residential facility was seen by elderly participants to be, at best, a forced choice [8].

#### 4.2 No Freedom

Elderly people expressed that they had lost their freedom by restricting them with the institutional rules and regulations. For instance elderly cannot go out the centre without accompaniment, registration of the accompanied person, and duration of the leave. "I'm in a jail. I don't feel free as before", "In winter time we should stay only inside, and we can't go out in the garden. We should have in door garden for such times". The old person in Bahrain community was referral for advices and wisdoms and privileged with respect. This position for elderly people had changed. Elderly role as spouse, parents and grandparents has diminished, as children and grandchildren reverse roles and begin to treat them as the children. The former roles would exacerbate this devalued sense of self [8]. Living with rules and regulations was also found to be a strong barrier to residential life adjustment and tend to exacerbate feelings of abandonment, leading to poorer prognoses [9, 17-19]. However, a study [17] regarded rule and regulation as "law of the country", as Chinese elderly participants expressed their understanding about meeting collective needs rather than individual needs. Traditionally, they prefer eating together as a big family, and their past experience in sharing a flat with many families had helped them accommodate the residential need of sharing common facilities with ease.

One attribute of autonomy is involvement of residents in decision making and negotiating care planning [20]. Elder participants expressed that their autonomy had been reduced after their relocation, in term of deciding for themselves and telling their opinions and suggestions. "I can't take shower the time that I wish, they used to wake us early morning and give us shower, and they will not leave the decision for

us in regard to bath timing." Elderly people who feel that they have at least been given the opportunity to be involved in decisions could perceive themselves to have more control after their relocation [8].

Additionally, elders may feel losing autonomy because of the disruption of their already established daily routine and relationships before admission [9]. Elderly lack of control started when feeling forced to leave home and enter a residential facility along with losing their identity and possessions such as losing home, neighborhood, and contact with friends and family [8, 17]. Elders may feel the loss is not so much for the material objects but rather for what they symbolized and the memories that they evoked [8]. The great differences in living arrangement before and after admission, as well as the lack of previous residential life experiences were identified as major causes of a painful transition experience [17]. "I have travelled to many countries before. Out is different. I can go everywhere and see people again. I can go to my favorite coffee shops and set with my friends. I missed the radio sound in there. I'm not annoyed to be here, but of course I prefer outside, although I can't see well."

Also, elderly losing of autonomy was expressed as lack of privacy, by elderly residents not feeling comfortable with their roommates. "Sometimes I quarrel with my roommate because we want different channel on TV, or because I want the light on, when I am praying, and she doesn't." The elders may feel inconvenient in the new environment together with the sick roommates, resulted in feeling very frightened and uneasy with the transition [9]. "I don't like keeping me with confused patient who are out of their mind. Some of them scream, laugh loudly beet themselves like crazy people and curse others. I can't sleep at night time because they used to disturb me."

#### 4.3 Killing Routine

Living in a routine was one important concern for elderly residents in this study. They had explained

feeling boring of similar daily activities, and living a routine life, was just like living in a cycle that ends where it starts. "There are limitations in inside activities. Every morning they used to take us to the main hall, have lunch, set for a while watching TV, having dinner and then back again to bed." "I remain alone even though I'm setting with all, and I feel bored." Elderly people previous roles as a home-maker and breadwinner have gone, along with employment and home, leaving in elders' life big space and loneliness [8].

Although the center in which the study was connected provides indoor and outdoor activities, it was recognized by participants as few and repeated. Such activities were gathering in TV hall, occasional celebrations and scheduled picnic. The place lack occupational and recreational therapy. In contrast, in a study conducted to investigate an elderly residence occupation activities [19], 20 programs were available in the residential facility in which the study held, including pet therapy, exercise, cooking classes, residents and family councils, church services, access to pastoral care, and interaction with preschoolers at an on-site daycare and occasional visiting entertainment. Residents usually desire to have more activities in their residence [21]. One participant suggested changing the routine by introducing new activities, "I know many elderly who have handcraft talent such as carpentry, pottery making, and sewing, but nobody offer them handcraft activities. If we were engaged in such activities the time will run fast and we would feel productive and worthy."

A researcher [7] identified satisfaction with the facility as a significant predictor of adjustment. The increase in routinization in daily life is associated with decreases the adjustment abilities of the elderly residents [22]. A group of researcher emphasized that activity programs improve elderly well being, empower residents in their new environment and foster the adjustment process [15, 21, 23-28]. Specifically, activities that promoted social involvement was

reported to be significant in leading healthy life and enhance feeling good about oneself, even if elderly people are experiencing illness and disability [4]. In residential facility, elderly people may spent less time in social events and community interactions, which were major part of their life. For some, this increases feeling of isolation and loneliness and decreases their satisfaction with the activities provided [29].

There has been very strong support for resident's relations with staff and peers as the most important aspect of their quality of residential life [7, 19, 30-32]. The relocated elderly losses such as home routines, sense of independence, physical and communicative abilities, made any relationship gained, critical for their sense of self worth [19]. Elderly residents relay on family members for connection to their previous lives, and for those without family or friends, the relay on staff and peers [19]. Some participants in this study verbalized adjusting being away from loved once. Hence, relationships with staff and peers seemed to be more important, to participants in this study, than the familial relationships. Similarly, it was found that the frequency visit from family was not associated with adjustment to nursing home life [7]. Rather, the social support from the people inside the facility, staff and other residents, is more influential to adjustment.

#### *4.4 Home of Negligence*

Elderly participants explained that negligence took the form of ignoring elderly residents, poor communication and delay in responding to their needs. "It is like slow death here because of staff negligence" stated by one resident. "They never ask us anything about our opinion, and if we talk or suggest, no one would listen or react. For example nobody will ask whether food is good or not, or do we like it or not. I suggested greenhouse, but nobody bother about it. If I complain about anything happened during shifts duty to the administration, they would simply tell that it is not their responsibility, because they were not on duty at that time. Who should follow our problems and

concerns?” It was found that elder residents complain that staff couldn’t understand their needs [9]. They expressed feeling as if they have become non-person, not because of their inability to do things, but because they are dependent on waiting for staff to take their concerns. Participants considered lack of touch as a type of negligence, and used the word unethical to label this behavior. “Nurses here will depend on aids in direct contacting elderly, and will not touch the patients at all. They treat elderly like dirty people who shouldn’t be touched, is this ethical? I do not call them “sisters”, but I call their names, because they don’t deserve this nomination.” Furthermore, some participants expressed that they had been verbally abused by care providers. “Staff here sometimes rude when dealing with us, but if any staff talk to me harshly I just forgive them, this makes me live peacefully,” “There are good and bad nurses. Bad nurses are not treating us well, I mean verbally they are harming us, and never use good words.” Elderly residents generally were reported by group of researcher to be prone for abuse within the residential facility as a result of their frailty [33, 34]. Such abusive behaviors were reported to be mostly disrespectful behaviors, humiliation and negligence [33]. Although participants, in this study, acknowledged availability of good services, they verbalized that these services will never compensate good treatment. “It’s good to have place to live and food to eat, it’s clean here, they are cleaning the floor with disinfectant daily, but all this will not compensate the good treatment for elderly.”

A group of researcher had emphasized that communication is vital for care provision especially for assessing the specific needs of elderly patients [9, 30, 35, 36]. Nurses should communicate knowledge, understanding, caring, kindness, sociability, and intuition [9]. Staff-patient superficial relationship may form because elderly may view staff as rulers of the country [17]. Furthermore, the residents may feel that they are merely a list of tasks for staff to attend [9]. Some residents may turn between the need for attention and

feeling guilty in increasing work pressure on staff [19]. Such conflict may increase anxiety, feeling of helplessness, and result in a loss of personhood and self value of residents [19]. They, therefore, may not actively seek to establish relations with staff and reveal their difficulties in settling in.

However, some other residents verbalized having good relationships with health care providers, and commented that staff were caring and treating them nicely. “Health workers here are very nice, they treat me well.” Anyhow, a researcher [37] clarified getting high percentage of positive residents’ responses when assessed elderly people residential care satisfaction, which did not correlate with the observation because some residents were reluctant to criticize staff or their behaviour. The residents may cooperate to avoid being seen as trouble maker [19].

#### *4.5 Elderly Refuge*

Elderly were generally satisfied about services provided such as food, clothing, bathing schedule, medical treatment, and inside and outside activities. “Aids are giving us bath daily early morning, they dress and groom us, keep us on chair and gather us in the main hall to take breakfast and lunch together and then take us back to bed. I generally like the daily schedule here.” Some participants said that the center was better than the outside world. One liked being there because he was alone outside, whereas in the center he was able to meet other elderly people to talk to them. Elder persons living alone are more likely to look for residential placement as a strategy to gain social contact with others [9]. Therefore, for elders how were living alone before the admission, positive consequences of the admission were anticipated. “I am very happy here. I was living alone and have nothing to do, now I have many relationships, and we used to gather and chat daily.” A researcher [17] found that the average waiting time in Hong Kong for nursing home placement is more than two years, and majority of elders’ Chinese people consider themselves very lucky

to have been given a place in a nursing home. They believed that they should, therefore, be thankful and try to be complainant to nursing home life as much as possible.

Some other elders' statements implied that they accepted living in the center with a sense of hopelessness. "I do not mind being here, and I believe that dying here or there is the same because death is the same anywhere. I'm in the hand of God." Generally, when people move from a place to another, there is a sense of future, but for elderly people moving to long term care meant the end of line [8]. They could no longer think in term of future. When elderly people feel that they have lost everything that they have spent their life building, and they know that they have neither the time nor the capacity to start again, they cannot perceive the future [8].

Anyhow, it was shown that the elderly residents are able, within the institutional environment, to construct the life in world of their own [17]. The elderly people may find new ways to live within the new environment even if they are totally not accepting their situation. This complex process involves a struggle to regain a life that is as close as possible to that lived before placement to residential facility [17]. Participants of this study expressed using their effort to gain understanding of every aspect of residential life. They explained observing very closely how other residents acted, behaved and spent their days, and identified them as a role model to help settled them in to the residential facility. The author had noticed that, for the physically able participants, who are unable to live independently in the community for social reasons, shelter and food were the main forms of care provision needed. Whereas more frail and dependent older people, who are unable to live independently in the community for physical reasons, activity of daily living were the form of care provision needed.

Regardless the variations in the elders' needs and services provided, the residential facility seemed to refuge them. It is a place that substitutes the community

in term of provision of basic life needs, rehabilitation, and socializing, but it does not necessarily be like home. A researcher found that most elderly define elderly care facility as their second home that is almost like their first home except that elders will not live with their families [17]. While elderly participants of the current study believed that this placement was unavoidable, it was an important source of conflict in their later years.

## 5. Conclusions

In conclusion, the researcher intends to develop recommendations that stand for the five developed themes:

### (1) Recommendation for clinical practice:

Nurses must develop a comprehensive assessment for elderly residents, prior, after the admission and throughout their residency to assess their adjustment in the new environment;

Nurses must plan and reinforce appropriate coping strategy for each elderly resident;

Nurses must promote effective communication with elderly people;

Nurses must consider ethical issues and adhere to code of professional conduct of the residential institution to preserve patients' rights and dignity.

### (2) Recommendation for education:

Health care planner must introduce educational programs for both health care providers and elderly people in order to educate, orient and prepare them regarding the transition process.

### (3) Recommendation for management:

Health care planner must plan for gradual relocation process to help elderly people and their families accept and adjust the residential life;

Health care planner must maintain adequate activities within the residential facility, in order to maintain elders' productivity, sense of worthiness, and break the life routine;

Health care planner must develop roles and regulations that maximize the elders' autonomy, as much as possible, within the residential facility;

Health care planner must develop policy and procedures that are coherent with the elder residents' needs, which were represented through the five emerged themes;

Health care planner must assess elderly quality of life within the residential facility by auditing the specific areas of needs represented through the five emerged themes.

#### (4) Recommendation for research:

It is worthy to study the transition to residential life with older resident whose admission is expected to be temporary. It is assumed that the experience will differ for elderly people whom admissions are temporary.

While this investigation focused on understanding resident's point of view, future research could extend understanding the issue of elderly residential life experience from nurses and significant others points of view.

It would be of value to compare the experience of those who live with families before their transition and those who live alone.

Future research may focus on studying each of the five emerged theme by using quantitative approaches to investigate variables such as gender, marital status, and health condition to validly and reliably sample elderly population.

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# Impact of Universal Precautions Training Program on Nurse Midwives Performance during Labor in Khartoum State—Sudan 2006-2009

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**Abstract:** Objective: evaluate the effect of training program on the nurse midwives performance during the process of labor to prevent HIV (Human immunodeficiency virus) transmission. Methods: the study utilized quazi-experimental design to elicit the effect of training program on the NMs (nurse/midwives) practices to prevent HIV transmission. An educational program drawn from six selected hospitals through stratified cluster random sampling method in universal precautions for nurse/ midwives was developed, implemented, and assessed ( $n = 180$ ). Official permission was obtained from the institutions and consent from participants. The study was implemented in three phases, pre-training base line assessment using a structured interview questionnaire to assess the knowledge, attitudes and practices towards HIV infection and prevention. Observation check list to assess application of universal precautions during the process of labor (two paths pre and post intervention). Training program is offered to participants and they were re-evaluated after six months. Results: universal precautions application scores increased significantly after training from 40.9%-52.2% ( $P < 0.005$ ). General NMs level of knowledge towards HIV infection and prevention were higher than 80% and 70%, respectively and their attitude level were higher than 60%. The study showed a high rate ( $> 80\%$ ) of needle stick injuries among nurse midwives which may prone them to HIV infection. Conclusion: training program is effective in increasing nurse midwives application of universal precautions. NMs were highly knowledgeable, and their attitudes were fairly appropriate. Recommendation resources needed for practice should always be made available, and creation of a body to supervise application of universal infection control precaution.

**Key words:** Universal precaution, nurse midwives performance, prevention of HIV transmission, infection control.

## 1. Introduction

HIV/AIDS (Human immunodeficiency virus and acquired immunodeficiency syndrome) problem is known to human-kind and has now affected virtually all the countries and continents in the world. The first cases were reported in the United States in 1981 and cases were reported from around the world in the immediate subsequent years. In 1983, the virus that caused HIV was discovered by scientists in France and the routes of transmission were identified [1].

HIV is the retrovirus that causes AIDS. AIDS infections includes opportunistic infections, growth problems, and developmental regression [2]. HIV has

been isolated from blood, vaginal and cervical discharge, wound secretion, breast milk, amniotic and other body fluids [1].

Midwives are prone to occupational infection (high risk group) from needle sticks or splashes from infected blood or body fluid to the mucus membrane or exposure of their broken skin, open cuts and wounds to blood or other body fluid from an HIV infected persons [3].

Midwives may transmit HIV to the patients by needles sticks or from patient to patient through contaminated instruments that are re-used without adequate disinfection or sterilization, so training program regarding infection control on nurse midwives practice is very important .

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### *1.1 Global Burden and National Burden (HIV/AIDS Situation in Sudan)*

The latest statistics on the world epidemic of HIV and AIDS estimate that more than 25 million people have died of AIDS since 1981. By December 2005, women accounted for 46% of all adult living with HIV worldwide, and for 57% in Sub-Saharan Africa [4].

According to the UNAIDS report, some 320,000 men and women are living with HIV/AIDS in Sudan. Unlike other countries in the region, Sudan decided to deal with this serious threat to the health and survival HIV was estimated as 1.4% among the adult population. The estimated number of affected women aged 15 or above is 170,000 with 25,000 affected children [5, 6].

From available data on the epidemic in Sudan, HIV started to spread in 1980s and the first AIDS case was reported in 1986. HIV/AIDS prevalence was low in the 1980s, but increased quickly through the 1990s and rose up to an estimated 2.6% of the adult population in 2002. A rate of 1% among pregnant women at sentinel surveillance sites in Juba. As no routine surveillance exists in rural areas, the level and trend of HIV/AIDS prevalence in these areas are difficult to estimate [7]. Since 2005, HIV has rapidly moved to the centre of government attention as a key health and development issue. A national response is being led by the SNAP (Sudan National AIDS Program) with the assistance of a number of national and international NGOs, with financial support and Global Fund to fight HIV/AIDS, tuberculosis and malaria over the periods 2005-2008.

According to SNAP, 94 free voluntary counseling and testing centers and 35 HIV/AIDS care and treatment centers have been established and equipped across 15 northern states, in addition to seven prevention mother-to-child transmission centers. So far, 2,100 HIV/AIDS patients are receiving free antiretroviral treatment [5-8].

Regionally, Sudan has the highest HIV/AIDS prevalence than any country in the Middle East [9]. The increasing prevalence of HIV increases the risk in health-care workers exposed to blood and other body

fluid from patients infected with HIV, especially when blood and body-fluid exposure precautions are not followed for all patients. Thus, nurse midwives must consider all patients as potentially infected with HIV and/or other blood-borne pathogens and to adhere rigorously to infection-control precautions for minimizing the risk of exposure to blood and body fluids of all patients. Application of the UPs (universal precautions) during the process of labor expected decreases spread of HIV infections on its all dimension from patient to patient, from midwives to patient, from patient to midwives, and from mother to child [3].

### *1.2 Role of Nurse Midwife at Labor and Delivery*

About 60% of HIV from MTCT (mother to child transmission) is thought to occur around the time of labor and delivery. So universal precaution should be followed by NMs (nurses/midwives) in all aspects of care regardless of the HIV status of the woman or the NMs at the time of labor and delivery to decrease the risk. The use of chlorhexidine 0.25% to cleanse the birth canal after each vaginal examination and during labor and delivery has been shown to be effective in reducing MTCT [10].

Vaginal deliveries are more likely to increase the risk of MTCT while elective caesarian sections have been shown to reduce MTCT. However, the potential benefits have to be balanced against the risk to the mother. Higher rates of post operative death in HIV positive women have been reported, especially from infective complications. In addition, elective caesarian sections are not available to the vast majority of women worldwide.

### *1.3 Universal Precautions*

Universal precautions is the name used to describe a prevention strategy in which all blood and potentially infectious materials are treated as if they are actually infectious, regardless of the perceived status of the source individual. HCWs (health care workers) do not have to make assumptions about people's lifestyles and

risk of infection. Health care workers should have the right to be able to protect themselves against infection, whether it is HIV, hepatitis or anything else. UPs must be applied in all invasive procedures. Delivery is one of the invasive procedures. An invasive procedure is defined as surgical entry into tissues, cavities, or organs or repair of major traumatic injuries in an operating or delivery room, emergency department, or outpatient setting. During a vaginal or cesarean delivery or other invasive obstetric procedure, bleeding may occur. Therefore, universal blood and body-fluid precautions should be the minimum precautions for all such invasive procedures [11].

The detailed components of UPs are as follows:

(1) Wash hands before and after every patient contact, and immediately if in direct contact with blood or body fluids, and avoid hand to mouth/eye contact.

(2) Use protective barriers such as gloves, gowns aprons, masks, goggles for direct contact with blood and other body fluids, mucous membranes or non-intact skin is anticipated and wash hands after their removal.

(3) Take precautions to prevent puncture wounds, cuts and abrasions in the presence of blood.

(4) Protect skin lesions and existing wounds by means of waterproof dressings and/or gloves.

(5) Avoid invasive procedures if suffering from chronic skin lesions on hands.

(6) Avoid use of or exposure to sharps and sharp objects when possible, but when unavoidable, take particular care in their handling and ensure approved procedures are followed for their disposal.

(7) Use of new, single-use disposable injection is highly recommended. Sterilizable injection should only be considered if single use equipment is not available and if the sterility can be documented with time, steam and temperature indicators. Discard contaminated sharps immediately and without recapping in puncture and liquid proof containers that are closed, sealed and destroyed before completely full.

(8) Protect the eyes and mouth by means of a visor,

goggles or safety spectacles and a mask whenever splashing is a possibility.

(9) Wear rubber boots or plastic disposable overshoes when the floor or ground is likely to be contaminated.

(10) Control surface contamination by blood and body fluids through containment and appropriate decontamination procedures.

(11) Use approved procedures for sterilization and disinfection of instruments and equipment.

(12) Clear up spillages of blood and other body fluids promptly and disinfect surfaces.

(13) Handle properly soiled linen. (Soiled linen should be handled as little as possible. Gloves and leak proof bags should be used if necessary. Cleaning should occur outside patient areas, using detergent and hot water.) Dispose of all contaminated waste safely.

(14) Disinfect instruments and other contaminated equipment. Document the quality of the sterilization for all medical equipment used for percutaneous procedures.

A “sharps” or “needle stick” injury is one in which blood or body fluid from one person is inoculated into another on the point of a needle, scalpel or other sharp object. However, the following advice also applies to spillage of blood or body fluids on to skin, especially broken or eczematous skin, mucous membranes or the eye. In case of any doubt seek advice (post exposure prophylaxis). The major health risks to someone receiving a sharps injury are Hepatitis B, Hepatitis C, and HIV [12].

#### *1.4 The Knowledge of Nurse Midwives*

The knowledge of NMs of HIV is important because it is the basis on which positive changes in behavior occur. It brings awareness, which in turn leads to action. Knowledge, training and experience in every aspect of one’s profession are very important. Knowledge is operationally defined in the study as the level or degree of information acquired by the nurse/midwife in relation to HIV/AIDS and UPs. On the other hand,

practice refers to direct goal oriented actions taken by the NMs in order to prevent the transmission of HIV from mother to others, from mother to midwives or vice versa, or from mother to child in the course of their professional duty as a nurse midwives. Behavior operationally refers to, the actions or reactions of the nurse/midwife, usually in relation to HIV/AIDS prevention and the working environment. Issues relating to HIV/AIDS have generated a lot of fear in the mind of health workers especially nurses and midwives. Some act inappropriately towards HIV sero positive clients. This makes one wonder if they had adequate knowledge about the mode of transmission of HIV and adequate knowledge and skill to prevent it [13].

## 2. Materials and Methods

### 2.1 Research Design

Aquasi-experimental research design was used to accomplish this study.

### 2.2 Setting

This study was carried out at labor rooms in six teaching hospitals, Khartoum state.

### 2.3 Sample

Out of 240, 180 nurse midwives providing direct care were randomly selected, followed stratified cluster sample design.

### 2.4 Tools

A structured interview questionnaire sheet was developed to assess nurse midwives knowledge attitude and skills. Observational checklist to assess their actual skills regarding Universal precautions pre intervention and post intervention.

### 2.5 Phases of the Study

#### 2.5.1 Pre Intervention Phase

Base line survey was conducted.

#### 2.5.2 Intervention Phase

A total of 176 nurse midwives were trained.

#### 2.5.3 Post Intervention Monitoring

Monitor and supervision were carried out monthly for six months after intervention to assess application of UPs, in the first two months they were observed in the morning shift, the second two months they were observed in the afternoon night shift and the last two months they were observed again in the morning shift. Post intervention data was collected after six months.

### 2.6 Validity of Instruments

The instruments were pre-tested in the pilot study ( $n = 30$ ) before final data collection.

### 2.7 Reliability

Test/re-tests of the research instruments were done before the actual administration of the questionnaire and check list. For reliability, the test-retest at a three week interval period yielded a Pearson correlation coefficient of 0.6713. This result confirmed that the instrument was suitable for the study.

## 3. Results

### 3.1 Demographic Characteristics of Respondents

The study was carried out in Khartoum state with 180 respondents, in six major civil teaching hospitals. The majority (148) of the respondents were married (82.2%). Apart from their midwifery certificate, their highest educational qualification was secondary school (28.3%). Additionally, they were mainly certified NMs and with more than 5 years experience in obstetrics and gynecology unit (91.1%). They received courses on HIV/AIDS as workshops/seminars and/or attended lectures on for the same purpose (77.8%), as presented in Table 1.

Analysis of the structured interview questionnaire showed that most of NMs knew the causative organism of HIV (88.3%). Their knowledge with regard to the pathophysiology is very weak (5.6%). Most of them knew the mode of transmission (91.4%). More than half of them knew that HIV has life-long incubation period (67.8%), but only 10% knew the window period

**Table 1 Socio demographic characteristics of the respondents (n, 180).**

	Percent (%)	
1. Marital status	Married	82.2
	Single	6.7
	Divorced	6.7
	Widowed	4.4
2. Education	Primary school	14.4
	Intermediate	57.2
	Secondary	28.3
3. Years in current occupation	Less than 5 years	8.9
	5 years and more	91.1
4. Previous courses on HIV/AIDS	Yes	77.8
	No	22.2

**Table 2 Knowledge of the study group about HIV/AIDS (n, 180).**

	Correct response percent (%)
Cause of HIV/AIDS	88.3
HIV attack the immune system	5.6
Mode of transmission blood or its products	98.9
Sexual intercourse	99.4
Non sterile syringe	96.7
Public toilets	84.4
Insect bite	73.3
Sharing sharp instruments	96.7
Mother to child transmission	90.6
HIV has incubation period	67.8
HIV/AIDS has a window period	10.6
Chronic cough is HIV sign	92.2
Enlargement of lymph node is HIV sign	92.2
Chronic diarrhea is HIV sign	98.9
Wt loss is HIV sign	100.0
Fever is HIV symptom	93.3
Skin cancer is HIV sign	94.4
Total mean	81.5

of HIV while most of them knew the signs and symptoms of HIV/AIDS (Table 2).

### 3.2 Knowledge of NMs towards Prevention of HIV and UPs

All NMs knew that isolation is one of the items of infection prevention. They also knew that prevention of HIV could be by avoidance of non marital sexual relationship which is the most common mode of transmission in Sudan. 79.8% of them knew that barriers are important but only 25% knew the importance of the use of goggles; less than half of them

knew the proper handling and disposal of sharps, thus, 80% of them had needle stick injury and few of them reported it (23%). Most NMs used gloves which were good strategies in prevention of infection. Concerning resources, more than half of NMs agreed that they were not adequate and sometimes were not available. 70% of NMs knew sterilization and disinfection while the proper handling of linen was known by 67.8% of them. 87.8% of the NMs knew the importance of the use of a septic technique when caring the airways for the newborns.

PMTCT (prevent mother to child transmission) were

also known by most of them (80%) (Table 3).

Result of pre-training test about knowledge of NMs towards prevention of HIV and used of UPs during labor was 70.6%, but after training increased to be 87%.

### 3.3 Attitude of the NMs towards HIV

72.8% of respondent mentioned that the way the patient acquired infection affected their attitude positively and negatively, also most of them said they would change the way of care if they discover positive HIV cases. More than half of them considered other HCWs, e.g., doctors as a good example for proper care while others considered them not following Ups, e.g., not wearing gloves for intravenous infusion or insertion of cannula. Approximately more than 76% of

the NMs are worried and have fear during their patients care, half of respondent think that clients accept the use of barriers while other considered it may interfere with midwives client relationship, and 56% of NMs accept the idea of screening while the rest were afraid from the results (Table 4).

Pre intervention or base line data was collected by observational check list regarding application of the universal precaution practices by NM was 40.9% (Table 5 and Fig. 1).

### 3.4 Results of Post Intervention Application of UPs First to Six Months in the Memory Gap

Analysis of the observation check list carried in the morning shift showed that in the first month there was marked increase of application and then started to

**Table 3 Knowledge of the study group about prevention of HIV transmission (universal precautions or safe labor) (n, 180).**

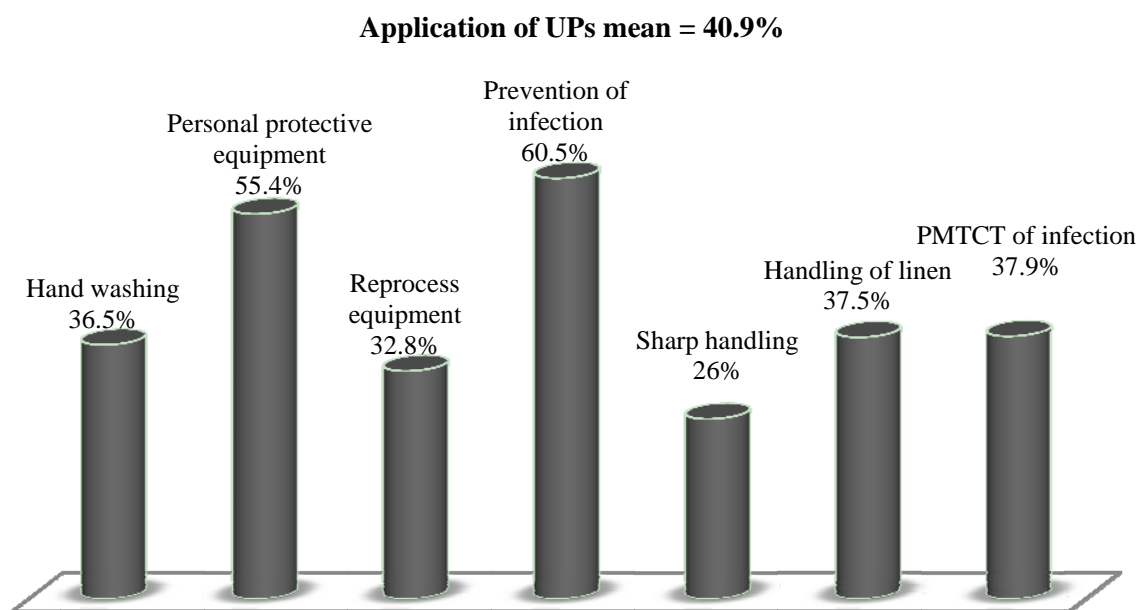
	Correct response %
Barriers prevent HIV transmission	54.4
HIV infection can be preventive by isolation of the infected cases	100.0
Abstinence non-marital relationship will prevent HIV transmit ion	99.4
Number of scissors used in the process of the normal labor	80.0
Type of needle used in suturing	56.7
Importance of using needle holder and forceps during suturing	57.8
To perform oral-nasal suction using a septic technique for each child	87.8
Action taken when having needle stick	78.9
Have a needle stick injury	80.6
Report needle stick injury	23.9
Using gloves for PV examination	100.0
Using gloves for enema	83.3
Using gloves when decontaminating the delivery set	99.4
Using gloves when inserting cannula	95.6
Using gloves for decontamination of the delivery table	98.9
Using gloves for cleaning the newborn	86.7
Wearing plastic apron when attending to child birth	97.8
Glove is one of the barriers	100.0
Mask is the one of the barriers	98.3
Goggle is the one of the barriers	25.0
Over shoes is the one of the barriers	18.9
Proper disposal of the sharps (syringes after used)	23.3
Number of the delivery set and other resources are adequate	43.9
Proper handling and decontamination of linen and surface contaminated with blood and body fluid	67.8
Sterilizations means destruction of all micro organism and their spores	77.8
Disinfection means destruction of micro-organism but not the spores	58.9
Boiling for 20 minutes means high level disinfection	12.2
Total mean	70.6

**Table 4 Attitude of the NMs towards HIV/AIDS (n, 180).**

	Responses %
Mode of transmission affects your behavior towards the infected person	72.8
Change your way of care if you discover HIV positive patient	37.2
consider other health providers example in taking the necessary preventive precautions	58.3
Protective cloth psychologically accepted by the client	54.4
Feel anxious and fear while conducting labor	77.8
Accept the voluntary screening of HIV	56.7
Total mean	60.0

**Table 5 Base line application of Ups items by the NMs during the process of labor (n, 180).**

Item applied	Mean	%
Hand washing	65.7	36.5
Use of protective equipment	99.7	55.4
Reprocessing of the equipments	59.1	32.8
Prevention of infection in the labor	108.9	60.5
Proper handling and disposal of sharps	46.8	26.0
Proper handling of the soiled linen	67.5	37.5
Prevention of mother to child transmission of HIV infections	68.2	37.9
Total mean		40.9



**Fig. 1 Base line application of Ups items by the NMs during the process, pre-intervention (n, 180).**

decrease in the second month. On the other hand, there was marked reduction of application in the third and fourth months that was observed in the afternoon night shift. The application increased again in the fifth and sixth months when they were observed in the morning shift but still there was rhythmic reduction compared to first and second months as shown in Table 6 and Figs. 2 and 3.

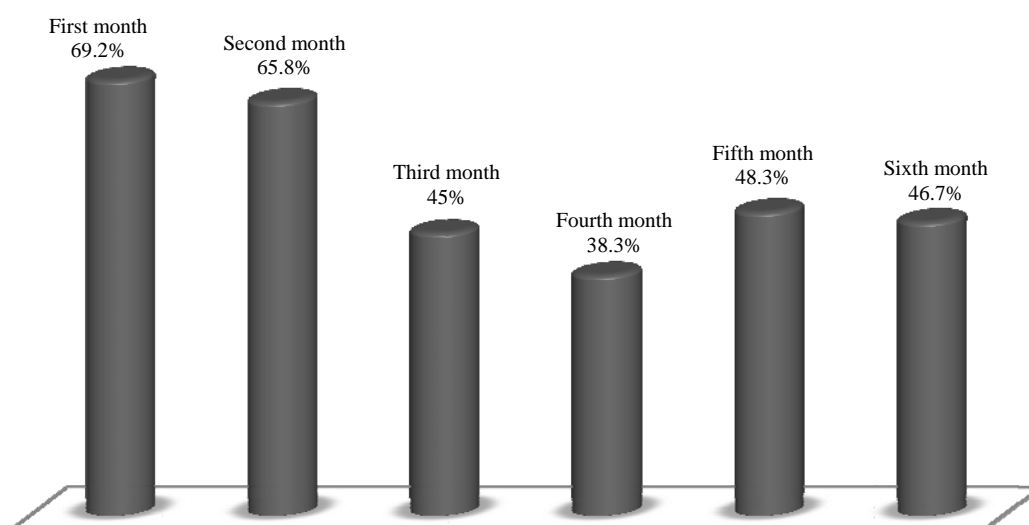
### 3.5 Application of Universal Precautions

Analysis of the observational check list of pre intervention compared with post intervention showed a significant increase, whereas pre intervention application of UPs was 40.9%, the post intervention was 55.2% with  $t = 5.877$  and  $P = 0.000$ . (Fig. 4).



**Table 6** Application of UPs post intervention data monitoring in the memory gap first to six months (*n*, 72).

Months	First	Second	Third	Fourth	Fifth	Sixth
UPs						
Hand washing	8.3 (69.2%)	7.9 (65.8%)	5.4 (45.0%)	4.6 (38.3%)	5.8 (48.3%)	5.6 (46.7%)
Use of protective equipment	6.4 (53.3%)	6.0 (50.0%)	6.1 (50.8%)	6.1 (50.8%)	6.3 (52.5%)	6.6 (55.0%)
Reprocessing of the equipment	4.5 (37.5%)	3.0 (25.0%)	4.0 (33.3%)	2.5 (20.8%)	3.0 (25.0%)	4.0 (33.3%)
Prevention of infection in the labor	8.5 (70.8%)	8.0 (66.6%)	8.0 (66.6%)	6.0 (50.0%)	9.5 (79.2%)	7.0 (58.3%)
Proper handling and disposal of sharps	3.0 (25.0%)	1.3 (10.8%)	4.3 (35.8%)	2.5 (20.8%)	2.1 (17.5%)	3.7 (30.8%)
Proper handling of the soiled linen	6.0 (50.0%)	8.0 (66.6%)	3.5 (29.2%)	6.0 (50.0%)	5.5 (45.3%)	6.5 (54.2%)
Prevention of MTCT of HIV	6.6 (55.0%)	6.6 (55.0%)	6.0 (50.0%)	4.8 (40.0%)	6.0 (50.0%)	5.0 (41.6%)
% of mean score of total application of UPs	51.5%	48.6%	44.4%	38.7%	45.5%	44.9%

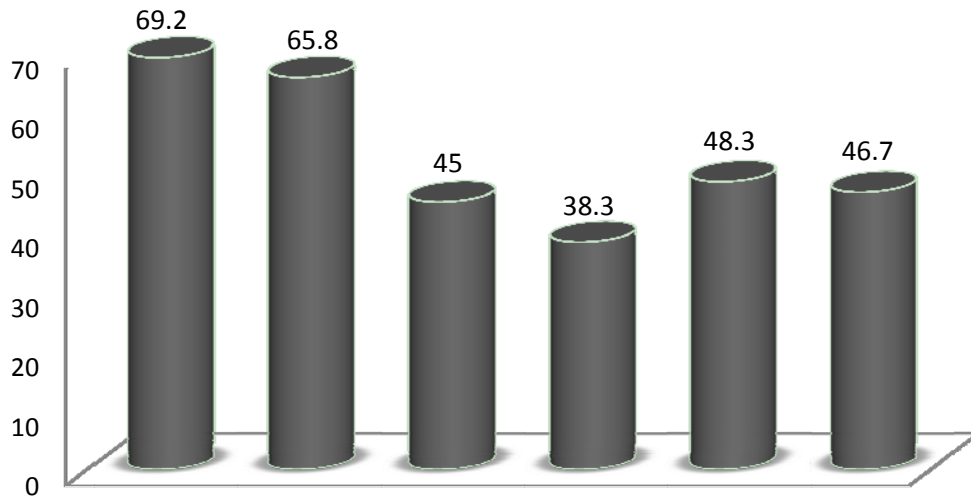


**Fig. 2** Hand washing post intervention monitoring in the memory gap 1-6 months (*n* = 72).

#### 4. Discussion

Education is a very crucial element for NMs, particularly in countries where there is lack of formal and well-organized infection control programs. Despite limited resources, developing countries, such as Sudan, still have to deal with complex issues related to enforcement of standard precautions. In this context, exposure to risk is increased, because of the inadequate supply of personal protective equipments, improper disposal of medical waste, and lack of effective sharp instrument disposal systems. In this study, despite these conditions, universal precautions application

scores increased significantly after training as shown in Figs. 4 and 5 and Tables 7, 8 and 9, this is supported by the study that mentioned universal precautions knowledge scores increased significantly after training ( $P < 0.0001$ ). Personal assessments of the risk of developing HIV due to patient care was significantly decreased ( $P < 0.0001$ ) and willingness to provide care for AIDS patients was increased ( $P = 0.004$ ) [14]. The study “Effects of Training Programme on HIV/AIDS Prevention among Primary Health Care Workers in Oyo State, Nigeria” showed that: at pre-test, only 30.8% of trainees could list at least four signs and symptoms of AIDS compared with 70.9% who could do so after the



First, second months were observed in the morning shift, third and fourth months were observed in the afternoon night shift and fifth and six months were observed again in the morning shift.

Fig. 3 Application of Ups by NMs in the memory gap post-intervention monitoring 1-6 months, (n = 72).

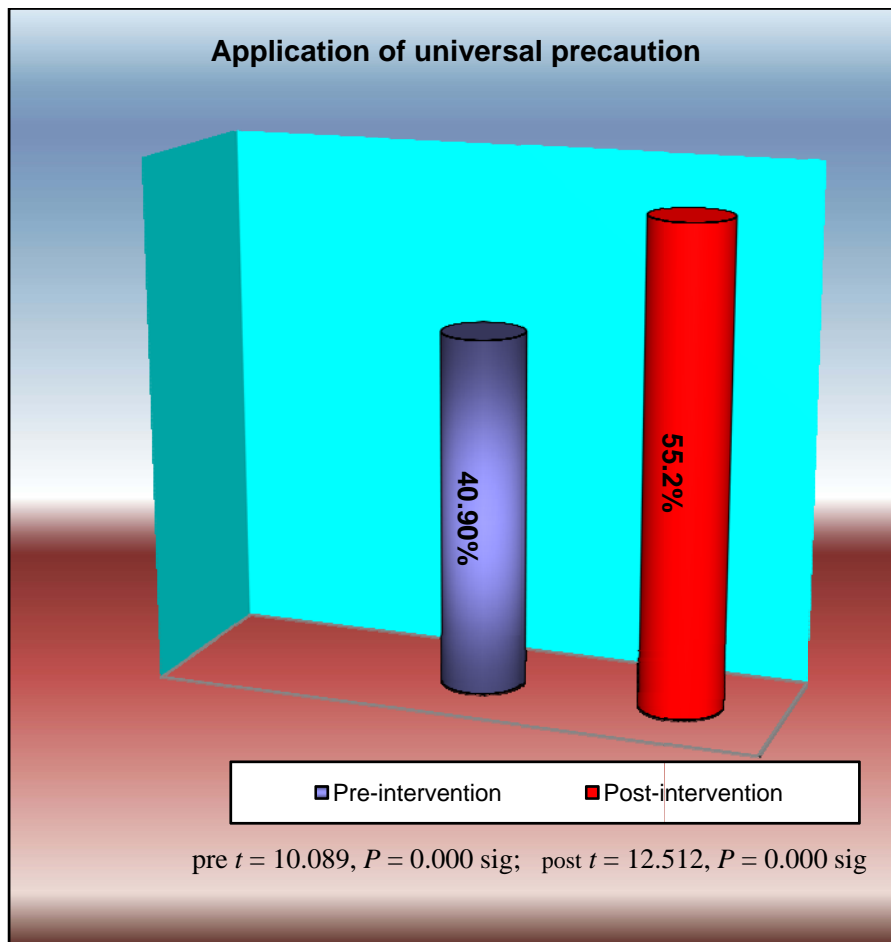


Fig. 4 Application of UPs by the nurse midwives during the process of labor pre intervention compared with post intervention.

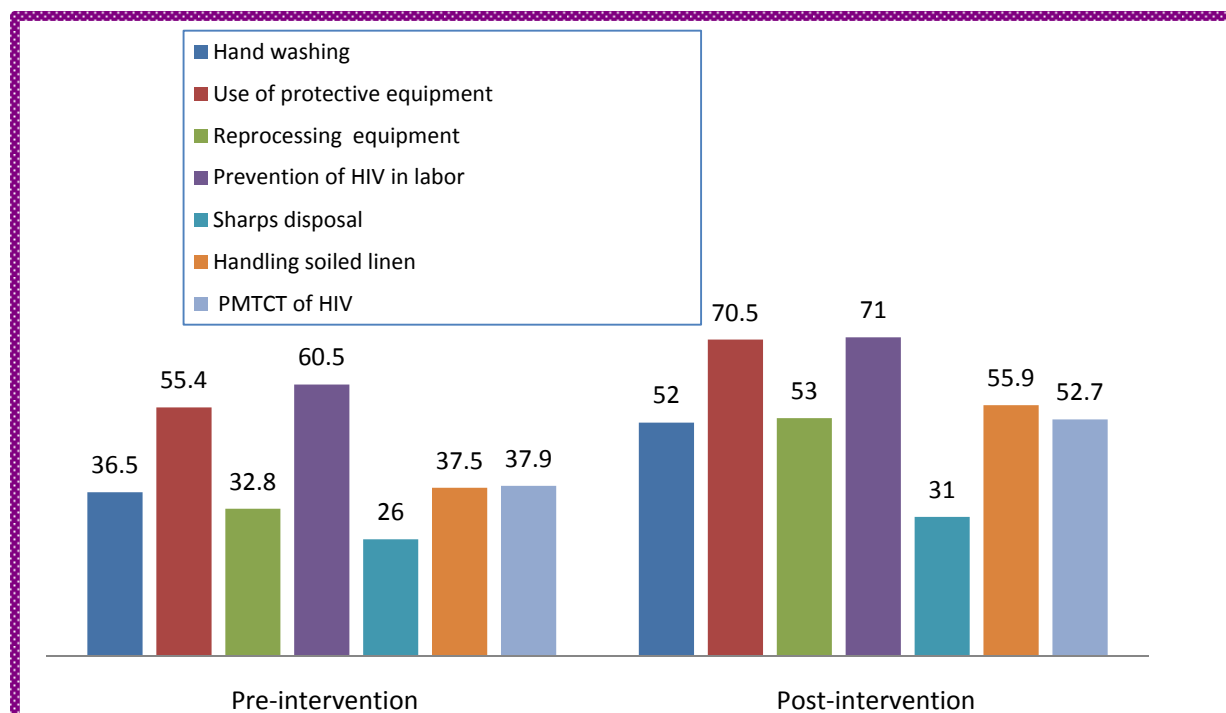


Fig. 5 Detailed results of application of universal precautions by the nurse midwives pre intervention (n, 180) compared with post intervention (n, 169).

Table 7 Application of UPs by NMs, post intervention after six months (n, 169).

Item applied	Mean	%
Hand washing	87.9	52
Use of protective equipment	119.1	70.5
Reprocessing of the equipment	89.6	53
Prevention of infection in the labor	119.9	71
Proper handling and disposal of sharps	52.4	31
Proper handling of the soiled linen	94.5	55.9
Prevention of mother to child transmission of HIV infections	89	52.7
Total	93.3	55.2

Table 8 Detailed results of application of universal precautions by the nurse midwives pre intervention (n, 180) compared with post intervention (n, 169).

Item applied	No. of responses Pre intervention	No. of responses Post intervention	$\chi^2$	P
Hand washing	65 (36.5%)	87.9 (52%)	21.75	0.0013
Use of protective equipment	99.7 (55.4%)	119 (70.5%)	54.42	0.000
Reprocessing of the equipment	59 (32.8%)	89.5 (53%)	0.35	0.5542
Prevention of infection in the labor	109 (60.5%)	120 (71%)	2.16	0.141
Proper handling and disposal of sharps	47 (26%)	52.3 (31%)	4.59	0.1000
Proper handling of the soiled linen	67.5 (37.5%)	94.5 (55.9%)	6.21	0.0127
Prevention of mother to child transmission of HIV infections	68.2 (37.9%)	89 (52.7%)	8.87	0.0644

Table 9 Result of compared mean one sample T test.

Item applied	Mean difference	T	P
Score of total application of universal precaution pre intervention	40.937	10.089	0.000
Score of total application of universal precaution post intervention	55.200	12.512	0.000

training. The evaluation conducted after four months of implementation of the community-based projects showed improvement in knowledge of HIV among all the target groups [15].

Other study showed that cases of injuries by sharp devices before and after the implementation of intervention methods, were markedly reduced, from 55.2% to 19.5% ( $P < 0.05$ ). The study concluded that a large number of injuries by contaminated sharp devices can be prevented by implementing suitable educational programs regarding disposal of sharp devices [16]. In rural Bangladesh, it is found that trained TBAs (traditional birth attendants) were significantly more likely to practice hygienic delivery than untrained TBAs [17]. The study of impact on knowledge, attitudes and compliance with universal precautions revealed that a number of positive changes occurred over the period of the study. Not only were the Nigerian nurses better informed about AIDS than previously, but their attitudes towards the disease and patient care had become considerably more liberal, as well as their disposition to comply with universal precautions [18].

The study conducted in the United Arab Emirates for prevention of sharps injuries training improve the situation resulting in underreporting of sharps injuries [19].

On the other hand, Goldman [20] argued that training midwives had little effect on the quality of midwife care.

The present study revealed that performance of the midwives in the first month immediately following intervention was very high compared with the following months in the memory gap and there were marked reduction of UPs application in the months monitored in the afternoon night shift (Table 6, Figs. 2 and 3). This may be due to lack of supervision and decreased number of the staff or supplies. Similar to this was mentioned in the Province of North Sumatera, knowledge and skill of midwives were significantly increased after given health promotion, but there was significant decrease of knowledge in the coming period

and after one month after promotion. Lund [21, 22] mentioned that during each of the three shifts, 88% of all high-level contacts to blood and body fluids were limited to the hands; 47% of these contacts occurred during the night shift.

For sustainability of the effect of this training program, we need a strong infra structure and effective midwives as highlighted by Maclean who showed the importance of the three “Es (education, environment and effectiveness)” of skilled attendance at birth: the education of the skilled attendant, the environment in which she practices and the effectiveness of the skilled attendant, as supported by O’Heir [23, 24] who concluded that Midwifery education for safe motherhood was relevant and easy to use, but will not be applied because of weaknesses in the health system infrastructure.

The nurse/midwives’ knowledge of HIV is important because it is the basis on which positive changes in behavior and practice will be adopted to enable them to prevent the transmission of HIV in the course of their professional duty. This study revealed that nurse/midwives had high level of knowledge towards HIV and UPs (Tables 2 and 3). This agreed with the study of compliance with UPs. Knowledge and behavior of residents and students in a department of obstetrics and gynecology were assessed; knowledge regarding UPs was nearly 100%, while overall observed compliance was only 89%. Compliance with UPs was better among students (96%) than among residents (88%). Compliance with universal precautions was inversely related to years of experience [25]. In Iran, similar study concluded that registered nurses and midwives had a significantly higher level of knowledge about HIV infection. Also the knowledge, attitude, and practice regarding infection control and standard precautions among a group of nursing and midwifery instructors and students in Iran were assessed, and they found that there was a linear positive correlation between knowledge, practice, and attitude scores for the group

of nursing and midwives [26, 27].

But on the other hand, Hani Nawafleh [28] concluded that lack of nursing leadership and role models, cultural beliefs and geographic isolation are factors that reduced the capacity of the primary care nurses to raise awareness, and therefore influence the prevention and control of HIV/AIDS.

This study showed that NMs level of knowledge towards HIV and Ups is always higher than their practices even after intervention as illustrated by Tables 2, 3, 5 and 7, and Figs. 1 and 6.

This was similar to what was mentioned in the South Thames Region of the UK conclusion of the study “Knowledge obtained within midwifery education may be difficult to be translated into clinical practice” [29].

The prevalence of needle stick injury, as shown by this study, was very high among midwives who were have needle stick injury while suturing because of the fact that they did not use a needle holder as shown in Table 3 and this is similar to what was mentioned by Ismail in Egypt, who found that exposure to needle stick injuries were common among the HCWs (66.2%). Also, in the study of needle stick injuries among nurses in Sub-Saharan Africa showed that 57% of the nurses and midwives had experienced at least one needle stick

injury a year. Only 18% had not experienced any such injury in their entire career. The study showed a high rate of needle stick injuries among nurses and midwives working in Uganda, and the strongest predictor for needle stick injuries was lack of training. Other important risk factors were related to long working hours, working habits and experience [30, 31]. Other study showed that 50% of injuries occurred in the first three years of employment. The middle hours of the shifts showed a significant association with injuries. The pattern of injuries was significantly associated with overloaded periods of medical practices. Deficiencies in implementing the standard recommendations for prevention and control of needle stick injuries were noted in the studied hospitals [32]. Other study showed that only 20.9% of the participants prone to needle stick injuries, and it is almost similar to the United States study. Although only a small percentage was unfamiliar with UPs, almost half did not use UPs in practice, and more than 20% had needle stick injuries [33, 34].

This study showed that NMs have good attitude towards HIV patients, the major factors that influence behavior were mainly the fear of getting infected; irregular supply of resources may be an important reason too, as shown in Table 4. Reda et al. [35] showed

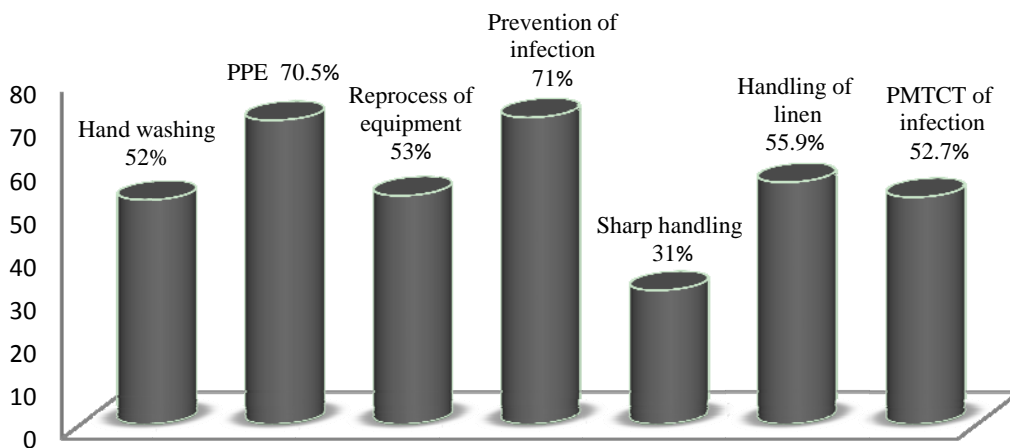


Fig. 6 Post intervention after six months application of Ups items by the nurse midwives during the process of labor (n = 169).

that there were no studies describing the attitude and behavior of HCWs.

A study in Owerri, Imo State, Nigeria, showed that NMs had moderate level of knowledge and behavior but still had gaps in certain areas. Their behaviors were fairly appropriate [13].

## 5. Conclusions

The studies concluded that the training program was effective in increasing NMs application of universal precautions. The NMs were highly knowledgeable, but their knowledge did not reflect on their performance. Their attitude towards HIV was fairly appropriate. High rate of needle stick injuries occurred among NMs.

## 6. Recommendations

Creation of qualified organs to supervise the application of the universal infection control precautions so as to guarantee the sustainability, viability and efficacy of the program.

Managers are to be made responsible for providing the appropriate protective measures to enable midwives to perform their duties safely, thus, the safety of the women they care for is ensured.

Further studies recommended on Performance of NM regarding UPs.

## Acknowledgments

The author gratefully acknowledges University of Khartoum and Ministry of higher education in Sudan for providing financial support. The author also thanks Dr. Magda Elhadi Ahmed from University of Gezira, primary Health Care and Health Education Center for her kind suggestions and advices.

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# The Undergraduate Medical Students' Approach to Learning in University of Latvia

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**Abstract:** Learning approach of medical students has been pointed out as one of the most important long term success factors for medical career. There are two major differences how students learn. One group of students use deep approach learning that is characterised by understanding of matter of studies. Other part of students using surface approach of learning that is based on memorisation of facts and knowledge. It has been recognised that deep approach learning is more used in modern medical schools but surface learning in traditional medical schools. The purpose of this study was to evaluate, describe and compare the learning approach of students at the University of Latvia in the academic year of 2009/2010. University of Latvia represents traditional medical school, which uses traditional curriculum. The assessment was done using Entwistle's Short Inventory of Approaches to Learning consisting of 30 items. The inventory was answered by a total of 345 students. Findings show that learning approach has trend towards surface approach of learning in six groups studied in the University of Latvia in year 2009/2010. It has been recognised that reproduction and learning pathology were found increased in all study years. Results might suggest that modernisation of undergraduate curriculum including assessment with target to improve the way how medical students learn could be topic of discussion in senior management of University of Latvia. Study allows compare findings of this study to similar studies where the same instrument is used for evaluation of learning approaches in longitudinal prospective. It gives opportunity to compare results within findings of medical schools of Europe.

**Key words:** Medical students, curriculum, educational measurement, learning approaches, learning style.

## 1. Introduction

One of the most outstanding features of teaching in traditional schools of medicine is that the cognitive aspects are the most emphasised ones. The curriculum is organised on pieces of information in different areas, which are considered necessary for students to learn in order to accomplish suitable practical work later during the postgraduate studies. Students use different strategies, tactics and skills in their studies [1-4]. There are three approaches to learning recognised: deep, surface and strategic [5, 6]. How medical students learn may have special implications for teaching and learning in a medical curriculum, particularly in activating and sustaining motivation. The current model of University of Latvia is centred in teaching where teacher is in the middle of educational process. Teachers controlling what, when and how will be done.

Little competence has been given to students themselves. Teacher's based educational process is based on the transmission of information to students [7-10]. Education is carried out through a defined way of delivering to the students some theoretical information through lectures and bringing them to the laboratory or to the hospital for practical work. It is known that teaching methods influence the way how students learn [10-17]. It has been also shown that assessments and examinations influence students' learning styles and approach to studying. Therefore, it can be expected that this definite way of teaching and evaluating has influence on the learning style of students who have undergone the same teaching model [18-20].

While the need for meaningful learning experiences for medical students has always been recognised, little enough is known about the learning tendencies lying within medical students for such experiences to be developed [21].

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The undergraduate curriculum of University of Latvia has been established in the Medical Faculty. More objective assessment forms were implemented [22, 23], such as objective structured clinical examinations. Library internet facilities have been implemented [9, 23].

This article will attempt to review the current information how students learn with particular relevance for the medical teacher and academic staff.

The purpose of this study is to describe the learning approach of students belonging of 6 different study years in the academic year of 2009/2010. It might be important to find out how students learn at the University of Latvia. Are they using deep approach with understanding or they tend to memorise subjects and pass exams? It will be highly interesting to see how learning approach changing during study period. How the learning styles of the University of Latvia Curriculum influence students. These findings might be taking into account when new curriculum created.

## 2. Approaches to Learning

The study followed the three approaches statement proposed by Newble et al. [24] and Entwistle et al. [25]. The three approaches to learning can be categorised by identifying the following characteristics: predominant factors, which motivate students, the primary intention of students, and the learning process, used to fulfil those intentions. According to these factors, the authors recognise three approaches to learning: deep, surface and strategic.

Students who adopt the deep approach are predominantly motivated by an interest in the subject material and/or recognition of vocational relevance; their study methods are addressed to understand meaning of a subject and relating it to previous knowledge and personal experiences.

Students presenting the surface approach are mainly motivated by either a desire simply to complete the course or a fear of failure; their goal is to pass the course and it is fulfilled mainly through memorising

and reproducing the material they believe is likely to appear in the evaluation tests.

The third approach to learning proposed by Newble et al. [24] and Entwistle et al. [25] is the strategic approach. The main motivation of the students who adopt this approach is competition and the achievement of high grades. They are prepared to use whatever means are necessary to achieve success, so they can elect to use either the surface or the deep approach, depending on which is necessary to get the most successful results.

## 3. Materials and Methods

### 3.1 The Subject

The curriculum of Faculty of Medicine of University of Latvia is a traditional one. According to the curriculum, the students' experience has a sequential, subject-based, teaching-centred model [26].

It is divided into two parts. The first part is the preclinical curriculum which deals with the basic sciences necessary for the understanding of medicine such as anatomy, histology, biology, chemistry, physics, physiology, etc.. This part of the course lasts for four terms. The students have no interaction with patients and the academic pressures are intense. Some adverse effect on motivation might be expected during these four terms because of lack of clinical input.

The second part of the curriculum starts in the fifth term although some subjects are continued to be preclinical ones. Medical students begin their clinical course. This is an important term as it forms as introduction to clinical studies and lays the basis forward work in the following terms. After these 6 years of undergraduate training, the graduates have to finish specialised internship and they can work as general practitioners.

### 3.2 The Instrument

A questionnaire was drawn up using the 30 items of Entwistle's Short Inventory of Approaches to Learning [5, 6]. This Inventory is a shortened version of Entwistle's

60-item Lancaster Approaches to Learning questionnaire and is answered on a scale from 4 (definitely agree) to 0 (definitely disagree). The Short Inventory was selected for an initial study to investigate students learning approaches in the Medical Faculty, University of Latvia. The same tool of assessment of learning approaches has been used in many of European and World medical schools [23, 27, 28]. It has been investigated in Riga Stradins University in years 1994/1995 and 1999/2000. The questionnaire was translated from English into Latvian and evaluated by language professionals [21].

Each factor and its definition are shown in Table 1.

### 3.3 Sample

The study was conducted in the Faculty of Medicine, LU, and Riga, Latvia. In the academic year 2009/2010, the questionnaire was distributed to the class of each study year at the beginning of a small group session. The sample consisted of first year, second year, third year, fourth year, fifth year and sixth year students. The total number of students was 339. Students were asked

to complete them on the spot anonymously. The 30 items of the questionnaire plus study year formed the variables. The response rate in the year 2009/2010 for the questionnaire was 100%. Table 2 shows the student number that responded in every study year.

High response rate is explained by distribution of questionnaires in small group sessions and due to involvement of teacher. Results for each student on each variable on the combined scores were computed using SPSS (Statistical Package for the Social Sciences). The data were obtained using the independent test. Data of eight scales were analysed by using the Kolmogorov-Smirnov test that shows normal distribution of collected data. This allowed further analysis to perform by parametric methodology that gave opportunity to see differences between study years—ANOVA (analysis of variance) was used to evaluate differences.

## 4. Results

The Entwistle's Short Inventory of Approaches to Learning was answered by a total of 339 students in the

**Table 1 Inventory scales and their meaning.**

Inventory scales	Meaning of scales
Achieving	“Strategic” approach Organised study methods Competitiveness Motivated to achieve
Reproducing	“Surface” approach Rote-learning and memorisation Extrinsically motivated Influenced by lure of qualifications
Meaning	“Deep” approach Interested in medicine for itself Intrinsically motivated
Comprehensive learning	“Holist” approach Broad perspective of learning task Relates concepts to wider context
Operation learning	“Serialist” approach Step-by-step sequential and detailed approach
Improvidence	Jumping to conclusions on insufficient evidence
Globetrotting	Over-emphasis on details
Versatility	Ability to adopt either approach according to demands of learning task
Learning pathologies	Failing to see how topics fit into overall picture Best prediction for overall academic success highly organised study methods with versatile approach
Prediction to success	Strong motivation Some tendency towards competitiveness Lack of doubts or fear to failure

**Table 2 Total No. of questionnaires distributed and returned in the academic year of 2009/2010 for students in six different study years.**

The year of study	Total number of students	Total number of distributed questionnaires	Total number of returned questionnaires	Returned questionnaires in %
Year one	143	102	102	100
Year two	86	59	59	100
Year three	106	54	54	100
Year four	73	56	56	100
Year five	60	43	43	100
Year six	31	25	25	100
Total	499	339	339	100

year of 2009/2010 at the University of Latvia, Faculty of Medicine. Each individual received a score according to his/her answers. The average of all individual scores for each category was obtained in each study year. Similar study has been performed by author also in 1994/1995 and 1999/2000 study years that allows give some insight by comparing learning approaches in longitudinal period (Table 3). Previous studies were carried out at Riga Stradins University.

Comparing overall differences statistically significant differences ( $P \leq 0.05$ ) were found in scales of reproduction, learning pathologies and prediction of

success. Other differences were not statistically significant ( $P > 0.05$ ).

Comparing achievement/motivation scale in Year 1 and in Year 6 scores indicates substantial differences at the 5% level of significance. It has been recognized that last year students have higher motivation comparing with learners in study Years 3, 2 and 1.

Comparing reproduction scale for Year 1, Year 3 and Year 5 scores indicates substantial differences at the 5% level of significance. Year 3 students has lower tendency to memorize knowledge to compare with Year 1 students but at their Year 5 reproduction score

**Table 3 Inventory data means and standard deviations (SD) for different year students in the year 1994/1995, 1999/2000 and 2009/2010.**

	Year 1			Year 2			Year 3			Year 4			Year 5			Year 6		
	94/95	99/00	09/10	94/95	99/00	09/10	94/95	99/00	09/10	94/95	99/00	09/10	94/95	99/00	09/10	94/95	99/00	09/10
Achievement motivation	16.36	16.18	15.88	16.14	15.79	15.93	14	15.08	15.24	15.43	15.09	16.38	13.98	15.06	16.44	14.14	15.69	17.6
SD	3.18	3.95	3.29	2.46	3.32	3.49	3.18	3.5	2.63	3.77	3.15	3.2	3.29	3.73	4.03	3.65	3.85	4.19
Reproducing	16.76	16.39	17.3	15.72	16.52	16.07	15	15.26	15.49	16.31	16.96	16.12	16.98	15.56	17.42	16.6	15.71	16.33
SD	3.77	4.18	3.91	4.8	3.34	3.32	4.18	3.92	3.79	3.42	4.03	3.46	3.45	3.53	4.62	3.46	3.6	4.55
Meaning	15.42	15.35	17.27	16.86	15.93	16.53	15.45	14.97	16.29	17.2	15.78	17.02	14.19	14.44	16.74	14.51	14.74	16.88
SD	3.73	4.27	3.02	3.15	3.31	3.32	3.05	3.34	2.96	7.87	2.88	3.41	4.03	2.96	4.11	3.59	3.94	3.8
Comprehensive learning	14.97	14.08	14.1	14.59	14.48	13.81	14.35	14.15	14.44	14.97	15.65	13.98	15.48	13.94	14.96	16.4	13.44	15.72
SD	3.14	2.91	2.77	2.5	2.52	2.61	3.67	3.06	2.82	3.25	2.79	2.85	3.49	2.15	4.16	2.93	3.75	3.61
Operation learning	13.48	12.85	13.5	11.27	11.93	12.49	11.15	11.18	13.33	12.68	11.63	13.38	12.1	12.53	13.52	12.8	11.07	14.36
SD	3.48	3.71	3.58	3.13	2.96	3.69	3.66	2.96	3.64	3.17	3.13	3.41	3.48	3.73	5.24	3.51	3.04	5.1
Versatility	32.79	31.48	34.17	33.93	32.64	34.29	34.7	31.92	34.47	35.14	33.35	35.4	30.53	31.31	33.79	31.94	30.58	35.4
SD	5.55	7.11	5.07	5.28	5.39	5.58	5.41	6.3	5.41	8.17	3.87	5.7	5.01	4.86	7.37	5.5	6.38	6.14
Learning pathologies	27.85	27.08	28	24.52	26.21	24.77	24.35	23.64	24.96	26.03	23.7	24.98	28.25	25.16	28.84	28.37	24.11	27.54
SD	6.05	6.91	6.45	7.15	5.52	5.76	6.72	6.51	5.96	5.95	5.34	5.74	6.42	4.86	9.45	5.26	6.43	9.4
Prediction of success	69.3	68.59	70.07	73.55	70.21	73.15	69.35	71.36	72.84	72.54	72.74	74.74	64.23	65.16	69.13	65.1	65.13	72.92
SD	9.42	12.9	9.54	9.96	10.26	9.52	10.52	11.4	10.03	11.04	7.69	9.05	10.21	4.86	12.31	7.27	6.35	7.35

rises back to the level of first year and stays high until the end of Medical School.

There were no statistically significant differences in scale of meaning for students in different study years.

Comparing scale of comprehensive learning results shows statistically significant higher scores for the Year 6 students to compare with learners from Year 1, 2 and 4. Last year students might be characterized as meaningful learners.

Comparing scale of comprehensive learning scores shows statistically significant higher meaning and comprehensive learning scores in Year 4 with a less orientation towards reproducing.

Comparing Year 4 and Year 5 scores shows non-significant higher reproducing score in Year 5 with a decrease of score for meaning.

Looking at learning pathologies significant differences have been recognized for study Year 1 and study Year 5 that shows statistically significant higher usage of learning pathologies.

Comparing prediction of success scale, it has been recognized that students try to achieve best results during their Year 4 of studies to compare with study Year 1 and the study Year 5. At the end of studies, students have attempting their best academic achievements above the average of study period.

## 5. Discussion

In the world of medical education the most attention has been given to the evaluation of the curriculum, conduction of assessment and implementation of modern technologies. Little attention is directed to the way students learn [8, 25, 29-31].

A finding of this study is that, the learning approach has trend towards surface approach of learning in six groups studied in the University of Latvia in year 2009/2010. Study results of 2009/2010 shows statistically significant improvement of achievement motivation and comprehensive learning in Year 6. This trend has not been identified in previous investigations 1994/1995 and in 1999/2000 [22]. It has been

recognised that improvement is only in last year of their studies. However, scores for reproduction and learning pathology were found high within all study years. It is reasonable to expect that the educational experiences of the students during their undergraduate education could have modified progressively by using deep approach of learning [1-3, 5, 28, 32-34].

The results reported in this paper showed a similar pattern of learning approach in 228 students of six different levels in two years of traditional school of medicine in Riga, who answered Entwistle's Short Inventory of Approaches to Learning with 30 items in 1994/1995 and in 1999/2000 [22]. The founding suggested that students tend to be surface and strategic learners more than deep approach users [22].

The achievement motivation in the academic year 1999/2000 is significantly higher in Year 6 compared with the academic year 1994/1995 that highlighted probability of positive results of elements of modernisation of curriculum. It should be pointed out that motivation is not decreasing during the six study years but stays on the same level compared with study conducted in the year 1994/1995. The same study was conducting in modernised medical schools. Students in modernised medical schools has been found as deep approach learners to compare with surface approach learners in traditional medical schools and during studies they adopt deep approach learning habits versus traditional medical schools [9, 27, 34].

Results might suggest that modernisation of undergraduate curriculum with target to improve the way how medical students learn could be topic of discussion in senior management of University of Latvia.

In this study, it was not possible to incorporate student assessment of important environmental factors such as the context of learning, influence of curriculum, new technologies and impact of stress, social circumstances and teaching styles.

A further study could be developed with a follow-up of students involved in the investigation. This should

allow the establishing a valid comparison for the same group of students in different stages of the curriculum.

The independent study in learning approaches could take place sometime after graduation from a medical school to see “deep” and “surface” approach students’ evaluation and their achievements of carrier.

The feedback session on the results of this study could help students to see what teachers expect from them.

It would be valuable to see further investigations of approaches to learning with a view to modifying education strategies for traditional medical schools as the Riga Stradins University and the Faculty of Medicine, University of Latvia.

## 6. Conclusions

Findings reported in this article showed a similar pattern of learning approach in 339 medical students of six years of studies of University of Latvia. Data suggests that progression through the traditional curriculum is associated with a more surface approach to learning than progression through modernised medical curriculum.

The study opens that study methods of learners are not constantly addressed to understand its meaning and to relating it to previous knowledge and personal experiences. Despite of fact that Inventory data highlights Year 6 students have more achievement, motivation and comprehensive learning to compare with beginning of the studies overall students tend to learn by memorisation and acquiring facts. It is more likely that students use surface approach to reach requests of academic personnel and to meet objectives of the curriculum. This article suggests continuing research to find in details importance of assessment, learning methods, impact of curriculum on way how students learn.

## Acknowledgments

The author would like to thank M. Davis and R. Harden from University of Dundee who opened spirit

of mind to medical education. Thanks to Prof. Ingrida Rumba-Reinfelde, Uldis Teibe, Andrejs Ivanovs, Inga Stabina, Ieva Zarina, Vilnis Dzerve and Valdis Pirags for their support without which the observation would not have been possible. The author wishes to thank students and teachers of University of Latvia who helped in the management of this study.

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