APPLICATION OF MOLECULAR BIOLOGY IN DETECTION PATHOGENS AND VECTOR BORN DISEASE AT NATIONAL INSTITUTE OF MALARIOLOGY - PARASITOLOGY- ENTOMOLOGY IN HO CHI MINH CITY

Pham Nguyen Thuy Vy[1], Ngo Thi Hong Phuong[1],
Nguyen Thi Minh Chau[1], Nguyen Thi Van Anh[1], Tran Minh Quy[1]

Summary

Molecular biology techniques are increasingly being used in scientific research and supply for medicine to contribute response the duty about taking care and protecting people's health. Institute of Malariology - Parasitology - Entomology in Ho Chi Minh City (IMPE HCM) has deployed the application about some molecular biology techniques to detect pathogens, vector born disease such as Nested-PCR, Real time PCR, LAMP…. These techniques give the high results, particularly the detection of pathogens with low density such as malaria parasites, with high sensitivity and specificity, predictive value is relatively accurate. In the identification of medical vectors such as mosquitoes, ticks, mites, chigger mites…it also give the exactly value when we compared with GenBank.

Especially, the previous study of the IMPE HCM assesses the prevalence of malaria parasites in the heavy malaria population, which tested by the Giemsa technique (the gold standard for malaria parasites test) detected parasite with low density (about 0.2% to 0.5%), but with LAMP and Nested PCR method detected higher rates (4.4% and 14.7%). Based on this research results, a number of malaria parasites may be missed without the application of molecular biology techniques in the pathogens detection. Beside that, the researches about genetic identification also contributed a new look with more accurate and needs more deep research in the field of parasites, helminths and vectors born disease.

Key words: *Molecular biology, pathogens, vector, IMPE HCM.*

INTRODUCTION

Molecular biology is a part of biology; based on physiology, genetic and biochemical. Molecular biology studies about the shape, structure, and function of macromolecules that have important roles in the life, such as nucleic acids, proteins…. Molecular biology techniques are very diverse and also applied in many different fields, such as molecular diagnostics (PCR, molecular hybridization); production of active substances detecting or treating diseases (producing monoclonal antibodies, making proteins with biological activity ...); gene therapy (recombinant...). The applications about molecular biology, the earliest and most visible, are the medical fields. Based on genetic engineering, medicine not only relies on clinical symptoms but also impacts to directly the true reason that cause the disease: genetic abnormalities. Molecular biology pervate almost the medicine field; inside, the most potents are diagnostic and preventive.

Historically, following the traditional methods, to determine the cause of the disease, the test will be did through direct or indirect methods, such as stain and examination specimen, chemical properties, culture, experimental disease, the presence of specific antibodies in the blood.... However, these techniques almost spend a lot time, and some cases, It is not only expensive but also low about the sensitivity and the specificity. An essential requirement for these techniques is that they require large amounts of microorganisms to be detectable under microscope or microorganism

---

[1] *Institute of Malariology - Parasitology - Entomology Ho Chi Minh City.*

Date of receipt: *August 16, 2021.*
Reviewed completion: *September 25, 2021.*

Responsible person: *Pham Nguyen Thuy Vy, Institute of Malariology - Parasitology - Entomology in Ho Chi Minh City.*

Reviewer: *MSc. MD Pham Van Tung.*

Tel: 0983092900. Email: thuyvy83.pntv@gmail.com.

grown on artificial media. In fact, many cases have the low density specimens, or the bacteria died, or grow very slowly..., the determination of these techniques encountered many difficulties, even impossible. Meanwhile, if the application of molecular biology techniques, the results of microorganisms will be faster, many cases will be cheaper. In particular, these techniques give high sensitive and high specificity. In addition, molecular biology techniques can also reveal the mechanisms of microorganisms by determining the presence of genes that regulate the microbial activity microorganisms and indirectly indicate their 'pathogenic mechanisms' or the resistance drug of microorganisms will also be identified. Beside, sequencing a nucleotide fragment and comparing on the GenBank, can provide information that microorganism has been studied or is a microorganism that is unknown.

In addition, molecular biology techniques have not only characteristics of accurate and fast results but also very convenient which difference sample such as blood, faeces, phlegm, urine... contribute to the taking care and protecting people's health is better and better.

APPLICATION OF MOLECULAR BIOLOGY TECHNIQUE AT IMPE HCM

With the current trend, the IMPE HCM has applied molecular biology techniques in pathogen testing and vector born disease in the scope management of Institue such as malaria, helminths, pathogens on vectors... to provide the National Program with the necessary data on disease prevention.

Research subjects applied molecular biology techniques in IMPE HCM are very diversity, depending on the purpose example pathogens or vectors, we will collect different types of samples, such as blood samples for parasites, fecal samples for helminth, external parasite...

For pathogen testing

Applying the protocol of IMPE about Nested PCR, Real time PCR and LAMP techniques to amplify the target genome for malaria parasites, the helminth such as tapeworms, the Ricketsia in the external parasite…. Thereby, databases have been provided to having the prevent plan to control the specific disease.

With agents that have low density in sample, molecular biology techniques have found infection rates higher than traditional techniques. In a study of active case detection (ACD) in the high risk malaria area in 2015, the Nested PCR technique detected infection rates to 14.7% compared with 0.7% the parasite be detected by Rapid diagnostic test (RDT). Another study in 2017, with Real time PCR, detected 4.4% parasites compared to 0.52% of RDT-detected parasites.

Also in 2017, the Institute also detected 4.43% parasite prevalence rate by LAMP technique compared to 0.5% parasite detected by the traditional method with the same sample collected in the field. .

In the investigation of pathogens on external parasite in the South Vietnam - Lam Dong, a 2015 - 2017 study, on pathogenic parasites including ticks, mite, chigger mite has been tested for Ricketsia bacteria cause scrus typhus, the results showed that the rate of 1.57% positive for DNA with *O. tsutsugamushi*.[1,2]

For identification

IMPE HCM has also successfully established a protocol to identify species of helminths, external parasites such as ticks, mites, chigger mites, etc. by PCR, LAMP, and sequencing.

In a study in 2015, LAMP technology was used to identify tapeworm species and recorded 100% the specimens collected as *T.saginata*. The results show that there is a difference in the identification morphology (*T.saginata* is 68.75%, *T.solium* is 31.25%).

In a study from 2015 to 2017, the Institute used PCR techniques and sequencing to identify vector such as ticks, mites, chigger mites. The research showed that identification vectors born disease by molecular biology techniques in Vietnam has not been applied much, so that provide a general view to further research into identification by genetics for external parasite in Vietnam.

FOR DETECTING SPECIFIC GENES OF MICROORGANISMS

In a 2014 study, involving the finding of mutations in the K13 gene associated with artemisinin resistance in *P. falciparum* by PCR and sequencing methods, detected four points mutation at positions 543, 553, 580 and 447.[3]

In another study in 2015, determining genetic polymorphisms on the MSP1 of *P. falciparum* by PCR, the K1 genotype was 34.43%, identifying five allenes. MAD20 accounted for 37.70%, identified six variants of allen. The RO33 genotype was 27.87%, identifying two alleles.[4]

THE EXPERIENCES LESSION

- To detect the rate prevlence malaria parasite by PCR, real time PCR, compared result with the traditional method shown that about 3,91% to 14% parasite omitted if not apply the molecular biology to detetct. With the LAMP technique, which have the threshold less than the PCR , have the sensitive and specificity higher than traditional technique (such as RDT, giemsa…) and can apply this method in the field. This difference may be caused by the high threshold of Giemsa and RDT, while this community belong to the high risk malaria area, persistent malaria, the asymptomatic patient carry out low density parasite, the people in this community have immune response who can't detect by giemsa and RDT.

- Tests to find the prevelence rate of Ricketsia bacteria (caused scrub typhus) in external parasites, have not been published in Vietnam yet. The research report that the medical arthropods have pathogens and this can be a source of disease causing diseases affecting people's health.

- In the identification test, the Institute has applied LAMP technology to identify helminth, that have not been applied in Vietnam example tapeworm. However, according to some authors in the world, although this technique is more sensitive than PCR, but to confirm the advantages LAMP technique in identification species, we need proposals to compare with LAMP such as Real time PCR for more additional assertion, and extending the application of molecular biology techniques in identification helminths in the diagnosis and treatment of patients.

- For external parasites, the results of gene identification, sequencing of ticks, mites and chigger mites are initially to give an overview of the identification external parasites in the South Vietnam compare the world. This report is also the premise for further research into the specific identification with ticks, chigger mites and mites in Vietnam both morphologically and genetically. And in the future, we need construct specific gene sequences of external parasite, that is confirmed by morphologically in Vietnam, can be published in the GenBank.[1,2]

- In the results of the analysis of mutant point on the K13 gene associated with artemisinin resistance, 4 mutation sites were detected. This result can not be confirmed as artemisinin resistance, however, it partly indicates the location of the mutation in the K13 gene of patients living in high risk area. Thereby, this research can investigate and contribute to the study of artemisinin resistance based on molecular biology techniques.[3]

- The study of genetic polymorphisms on the MSP1 locus gene of *P. falciparum* by Nested PCR technique as a first step towards researchers analyzed genetic polymorphism of the gene locus other of *P. falciparum* (MSP1, MSP2, GLURP) to study the genetic structure and the genetic variation of the parasite populations in different endemic areas. This technique that can detect rerudescent and reinfections by comparing the difference in genotype and allelic variations between type before and after treatment to evaluate the effectiveness of malaria drugs in the community, especially the malaria heavy areas.[4]

CONCLUSIONS

In conclusion, the previous study of the IMPE HCM assesses the prevalence of malaria parasites in the heavy malaria population, which tested by the Giemsa technique (the gold standard for malaria parasites test) detected parasite with low density (about 0.2% to 0.5%), but with LAMP and Nested PCR method detected higher rates (4.4% and 14.7%). Based on this research results, a number of malaria parasites may be missed without the application of molecular biology techniques in the pathogens detection. Beside that, the researches about genetic identification also contributed a new look with more accurate and needs more deep research in the field of parasites, helminths and vectors born disease.

REFERENCES

1. Lê Thành Đồng et al (2017). Research the prevelence bacteria on ticks, chigger mites, mites in South Viet Nam - Lam Dong, Journal of Malaria and Paratite Diseases Control No. 4/2017, ISSN 0868-3735.
2. Lê Thành Đồng, Đoàn Bình Minh, Phạm Nguyễn Thúy Vy, et al (2017). Determination of the presence of viruses Bunyaviridae and Orientia tsutsugamushi families on ticks, mites and Chigger mites in the South Vietnam - Lam Dong, Journal of Malaria and Paratise Diseases Control No.5/2017, ISSN 0868-3735.
3. Nguyễn Thị Minh Châu et al (2016). Applying Nested PCR technique to identify mutations K13 gene in *P.falciparum* in Bu Gia Map district, Binh Phuoc province 2014, National Conference of Parasitology.
4. Nguyễn Thị Vân Anh, Phạm Ngyễn Thúy Vy et al (2015). Determination polymorphism of Plasmodium falciparum in the malaria-endemic area of Binh Phuoc province 2015, Journal of Malaria and Paratise Diseases Control No.(96)/2017.