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## Azerbaijan Medical Journal

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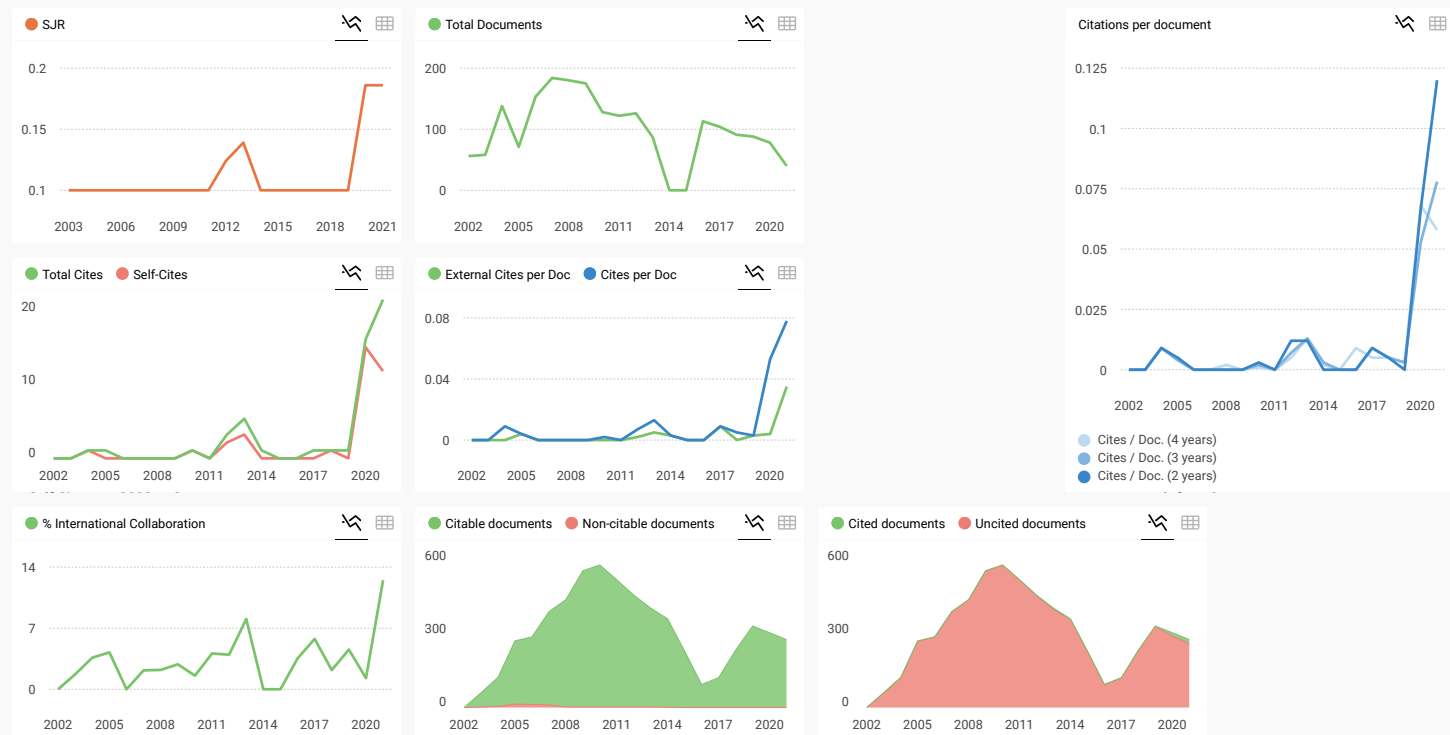
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## Evaluation of the locally applied doxycycline on alveolar osteitis incidence following surgical removal of impacted mandibular third molars

Journal ID : **AMJ-23-08-2022-10466**; Author : **Rasheed Musleh Ibrahim, Assist. Prof. Dr. Ali H. Abbas Al Hussaini,**

**Abstract** : One of the most common oral procedures carried out every day in oral and maxillofacial surgery units is the surgical removal of impacted mandibular third molars. After the impacted mandibular third molar has been removed, postoperative morbidity is unavoidable. Alveolar osteitis, a painful, unhealed socket, is one of the most frequent surgical sequelae. By introducing and applying a new material inside the extraction socket, many researches were attempted to prevent the development of alveolar osteitis. This study aims to make a comparison between the effect of the locally applied Doxycycline and the systemic Doxycycline on the occurrence of alveolar osteitis after surgical removal of impacted mandibular third molars. A randomized prospective clinical study was conducted on 50 patients who had undergone surgical removal of impacted mandibular third molars under local anesthesia. The patients were distributed in 2 groups: Study group; included 25 patients treated by local application of Doxycycline inside the socket immediately after surgical removal of impacted tooth. Control group: consisted of 25 patients who received systemic Doxycycline one hour before surgical removal of impacted tooth. Alveolar osteitis was evaluated on the 2nd and 7th day after surgery. There was a significant reduction of alveolar osteitis in the study group in comparison to the control group ( $p < 0.05$ ). The local application of Doxycycline significantly reduces the incidence of alveolar osteitis.

## Effectiveness of locally applied clindamycin on the occurrence of alveolar osteitis following surgical removal of impacted mandibular third molars. A comparative study

Journal ID : **AMJ-23-08-2022-10465**; Author : **Dashti Ali Mohammed, Assist. Prof. Dr. Ali H. Abbas Al Hussaini,**

**Abstract** : One of the most frequent procedures in oral surgery units is the removal of a partially or completely impacted mandibular third molar under local anesthesia and it directly affects the patient's quality of life. The aim of this study is to make a comparison between the effect of the locally applied clindamycin and the systemic clindamycin on occurrence of postoperative alveolar osteitis following surgical removal of impacted mandibular third molars. Fifty patients who had undergone the surgical removal of an impacted mandibular third molar under local anesthesia participated in a randomized prospective clinical study. The patients were distributed in 2 groups. Study group included 25 patients treated with local application of clindamycin inserted inside the socket after surgical removal of impacted tooth, while control group comprised of 25 patients treated with systemic clindamycin one hour before surgical removal of the impacted tooth. The occurrence of alveolar osteitis was evaluated on the 2nd and 7th day postoperatively. Alveolar osteitis occurred on the 48 hours postoperatively. The total incidence of alveolar osteitis was 10%. The incidence of alveolar osteitis was more in control group (16%) when compared to study group (4%) and the difference was statistically significant. Local application of saturated gelfoam by clindamycin reduces the incidence of alveolar osteitis to a significant level when compared to systemic clindamycin.

## Association between Bsm1 (rs1544410) and Taq1 (rs731236) VDR Gene Polymorphisms with Vitamin D Status in Type 2 Diabetes Mellitus Patients

Journal ID : **AMJ-22-08-2022-10464**; Author : **Sufitni Sufitni, T. Helvi Mardiani, Yunita Sari Pane, Siti Syarifah, Mutiara Indah Sari,**

**Abstract** : Type 2 Diabetes Mellitus (T2DM) arises due to combination of modifiable and non-modifiable. One of the modifiable risk factors is vitamin D concentration, which is associated with the incidence of T2DM and mediated by the vitamin D receptor (VDR). The aim of this study is to see the association between Bsm1 (rs1544410) and Taq1 (rs731236) VDR gene polymorphisms with vitamin D status in T2DM patients in the city of Medan, Indonesia. 76 T2DM patients were enrolled in this study. The vitamin D levels are analyzed with enzyme-linked immunosorbent assay (ELISA). Genotyping of Bsm1 (rs1544410) and Taq1 (rs731236) VDR gene polymorphisms are done with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique. In this study, 72.4% of T2DM patients have insufficient and deficient vitamin D status (35.50% and 36.9% respectively). Fisher's exact analysis of the association between vitamin D status with Bsm1 (rs1544410) and Taq1 (rs731236) VDR gene polymorphisms is at  $p > 0.05$ , respectively. This study shows that genotype Bsm1 and genotype Taq1 are not associated with vitamin D status in T2DM patients.

## Bitter Gourd (*Momordica charantia* L.) Affects the Pharmacokinetics Profile of Metformin in Rabbits' Plasma

Journal ID : **AMJ-22-08-2022-10463**; Author : **Asri Dwi Endah Dewi Pramesthi, Agung Endro Nugroho, Endang Lukitaningsih,**

**Abstract** : The drug-herb combination between metformin and bitter gourd needs further study of the benefits and risks. This study aims to explore metformin in plasma samples using the HPLC method and its pharmacokinetic profiles affected by the bitter gourd. This experimental method study with a post-test randomized controlled group design. Healthy albino rabbits were divided into three groups ( $n=3$ ). They were administered with bitter gourd juice 100% (4 ml/kg BW) and metformin (BM1), bitter gourd juice 50% (4 ml/kg BW), and metformin (BM2), and metformin 26 mg/kg (M). BM1 and BM2 were given bitter gourd juice for 14 days, and then a single dose of metformin was given to all groups on the 15th day before metformin pharmacokinetic parameters were measured. Blood samples were collected from marginal ear vein punctures at 0, 10, 30, 60, 120, 240, 360, and 480 minutes. The plasma was analyzed using HPLC methods, and the concentration vs. time was used for a 1-compartmental open model pharmacokinetics analysis. Bitter gourd juice with 100% (4 ml/kg BW) concentration decreased the V/F and CL/F, also increased  $K_a$ ,  $T_{1/2}$ ,  $C_{max}$ , MRT, and  $AUC_{0-inf}$ , also



significantly increased AUC0-480, and decreased Tmax ( $p < 0.05$ ). The pharmacokinetic interaction of metformin and Momordica charantia L. is presumably because of the competitive interaction between phytochemical constituents of bitter melon and metformin on the OCT and MATE transporter.

## **The Effectiveness of Education on Farmer Knowledge and Behaviors towards COVID-19 Prevention: A Study in an Agricultural Area**

Journal ID : **AMJ-22-08-2022-10462**; Author : **Yarmaliza, Teungku Nih Farisni, Fitriani, Zakiyuddin, Fitrah Reynaldi, Veni Nella Syahputri, Andi Imam Arundhana,**

**Abstract** : Following the healthcare and sanitation protocol has been widely used to prevent COVID-19 transmission. The research objective was to determine the effectiveness of WASH educational collaboration on farmers as an attempt to COVID-19 prevention. This study was an observational pre-experimental pretest-posttest design study without a control group. This research was carried out in March 2021, located in two districts with high COVID-19 positive cases, Johan Pahlawan and Meureubo Districts, West Aceh, Indonesia. A total of 37 farmers were recruited as the study participants. Data were analysed using logistic regression. The study result shows a significant influence between the collaboration of WASH education on farmers to prevent COVID-19. The statistical test was significant at a p-value  $< 0.05$ . Collaboration on WASH education for farmers is very influential in preventing COVID-19. It is believed that cross-sectoral involvement and roles can be maintained so that COVID 19 prevention behaviours through WASH can continue to be applied. Educative collaboration of WASH impacts the alteration of the behaviour toward farmers who live in the area confirmed to be infected by COVID 19.

## **PERFORMANCE OF MULTIPLEX REAL TIME PCR FOR DIAGNOSIS MALARIA IN FOREST ENDEMIC AREAS IN TANAH BUMBU REGENCY SOUTH KALIMANTAN PROVINCE INDONESIA**

Journal ID : **AMJ-22-08-2022-10461**; Author : **Istiana Istiana, Usman Hadi, Heny Arwati,**

**Abstract** : Malaria incidence has declined in Indonesia for years, but it is still found a foci malaria in forest areas. Rapid Diagnostic Test (RDT) has been used as a diagnostic tool in remote forest areas because other tests are difficult to carry out due to poor infrastructure. Microscopic examination requires expert personnel to determine the right interpretation. The disadvantage of both methods of diagnosis is that they cannot detect the low density of parasites, causing the diagnosis to be missed. For this reason, it is necessary to examine the molecular diagnosis to determine the right species. This cross-sectional study was conducted on 105 people aged 15-64 years in forest malaria endemic areas who were randomly selected. The diagnosis of malaria is established through rapid diagnostic test, microscopic, real-time PCR examination for identification of genus, and multiplex real time PCR for species identification. The performance of multiplex real time PCR, microscopic and RDT was compared and evaluated each other. From 105 blood samples, RDT identified 38 malaria cases, consist of 9 as Plasmodium falciparum infection, 9 as Plasmodium vivax and 20 as mixed infection. Light microscopy identified 9 as P. falciparum infection, 5 as P. vivax infection and 5 as mixed infection. Multiplex real time PCR detected 7 as P. falciparum infection, 6 as P. malariae infection, and 4 as mixed P. vivax and P. malariae infection. Using microscopy as a reference test, real time PCR showed an overall sensitivity of 100% and specificity of 91,9%. Multiplex real time PCR for species P. falciparum had 77,8% sensitivity and specificity of 91,83%. RDTs sensitivity of 94,7% and specificity of 76,4%. Using multiplex real time PCR as a reference, microscopy had shown better performance than RDT with sensitivity 73,7% and specificity 100% but RDT had low sensitivity with 14,3% (95% CI 11,45 – 26,87) and specificity of 70,5% (95% CI 65,78-79,67). All three tests performed well to diagnose malaria in this area, but multiplex PCR performed better for detecting Plasmodium species, especially species that produce submicroscopic infections or subpaten infections. Multiplex real-time PCR is the most sensitive malaria diagnostic method that can be used in malaria elimination programs. It has better performance in species identification, species detection with low parasitic density and mixed infection.

## **Molecular Co- Localization of Human Papilloma Virus 6 / 11 in Combined Resected Tissues from Non-oncologic Palatine and Pharyngeal Tonsillar Hypertrophies**

Journal ID : **AMJ-22-08-2022-10460**; Author : **Saad Hasan Mohammed Ali, Ameen Abdul Hasan Al-Alwany, Khalil Ismail A. Mohammed, Wifaq M. Ali, Suha A. AL-Fakhar, Shakir H. Mohammed Al-Alwany, Jinan M. Mousa,**

**Abstract** : More than 450 distinct types of human papilloma virus recognized via recent molecular techniques. The low and high oncogenic risk-HPV genotypes have an association with a variety of benign and malignant tumors in the oropharyngeal and nasopharyngeal localizations. This study aimed to determine the rate of DNA detection of HPV genotype 6/11 in non-oncologic nasopharyngeal and palatine tonsillar tissues from pediatric patients subjected to adeno-tonsillectomies. A total number of 64 tissue specimens enrolled; 44 non-oncologic nasopharyngeal and palatine hypertrophied tissue specimens from 22 pediatric patients sustained combined adeno-tonsillectomies and compared to 20 nasal trimmed tissues with unremarkable pathological changes (included as apparently healthy control tissues). Recent version of chromogenic in situ hybridization method used for HPV 6/11 DNA detection performed via DNA probes specified for HPV 6/11 low oncogenic - risk genotypes. Among a total 44 tissue specimens obtained from 22 pediatric patients who sustained surgical operations for combined non-oncologic nasopharyngeal and palatine tonsillar hypertrophies, 10 combined nasopharyngeal as well as palatine tonsillar hypertrophied tissues out of 22 combination of such tissues found to contain positive CISH reactions results for HPV 6 / 11 DNA, constituting 45.5% of the total screened tonsillar tissues. No positive- CISH reactions detected in the control tissues. Statistically, a significant difference showed when compared to the control apparently healthy nasal tissues. The significantly detected rates of these low- oncogenic HPV genotypes in both nasopharyngeal and palatine tonsillar hypertrophied tissues are pointing for a one important reservoir tissue site for such infection. In addition, indicating herald mark for exploring the transmission and pathogenesis dilemma of such an important sexually transmitted infection among, at

## **Effect of Phytosterols Fraction of Iraqi Cicerarietinum In Experimentally Induced Hyperlipidemic Mice**

Journal ID : **AMJ-22-08-2022-10459**; Author : **Nihal Ramadhan Hwerif, Ahmed Rahmah Abu Raghif,**

**Abstract** : The aim of this study is to evaluate the antihyperlipidemic effect of Phytosterols fraction of Iraqi Cicerarietinum in high-fat diet (HFD)-fed mice. Animals were divided into (4) groups, for each group (n =8). The total duration of the study was (56) days split into two intervals. During the first 28-days interval, mice were administered with HFD, whereas during the second 28-days interval, they were co-administered HFD plus Phytosterols (500mg/kg: p.o.) or the standard drug Atorvastatin(10mg/kg: p.o.). Phytosterol treatment to HFD-induced hyperlipidemic mice caused a high significant decrease in the levels of total cholesterol (TC), triglycerides (TG), LDL-C and VLDL-C. Moreover, Phytosterols resulted in a high significant increase in the levels of HDL-C, whereas it caused remarkable decreases in ALT, AST and ALP enzymatic activities also in total serum bilirubin (TSB) levels among hyperlipidemic mice. Besides that, Phytosterol treatment showed significant improvement in levels of tissue MDA and GSH in hyperlipidemic mice. Histopathological examination of hyperlipidemic mice group showed marked and diffused cytoplasmic fatty infiltration which was all ameliorated by Phytosterols administration. The results revealed that Phytosterols(500mg/kg; p.o) possess potential ameliorating benefits against hyperlipidemia induced by HFD on lipid profile, liver function enzymes, oxidative stress parameters, hepatic and aorta histoarchitecture. Further investigations are recommended to assess the efficacy and to fully dissect the mode of action the observed anti-hyperlipidemic effect of Phytosterols.

## **Study the relation between IL-17 rs2275913 gene polymorphism with H. pylori infection in Babylon province**

Journal ID : **AMJ-20-08-2022-10454**; Author : **Zahraa Alaa Abd Alhassan Hadi, Nktel Faaz Nassir AL-saad,**

**Abstract** : The current investigation aims to establish a connection between IL17 gene polymorphisms and H. pylori infection. Seventy-eight (78) cases were gathered with H. pylori infection-related clinical symptoms. Urea breath testing and rapid Ab immunoassay, two assays, identified 45 patients with H. pylori infection. As a control group, samples from 45 people were collected who appeared to be in good health. It has been determined that the gold standard method for non-invasive diagnosis is the urea breath test. Additionally, the interleukin 17 gene was found using ELISA, sequencing, and conventional PCR methods. It was discovered that genotype A/A was more closely related to infection, but genotype G/A provided carriers with a lesser susceptibility to infection. This demonstrated the link between H. pylori infection and phenotypes. The risk allele is represented by allele A, with a 95% confidence interval (CI) of 2.30 (1.25–4.21), whereas the protective allele is represented by allele G, with a CI of 0.43 (odd) (0.24–0.80). The mean concentration of interleukin 17 in the H. pylori infection group was 97.85±39.54 pg/ml, which was higher than the 75.58 ±22.74 pg/ml in the control group. The A/A genotype in the IL-17 gene (rs2275913) showed the highest concentration of IL-17 (114.24 pg/ml) in the serum of these studied groups, while the average serum level of the G/A (78.37 pg/ml) and G/G groups (76.13 pg/ml) was obtained from the comparison between the level of IL-17 and the genotypes of the IL-17 gene.

## **Effect of Zirconium oxide nanoparticles (ZrO2NPs) on liver function in mice infected with Leishmania donovani**

Journal ID : **AMJ-18-08-2022-10452**; Author : **Mohammed H. Mohammed, Hadeel A. Majeed, Nadheer J. Mohammed,**

**Abstract** : Visceral leishmaniasis consider a great health problem in Iraq, the traditional antileishmanial therapies are toxic and most are costly. For this reason, new approaches in the treatment of leishmaniasis are requested, zirconia nanoparticles ZrO2NPs, is a nano-sized and hollow colloidal metal oxide, it was recorded as anticancer agent, the approach was to prepare 17.4 µg/ml of ZrO2NPs by pulsed laser ablation. These nanoparticles are described to confirm the shape, size and other physical properties of the crystal using the following techniques: X-Ray Diffraction (XRD), results were indicated diffraction peaks at (28.2°, 31.5°, 38.5°, 50°, and 59.8°) were correspond to the (111, 111, 120,022 and 131), respectively. The Transmission Electron Microscopy (TEM) shown confirms the formation of nanostructures with granular size by 50 pulses of laser. The effect of ZrO2NPs on the parasite was tested in vivo by observation the levels of liver enzyme (aspartate aminotransferase AST, alanine aminotransferase ALT and cholesterol CL) in serum of infected mice and dosed with ZrO2NPs (0.1 ml / day) and comparing with control positive group after 7,14 and 21 days of dosed. The results showed that the level of AST in treated mice with ZrO2NPs significantly decreased at second and third week (49.17±0.70, 46.83±0.70 U/L) respectively, compared with the positive group was (61.83±1.33, 84.00 ±2.63U/L), there is significantly decrease at second and third week in level of ALT (50.00±0.58, 45.33 ±0.80) respectively, compared with the positive group (66.33±0.92, 79.67±2.22 U/L), respectively. As well as the results showed a significant increase in the cholesterol level within second and third weeks when mice were dosed with ZrO2NPs (164.83 ±1.70, 170.33 ±1.31 mg/dl) compared with positive group (139.83±1.35, 98.33±4.62 mg/dl).

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# PERFORMANCE OF MULTIPLEX REAL TIME PCR FOR DIAGNOSIS MALARIA IN FOREST ENDEMIC AREAS IN TANAH BUMBU REGENCY SOUTH KALIMANTAN PROVINCE INDONESIA

Istiana Istiana<sup>1</sup>, Usman Hadi<sup>2\*</sup>, Heny Arwati<sup>3</sup>

Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga, Indonesia<sup>1</sup>

Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Indonesia<sup>2</sup>

Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Indonesia<sup>3</sup>

Corresponding author: 2\*



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## Keywords:

performance – multiplex real time PCR – forest malaria

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## ABSTRACT

Malaria incidence has declined in Indonesia for years, but it is still found a foci malaria in forest areas. Rapid Diagnostic Test (RDT) has been used as a diagnostic tool in remote forest areas because other tests are difficult to carry out due to poor infrastructure. Microscopic examination requires expert personnel to determine the right interpretation. The disadvantage of both methods of diagnosis is that they cannot detect the low density of parasites, causing the diagnosis to be missed. For this reason, it is necessary to examine the molecular diagnosis to determine the right species. This cross-sectional study was conducted on 105 people aged 15-64 years in forest malaria endemic areas who were randomly selected. The diagnosis of malaria is established through rapid diagnostic test, microscopic, real-time PCR examination for identification of genus, and multiplex real time PCR for species identification. The performance of multiplex real time PCR, microscopic and RDT was compared and evaluated each other. From 105 blood samples, RDT identified 38 malaria cases, consist of 9 as *Plasmodium falciparum* infection, 9 as *Plasmodium vivax* and 20 as mixed infection. Light microscopy identified 9 as *P. falciparum* infection, 5 as *P. vivax* infection and 5 as mixed infection. Multiplex real time PCR detected 7 as *P. falciparum* infection, 6 as *P. malariae* infection, and 4 as mixed *P. vivax* and *P. malariae* infection. Using microscopy as a reference test, real time PCR showed an overall sensitivity of 100% and specificity of 91,9%. Multiplex real time PCR for species *P. falciparum* had 77,8% sensitivity and specificity of 91,83%. RDTs sensitivity of 94,7% and specificity of 76,4%. Using multiplex real time PCR as a reference, microscopy had shown better performance than RDT with sensitivity 73,7% and specificity 100% but RDT had low sensitivity with 14,3% (95% CI 11,45 – 26,87) and specificity of 70,5% (95% CI 65,78-79,67). All three tests performed well to diagnose malaria in this area, but multiplex PCR performed better for detecting Plasmodium species, especially species that produce submicroscopic infections or subpaten infections. Multiplex real-time PCR is the most sensitive malaria

diagnostic method that can be used in malaria elimination programs. It has better performance in species identification, species detection with low parasitic density and mixed infection.



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## 1. Introduction

Malaria is still a problem in the world. More than 200 thousand cases of malaria occur annually in the world and 95% occur in sub-saharan africa. Malaria cases have increased from 227 milion in 2019 to 241 million in 2020 in the world. its occurred due to the Covid-19 Pandemic which caused all health resources to be deployed to overcome this pandemic [1]. Indonesia has the second most malaria cases after India in Asia. Although most areas in Indonesia are malaria-free, there are still focus areas of malaria, most of which are located in remote forests and referred to as forest malaria [2], [3].

An accurate diagnosis followed by effective treatment is essential to eliminate malaria. The methods used for malaria diagnosis are microscopy which is the gold standard, rapid diagnostic test (RDT), and nucleic acid-based amplification assays such as polymerase chain reactions (PCR) and loop-mediated isothermal amplification [4]. Microscopes are still the main method of malaria diagnosis in many countries including Indonesia because it can identify species through thick or thin peripheral blood smears. However, the sensitivity and specificity of microscopy depends on the quality of the stained slides and the microscopic competence [5]. In addition, microscopy cannot detect sub-microscopic malaria (infections with a very low density of parasites) and mixed infections [5- 7].

Rapid diagnostic tests are easy to use, fast, cost-effective, and a tool that can be used in the field for the diagnosis of malaria [8]. Three antigens are typically targeted for commercially available RDTs, namely *P. falciparum* histidine-rich protein 2 (Pf-HRP2), lactate dehydrogenase (LDH), which can be either pan-specific (i.e., genus-specific) or species-specific (P.f.-specific or P.v.-specific), and genus-specific aldolase which detects all Plasmodium species [9]. However, RDT has limitations when detecting asymptomatic or low-density infections as well as from parasitic strains that have removal in genes encoding HRP2 or HRP3, their structural homologs [10], [11].

PCR is very sensitive and has the ability to detect 2-5 parasites/ $\mu$ L compared to RDTs that can detect if the number is more than 100 parasites/ $\mu$ L and a microscope with a limit of detection of 50-500 parasites/ $\mu$ L [12], [13]. Therefore, PCR is increasingly used for malaria diagnosis and is a useful tool for epidemiological studies that map sub-microscopic malaria [14]. However, PCR is expensive, requires qualified personnel, and has a long turn-around time; thus, they are impractical for use in the field or clinical setting [15].

To evaluate efforts to implement targeted malaria elimination requires highly sensitive malaria diagnostic tools, especially for screening and mass treatment strategies [16]. This effort can be made with the use of molecular tests for the diagnosis of malaria. Currently molecular tests for the diagnosis of malaria have developed. One of them is the development of real time PCR and multiplex real time PCR. This method can increase the processing time, which is faster than conventional PCR. This method is quantitative and closed which reduces time, labor, reagents, costs, and the risk of contamination [17]. Multiplex real-time PCR has improved the ability to detect mixed Plasmodium infections and detection of Plasmodium species

in cases of low parasitemia, with a detection limit of less than 2 parasites per ml and has the advantage of simultaneous detection of multiple targets in one go to improve sensitivity and specificity compared to microscopy and RDTs [18].

## 2. MATERIALS AND METHODS

### 2.1 Study area and study design

Teluk Kepayang sub-district in Tanah Bumbu Region still has a forest malaria endemic village. Some areas in that sub-district are still in the form of primary rain forest (20o 57' 0" LS – 30o 38' 24" LS and 115o 24' 0" BT – 116o49' 12" BT) [19]. Based on data from the Central Bureau of Statistic For Tanah Bumbu Regency, the population in that district was 8.154 in 2020 [20]. Data were collected in September 2020 until July 2021. All participants in this study were resided in forest villages (Batu Bulan and Batu Paha), with annual parasite index in 2017 was 80,8 [19]. Sampling was done using simple random sampling. The study was conducted on 105 male and female participants between the ages of 15 and 60 years, are not pregnant, are not experiencing severe pain and are willing to be research subjects. The research involved the health office, the malaria program manager at the local public health center, and the village authorities.

### 2.2 Data collection procedure

Researchers and village officials will visit every house to conduct interviews, collect venous blood, and observe the surrounding environment. Blood samples were taken through the median cubital vein and put into an EDTA anticoagulant tube. We used a standardized questionnaire to collect data from participants. For validity, it is based on the calculation of corrected item total correlation. The statement is valid if the R value > 0.483. Reliability test using Crohnbach's alpha with  $p=0.806$  The questionnaire was administered by trained interviewers to collect data on patient age, gender, the residence of villages, education, occupation, and ethnicity. Using venous blood taken from each patient, a thick and thin smear is prepared for a microscope. RDT testing was performed on-site and blood in EDTA tubes was collected and transported by cold chain to Eijkman Laboratory for molecular tests.

## 3. MALARIAL DIAGNOSIS

### 3.1 Rapid Diagnostic Test

RDTs testing was performed as per manufacturer instruction using SD Bioline Malaria Ag Pf/Pv (Standard Diagnostic Inc, Abbot, US). This kit will detect HRP2 Ag specific to *P. falciparum* and pLDH specific to *P. vivax* and differentiate mono infection and mix infection using whole blood. Using a capillary tube (5  $\mu$ L), the whole blood sample is collected until it reaches the black line and transferred to the cassette sample well. Four drops of diluent are added vertically for testing in the well. The result is read after 15–30 min. A result is considered negative if a color band is observed in line “C;” it is *P. falciparum*- positive if 2 color bands are observed inline “P.f” and C, or 3 color bands in lines P.f, “Pv” and C. The tests were performed in the field laboratory as a routine malaria testing in mass blood survey (MBS).

### 3.2 Malaria Microscopy

After made a thick and thin blood films in the field, slides were allowed to air-dry at room temperature. The thin smear fixed with absolute methanol and stored at 2-8oC until being transported to Parasitology Laboratory Faculty of Medicine, Universitas Lambung Mangkurat. All slides were stained with 10% Giemsa solution for 10 minutes. Both thick and thin smear were examined by an experienced laboratory analyst. An expert microscopist re-checked all positive slides and 10% negative slides. The microscope used 100 high power fields (HPFs) for parasite detection [21].

### 3.3 DNA Extraction

Genomic DNA extraction was performed using QiagenQIAamp® 96 DNA Blood Kit (QIAGEN Inc.) from whole blood. Briefly, 200 µl whole blood add into a 1.5-ml tube for processing as per manufacturer instructions. Finally, with 200 µl volume of elution buffer the DNA was eluted and stored at – 20 °C until assayed [22].

### 3.4 Real time PCR

The real-time PCR method according to the steps carried out by [23]. DNA was extracted from whole blood with EDTA anticoagulants using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia CA). The primary and probe set is based on the 18S rRNA sequence with u *primary forward* detection center is 5-GCTCTTTTGATTTTGGATG-3, and *the primary reverse* is 5-AGCAGGTTAAGATCTCGTTCG-3. The probe sequence is 5-ATGGCCGTTTTTTTTTCGTG-3 and is labeled 5 FAM (6-carboxyfluorescein) as reporters and 3 TAMRA (6 carboxytetramethyl-rhodamin) as quenchers. Real time amplification and measurements were performed using PCR Applied Biosystems 7500 with thermal profile for qPCR: 10 min at 95°C, 40 cycles 15 seconds at 95°C, and 1 minute at 60°C. For qRT-PCR, a 30-minute cycle at 50°C is added as a first step for the reverse transcription process. For reactions, 1 µl of template was added at 9 µl of the master mix mixture containing 1 QuantiTect Probe RT-PCR Master Mix (Qiagen), 0.4 M primer, 0.2 M probe, and 4 mM MgCl<sub>2</sub> respectively. For qRT-PCR examination, QuantiTect RT Mix (a mixture of Omniscript and Sensiscrip Reverse Enzyme) was added to the master mix mix as recommended by the manufacturer at a rate of 1µl per 100 µl of reaction parent mixture [23].

### 3.5 Multiplex real time PCR

Multiplex real-time PCR was performed under universal conditions (95°C for 15s, 60°C for 1 min) with the ABI TaqMan 7500. The reaction was performed with a final volume of 25 µl containing 5 µl of DNA, 12.5 µl of TaqMan universal master mix (Applied Biosystems), primers, and probes. The primers and probes used in this study are listed in Table 1 with the respective concentrations for each reaction. The Plasprobe, Ovaprobe, and Malaprobe were synthesized by Applied Biosystems, and the Vivprobe, Falcprobe, and β2 M probe were synthesized by Biosearch Technologies. All probes were purified by high-performance liquid chromatography. Samples were confirmed *Plasmodium* positive with genus-specific primers Plasmo1 and Plasmo2 and the 6-carboxyfluorescein (FAM)-labeled Plasprobe to detect a region of the *Plasmodium* 18S gene that is conserved across all five species [24].

**Table 1.** Primers and probes used for identification species Plasmodium.

Species	Primers or probes	Sequence (5'-3')
<i>P. falciparum</i>	FAL-F Primer Falc Probe	CCG ACT AGG TGT TGG ATG AAA GTG TTA A Quasar 670-AGC AAT CTA AAA GTC ACC TCG AAA GAT GAC T-BHQ-2
<i>P. vivax</i>	Viv-F Primer Vivprobe	CCG ACT AGG CTT TGG ATG AAA GAT TTT A Tamra-AGC AAT CTA AGA ATA AAC TCC GAA GAG AAA ATT CT-BHQ-2
<i>P. malariae</i>	Mal-F primer Malaprobe	CCG ACT AGG TGT TGG ATG ATA GAG TAA A FAM-CTA TCT AAA AGA AAC ACT CAT-MGBNFQ
<i>P. ovale</i>	Ova-F primer Ovaprobe	CCG ACT AGG TTT TGG ATG AAA GAT TTT T VIC-CGA AAG GAA TTT TCT TAT T-MGBNFQ

### 3.6 Data quality assurance

Data was collected by researchers and team who is skillfull. All blood films and RDT testing were

performed based on standard operating procedures. The quality of each reagen was checked before laboratory analysis was performed. Samples and reagens were stored at appropriate temperature as indicated on manufacturer's belonging. Internal and external quality were run as required during analysis. All samples stored at -20°C.

### 3.7 Data analysis

The data was analyzed by using SPSS version 28.0 for window. The descriptive analysis will be carried out in the form of percentage and frequency to describe patient sociodemographic. The sensitivity, specificity, predictive value and Kappa coefficient were estimated by comparing results from three assays and 95% confidence interval was computed.

### 3.8 Ethical Consideration

This study was reviewed and approved by the ethical commission research at the Faculty of Medicine, Lambung Mangkurat University Banjarmasin, Indonesia with number 603/KEPK- FK ULM/EC/V/2021.

## 4. FINDING AND DISCUSSION

### 4.1 Sociodemographic characteristic of study participants

A total of 105 participants in forest malaria endemic areas were recruited into the study. Most of the inhabitants work in the forest as forest workers. The lowest age was 16 years old, the highest was 60 years old and mean age was 32 years. The mean of length of stay in the village was 83.81 month ranging from 1 month - 30 years. The lowest body temperature was 36.1°C, the highest was 37.8°C and average temperature was 36.6°C. The complete data can be seen in table 2 below.

**Table 2** Demographic and risk factors of malaria infection

<b>Variables</b>	<b>Total (N=105)</b>
Age (years) (median, range)	32 (16-60)
15 – 45	90 (85. 7%)
more than 45	15 (14. 3%)
Sex	
Male	67 (63. 8%)
Female	38 (36. 2%)
Ethnicity	
Banjar	100 (95. 2%)
Dayak	5 (4. 8%)
Villages	
Batu Bulan	67 (64. 5%)
Batu Paha	38 (35. 5%)
Education	
No school	46 (43. 8%)
Elementary	44 (41. 9%)
Middle school	10 (9.5%)
High school	5 (4. 8%)
Occupation	
Forest workers	79 (75. 2%)

Non-Forest workers	26 (24. 8%)
Temperature (°C) (mean, range)	36.66 (36. 1 – 37.8)
History of malaria	
Yes	70 (66. 7)
Not	35 (33. 3)
Interval of previous malaria infection (month) (total; mean, range)	(70; 6; 3-14)

#### 4.2 Laboratory result of study participants for diagnosis malaria by RDTs, microscopy, real time PCR, multiplex real time PCR.

Among 105 participants, malaria positive cases by RDTs were 38 (36,19%), by microscopy were 19 (18,1%), by real time PCR (for genus) were 26 (24,8%). Malaria positive cases by multiplex real time PCR was 17 (16,1%). Malaria positivity rate was highest for RDTs. The positivity rate was increased among the age groups 15-45 years old, in male participants, and in forest workers. Malaria positivity rate for RDTs was higher in participants with history or previous malaria, but in microscopy, real time PCR and multiplex real time were higher in participants with no previous malaria infection. The complete data can be seen in table 3.

**Table 3.** Malaria positivity rate by RDTs, microscopy, real time PCR, Multiplex real time PCR in forest malaria areas

Characteristic	Malaria positivity			
	RDTs (n=38) %	Microscopy (n=19) %	Real time PCR (n=26) %	Multiplex real time PCR and AbTes real time PCR (n=17) %
Age Group				
15-45 (n=90)	33 (86,8)	17 (89,5)	22 (84,6)	15 (88,2)
> 45 (n=15)	5 (13,2)	2 (10,5)	4 (15,4)	2 (11,8)
Sex				
Male (n=67)	25 (65,8)	12 (63,2)	19 (73,1)	10 (58,8)
Female (n=38)	13 (34,2)	7 (36,8)	7 (26,9)	7 (41,2)
Occupancy				
Forest Worker (n=79)	31 (81,6)	19 (100)	26 (100)	17 (100)
Non-Forest (n=26)	7 (18,4)	0	0	0
History Of Malaria				
Yes (n=70)	27 (71,1)	5 (26,3)	10 (38,5)	6 (35,3)
Not (n=35)	11 (28,9)	14 (73,7)	16 (61,5)	11 (64,7)

#### 4.3 Diagnostic performance of RDTs, microscopy, real time PCR and multiplex real time PCR

Using microscopy as a reference test, real time PCR showed an overall sensitivity of 100% (95% CI 96-100), specificity of 91,9% (95% CI 93,54-96,87). Multiplex real time PCR for species *P. falciparum* had 77,8% sensitivity (95% CI 71,47-81,67) and specificity of 91,83% (95% CI 86,57-95,87). RDTs sensitivity of 94,7% (95% CI 92,57-99,67). Using multiplex real time PCR as a reference, microscopy had shown better performance than RDT with sensitivity 73,7% and specificity 100% but RDT had low sensitivity with 14,3% (95% CI 11,45 – 26,87) and specificity of 70,5% (95% CI 65,78-79,67). Real time PCR had shown good sensitivity and specificity for detection genus Plasmodium but Multiplex real time PCR had good performance to identified other species non falciparum. RDT showed low sensitivity and specificity to detect *P. falciparum* (33,3% and 76,7%). Other species had identified by RDT like *P. vivax* and mixed *P.*

*falciparum* and *P. vivax* infection. Microscopy had identified *P. vivax* and mixed infection but multiplex real time PCR had not found *P. vivax* mono-infection, but in mixed infection with *P. malariae*. Interestingly, multiplex real time PCR had identified *P. malariae* mono-infection in this study, which was not found in that areas in previous study. This species was not computed performance because it was not found in others assay. Kappa value for microscopy and multiplex real time PCR was 0,78, RDT and multiplex PCR was 0,68. Kappa value for RDT and microscopy was 0,74.

**Table 4.** Performance of multiplex real time PCR, RDTs and microscopy

Assay	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
<b>RDT vs microscopy</b>				
overall	94,7(92,57-99,67)	76,4 (71,57-79,46)	47,4 (43,6-54,76)	98,5(94,3-100)
<i>P. falciparum</i>	33,3(31,46-43,67)	76,7(70,34-78,54)	33,3 (29,76-41,45)	95,2(93,8=99,2)
<b>Real time PCR vs microscopy</b>				
overall	100(96-100)	91,9(93,54-96,87)	73,07 (70,45-84,23)	100 (93,0-100,0)
<b>Multiplex real time PCR vs microscopy</b>				
<i>P. falciparum</i>	77,8(69,8-88,45)	91,83(89,78-100)	100(94,45-100,0)	98,4(93,24-100,0)
<b>RDT vs multiplex real time PCR</b>				
overall	96,2 (93,0-100,0)	83,5(76,78-85,43)	65,8(61,78-72,87)	98,5(94,23-100,0)
<i>P. falciparum</i>	14,3 (11,45-26,87)	70,5 (65,78-79,67)	11,1 (9,87-21,56)	92,5 (89,57-98,23)
<b>Microscopy vs multiplex real time PCR</b>				
overall	73,7 (70,97-100)	100 (93,7-100)	100 (94,3-100)	91,86 (89,67-96,23)
<i>P. falciparum</i>	87,67 100 (93-100)	97,7(91,77-100)	77,8 (71,67-86,45)	100 (92,78-100)

## 5. DISCUSSION

An accurate and fast diagnosis is essential to control malaria infection. This study evaluated the performance of RDT, microscopes, and real-time PCR in diagnosing malaria in forest malaria areas that are low transmission. This study obtained the prevalence of forest malaria based on microscopic examinations of 18.1%, based on RDT of 36.19%, real time PCR of 24.8% and based on multiplex real time PCR and AbTes real time PCR of 16.1%. Positivity rates reported in this study were highest for RDTs followed by real time PCR, microscopy and multiplex real-time PCR. Real-time PCR detected all positive tests in a microscope and 10,36% on a positive RDT. The level of positivity by all diagnostic methods increases among the younger age group and decreases or becomes zero in the older age group. These results are in line with research conducted in the Western Arsi Zone, Ethiopia that positivity rates among younger age groups are higher when determined by microscopes, RDTs and molecular tests [13].

In this study, the results of species identification between RDT, microscopic and multiplex real time were different. In RDT and microscopic no *species of P. malariae* nor mixed infections of *P. vivax* and *P. malariae* were found. This suggests multiplex real time PCR is able to detect infections with low parasite levels as often found in *P. malariae* infections. Although this PCR detection capability is very good in detecting submicroscopic infections, the discrepancy between RDT, microscopic and multiplex real time PCR can be caused due to the limit of detection of each different examination [25- 27].

Unlike previous studies which showed that multiplex PCR has the best sensitivity and specificity [28] but in this study the results were not satisfactory. This is because the quality of DNA for PCR examination is not



enough good and the amount of DNA is small due to dilution with anticoagulants that are not appropriate so as to affect the results of the examination [30], [31]. In real time PCR obtained 26 samples that were positive for the genus Plasmodium, and then forwarded with multiplex assay for species identification. Due to dubious results such as due to the threshold cycle of more than 36 and low T<sub>m</sub> peak, this examination is continued with multiplex AbTes real time PCR examination. The final results were obtained *P. falciparum* infection as much as 41.2%, *P. malariae* as much as 35.3% and mixed infections as much as 23.5%.

Although the performance of multiplex real time PCR in this study is different from previous studies, the molecular diagnosis method remains the best choice for determining the diagnosis of malaria, especially in low-endemic areas with low parasitic density [32]. Multiplex real-time PCR can be an alternative diagnostic tool for epidemiological studies and verification of elimination in malaria elimination settings. Multiplex real-time PCR has the advantage of simultaneous detection of several species of Plasmodium in a single reaction [33- 35]. In addition, this method can also identify mixed infections that are often undetected and poorly reported by RDTs and microscopes. Mixed infection detection provides accurate information for the treatment of patients, and for epidemiological studies regarding the transmission of malaria. In an Israel study, of the 10 mixed infections identified by real-time PCR, only one was identified with a microscope and RDT testing [36]. In another study conducted in Switzerland to evaluate microscopy and real-time multiplex PCR, the multiplex qPCR test correctly identified mixed species and infections with low-level parasitemia [37]. A study to determine the prevalence of mixed infections using real-time PCR in northern Ethiopia from 168 samples, found the prevalence of mixed infections was 1.8% with a microscope and 12.5% with real-time PCR [17].

The study found the negative predicted value of multiplex real-time PCR was 98.4% using a microscope as a reference test. This means multiplex real-time PCR can used for diagnosis of malaria in both of genus and species. With the performance and quality of multiplex real time PCR in this study, this method becomes an ideal diagnostic tool for use in malaria elimination. In this study, RDTs and microscopes have the possibility of errors in diagnosis, especially in the identification of species that will negatively affect the elimination program, and potentially contribute to ongoing transmission. Multiplex real-time PCR have identified 17 cases of malaria in this study, consist of 7 infections of *P. falciparum*, 6 infections of *P. malariae* and 4 as a mixed infection of *P. vivax* and *P. malariae* from a total of 105 individuals living in forest malaria endemic areas in this study. Of these malaria cases, *P. malariae* was not detected by the RDT nor light microscopy tests. However, microscopy and multiplex real time PCR methods showed a good performance to identification of *the species P. falciparum*.

## 6. CONCLUSION

Multiplex real-time PCR is the most sensitive malaria diagnostic method that can be used in malaria elimination programs. It has better performance in species identification, species detection with low parasitic density and mixed infection detection than microscopes and RDTs especially in low malaria transmission settings. The test can be used for epidemiological and community-based prevalence studies and for verification of elimination in areas where malaria elimination is launched. However, microscopes and RDTs still have acceptable performance to be used as malaria diagnostic tools in health facilities due to affordability and easier performance diagnostic methods.

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AbTes real time PCR examination, and the Parasitology Laboratory for microscopic examination.

#### AUTHOR'S CONTRIBUTIONS

II: conceptualization of work, analysis of data, drafting and revising, final report:

UH: drafting and revising, final approval of the version to be published

HA: analysis of data, revising draft

#### DATA AVAILABILITY

All relevant data are within the paper

#### FUNDING

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#### COMPETING INTEREST

The authors have declared that no competing interests exist exist

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