“Evaluation of LAMP for Point-of-Care Detection and Surveillance of *Wuchereria bancrofti* at the Post-Elimination phase of Bangladesh”

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Background of the study

Lymphatic filariasis (LF) is a vector-borne neglected tropical disease, characterized by long-term disabilities worldwide including hydrocele and limb lymphedema[1]. The disease is caused by three parasitic nematodes - *Wuchereria bancrofti*, *Brugia timori*, and *Brugia malayi*. While *Wuchereria bancrofti* contributes to about 90% of filarial infections, *Brugia timori*, and *Brugia malayi* are responsible for the remaining cases[2]. Following ingesting microfilaremic blood from infected patients, mosquitoes of multiple genera Culex, Anopheles, and Mansonia transmit filarial parasites to uninfected humans[3].

At present, more than 36 million people are infected and almost 882 million people in 44 countries are at risk of developing LF infection globally. As Bangladesh is currently at the post-elimination stage, recrudescence of filarial infection is possible and identification of such zones is the main priority based on the National Strategic Plan 2018-2025 [4] [5]. For this purpose, a strong surveillance approach is now vital for the detection of filarial infection and the maintenance of the elimination status.

In the case of diagnosing human filarial infections, Loop-mediated isothermal amplification (LAMP) has high levels of specificity and sensitivity, and is more tolerant to inhibitors found in clinical specimens, making it useful for low-resource settings[6]. LAMP offers field-friendly, time-intensive alternatives to other molecular diagnostics and can be performed using heat-blocks, with results read by the eye under UV light[7].

As of yet, no filarial-specific LAMP-based surveillance study has been conducted in Bangladesh at the post-elimination phase of lymphatic filariasis. Therefore, in this study, LAMP assay will be carried out on the finger-pricked blood samples according to the standard operating procedure (SOP). The filarial infection rate will be determined for the regions within Bangladesh and graded as zones with high, moderate, and low transmission risk accordingly.

Aims and Objectives

Aims

- To determine the infection rate based on the results of the LAMP assay.
- Monitoring and maintenance of elimination status of lymphatic filariasis at the post-elimination phase of the disease in Bangladesh.

General objective:

- To develop a LAMP based surveillance approach in LF endemic regions at the post-elimination phase of Bangladesh.

Methodology

Study site and Study duration:

A subdistrict of Rangpur district, Gangachara, was chosen as the desired field site since the zone is highly endemic for LF. The laboratory activities will be carried out at the ‘Clinical Biochemistry and Immunology laboratory’ of the University of Dhaka. The study will span over one year only.

Sample Size

We will be using a total of 2,085 archived finger pricked blood samples of subjects at the post-elimination phase of lymphatic filariasis for carrying out the LAMP assay.

LAMP assay

All assay components will be thawed, followed by a brief vortex to ensure thorough mixing. The mixture will then be centrifuged to collect the material, after which it will be placed on ice. A reaction mixture will be prepared using the Colorimetric LAMP Master Mix, specific LAMP primers, and nuclease-free water. The reaction mixture will be vortexed again and centrifuged to collect the material. A volume of 24 µl of the prepared reaction mixture will be pipetted into the designated reaction vessels, followed by the addition of 1 µl of the extracted DNA sample. The contents will be mixed by vortex or pipetting if using similar plates or vessels, and then centrifuged to collect the solution. The reaction solution will be checked for a bright pink color, indicating the initial high pH required for successful pH-LAMP reactions. After incubation, the tubes or vessels will be removed and examined visually. Positive reactions will exhibit a color change to yellow, while negative controls should remain pink.

Data analysis

Normality test will be carried out on data of continuous variables using the Shapiro-Wilk test. The receiver operating characteristic (ROC) analysis will be performed to evaluate the diagnostic performance of the LAMP assay. An algorithm will be set up based on the amplification result based on the LAMP assay from the subjects and accordingly, the regions of the endemic zone will be stratified as areas with high, moderate, and low filarial transmission risk. All the statistical analyses will be conducted via IBM SPSS Statistics (Version 26.0) and GraphPad Prism (Version 8.0.1).

Socio-economic importance

The study based on LAMP-based surveillance of lymphatic filariasis will help us to identify regions with ongoing filarial transmission within the LF-endemic subdistrict of Gangachara. The filarial infection rate, as determined following the surveillance, will allow us to assess whether a resurgence of the infection has occurred or not. If the infection is found to be high, the National Programe for Elimmination of Lymphatic Filariasis (NPELF) will take necessary steps, e.g. implement MDA in some regions for reducing LF incidences and maintaining a constant rate of elimination at the post-elimination phase. Based on the results of the study, we will use this surveillance tool in different regions of Bangladesh to create a nationwide risk map for LF.

Conclusions

The study would allow us to determine the prevalence of lymphatic filariasis at the post-elimination phase of the disease based on LAMP assay. Assessing the presence of filarial infection would aid us in taking necessary measures for reducing cases and limiting further transmission of the disease. The activities in the study would help us to achieve the targets of Section 3 named “Good Health and Well-being” of The Sustainable Development Goal in Bangladesh.

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