ABSTRACT

Cancer is one of the leading causes of human morbidity and mortality. Timely diagnosis is critical to successful treatment, and the stage at which the disease is diagnosed determines prognosis. Devices for point-of-care diagnosis are being developed for many life-threatening diseases like diabetes, however, the complexity and diversity of cancer hinders the development of such devices. Moreover, health monitoring using advanced technology remains inaccessible and unaffordable to majority of the population, particularly in developing, or underdeveloped economies.

The objective of this work was to develop and use an affordable microfluidic platform for cancer screening using micro RNA (miRNA) in the blood as a biomarker. To this end, a device was designed and developed in which miRNA sequence-specific molecular beacon probes are allowed to hybridize to serum miRNA on a microfluidic platform, followed by visualization of the result on an easy-to-use fluorescence detector. miRNA-18a and miRNA-21 have been reported to be overexpressed in the blood of patients with retinoblastoma and breast cancer respectively. Molecular beacon probes for these miRNAs were used to test the microfluidic device.

Ethical clearance was obtained from Institutional Human Ethical Clearance Committee (IHEC no.16/180 dated 7-9-2016 and 14/173 dated 26-6-2014). Twenty blood samples were used to prove the concept for retinoblastoma. In the case of breast cancer, 51 blood samples altogether from healthy and breast cancer positive patients were collected for the study Experiments were designed to prove the simple concept of measurement of miRNA expression for screening cancer. COMSOL Multiphysics was used to design and evaluate mixers of different configuration to arrive at an efficient

passive mixer using split and recombine principle. A suitable microfluidic mixer was finalized for the purpose of screening that has effectively reduced the cost of screening by way of minimizing volume, miniaturization and at the same time allowing efficient hybridization. The passive mixer realized using pillar structures making the fluid split and recombine (SAR) while in flow is novel, cost effective and compatible for mass production. The mixing index of the SAR mixer is 0.78 at a length of 50µm with fine meshing which is superior (ease of fabrication) to passive mixers listed which was 0.96 at 10 mm for a complex 3-dimensional zig-zag channel. Thus, the concept was converted into a prototype by developing microfluidic platform for efficient hybridization of probe and target, and a simple to use fluorescence reader with user friendly display. The performance of the newly developed method was validated against the gold standard for screening, viz, quantitative Real Time Polymerase Chain Reaction (q-PCR). The significance of the method was analysed using a statistical tool to evaluate the sensitivity and specificity in comparison to existing screening method which is mammography for breast cancer.

The device developed allows easy, frequent screening of high-risk populations without the additional risks associated with radiation-dependent screening procedures such as mammograms. In the case of retinoblastoma, there is no recommended screening method other than collecting inputs from the parents upon an observed symptom. Hence, the screening for retinoblastoma can be easily done for new born babies, if they are at high risk. The simplicity of the test also makes it highly scalable for large-scale screening programs. The most important aspect is that there is no bias on the measured status which is very much reported as a drawback for mammography. Also, the sensitivity and specificity were reported to vary among symptomatic and asymptomatic women. Statistical analysis and

evaluation of the method developed revealed a significance level of 99.8% for retinoblastoma when a threshold level for detection was kept at 2.71 Volts. In the case of breast cancer, the significance level was 96% for a threshold value of 2.6 Volts with CI >95%.

Therefore, this device provides a low-cost, minimally invasive, versatile platform for the detection of one or more circulating biomarkers with high sensitivity and specificity, with customized probes for each biomarker. A patent application was filed for the microfluidic device and the method of detection vide application number 3035/CHE/2015 dated 17/06/2015.