8. INTRODUCTION

Breast cancer has the highest incidence among cancers in women worldwide [1]. An estimated 2.08 million women worldwide had been diagnosed with breast cancer in 2018, and in a five-year prevalence period, 6.8 million women have breast cancer [1]. In India, the incidence is 162 thousand, with a five-year prevalence is 405 thousand in 2018 [1]. Breast cancer is curable if diagnosed in the early stage. Hence, screening is important for early detection. Mammography is the current gold standard for breast cancer screening. However, mammography is less sensitive on younger women due to denser tissue. Further, the radiation effects due to the X-rays may be harmful enough to cause cancer in younger women [2]. The reported sensitivity of mammography ranges between 64% in extremely dense breast tissue to 90% in less dense tissue [2].

The feasibility of using thermography as an alternative breast imaging modality to current modalities is being evaluated with a goal towards screening of the patients to detect early breast cancer. Niramai has pioneered an advanced method of screening for breast cancer by enhancing thermography with machine intelligence. This combination of thermography and computer aided diagnostics is called Thermalytix™. Patented Niramai's Thermalytix™ solution uses machine learning (advanced data analytics and an upcoming research theme in Computer Science) consisting of probabilistic mathematical modelling of a decision process and statistical analysis of historic data to predict whether the subject belongs to malignant class or benign/normal Class.

RATIONALE FOR STUDY

The purpose of this study was to evaluate the effectiveness of Thermalytix™ algorithm over popular methods of screening used as the current standard of care.

9. STUDY OBJECTIVES

9.1 PRIMARY OBJECTIVE

1. To evaluate the effectiveness of Thermalytix™ for breast cancer screening as compared to standard screening modalities for breast cancer
2. To correlate the results of Thermalytix™ with digital mammography for screening diagnostics on patients walking in for health check as measured by:
    - Sensitivity of Thermalytix™ as compared to sensitivity of mammography
    - Specificity of Thermalytix™ as compared to specificity of mammography

9.2 SECONDARY OBJECTIVES

1. Influence of patient characteristics on diagnostic accuracy of Thermalytix™.
   Patient characteristics will include:
    - Age
    - Lesion type
    - Pathologic diagnosis
    - Menopausal and hormonal status
    - Breast density
    - Family history
2. Recommend how Thermalytix™ can be used to complement standard breast cancer screening procedure.

10. INVESTIGATIONAL PLAN

10.1 OVERALL STUDY DESIGN AND PLAN

This was a prospective, comparative study to evaluate the effectiveness of Thermalytix™ when compared to the standard screening modalities in females of age 18 years and above. The study was initiated after appropriate clearance from the ethics committee.

The study visits included a screening visit (Visit 1) followed by visits at Day 2-5 (Visit 2), Day 6-10 (Visit 3) and Day 10-14 (Visit 4):

1. The Visit 1 procedures included informed consent process, collection of information regarding current complaints, past medical history and treatment, family history, physical examination, record of risk factors, followed by Thermalytix™ procedures, and mammography as part of routine screening for all woman enrolled in the study. The Mammography machine used was (GE Dmr Plus analog Mammography and Prodigy). Thermalytix employed Algorithm ver 3.0.
2. The Visit 2 procedures were based on the results of Thermalytix™ and mammography and were conducted only for the women who had an inconclusive mammography report or who were reported as positive by either or both Thermalytix™ and mammography.
    - If mammography was inconclusive, a confirmatory test which included USG, or MRI scan was recommended by the clinician. The breast ultrasound (USG) was done using Samsung RS85 and MRI was performed on GE 3.0T.
    - If either or both Thermalytix™ and mammography reported the case as positive, a confirmatory test which included USG, MRI, or PET scan was recommended by the clinician. If any of the three tests identified a suspicious lesion, FNAC/biopsy was recommended in the same or follow-up visit.

3. The Visit 3 procedures included FNAC/biopsy and related laboratory work-up. Women underwent MRI guided biopsy in cases where results were positive by MRI.
4. The final visit or the Visit 4 was done by telephonic contact and all the procedures were completed for all the subjects enrolled into the study.

Women reported as negative for cancer by both tests (Thermalytix™ and mammography) would complete visits 1 and 4 only. Whereas positive cases and suspected cancer cases completed all visits as applicable. The patients were expected to go through the entire screening process within a duration of 2 weeks from enrollment on the study.

Efforts were made to conclude the screening procedure as early as possible for the subjects. The screening process was completed on the same day when subjects were enrolled in the study. No subject had a screening process that went beyond the allocated time of 14 days.

10.2 DISCUSSION OF STUDY DESIGN

The prospective and comparative nature of the study design was the most appropriate approach to evaluate the objectives of the study because the specificity, sensitivity, and accuracy of Thermalytix™ would be beneficial in clinical practice.

This study was done as a comparison to the standard method of mammography. The sensitivity of Thermalytix™ (an automated thermographic screening algorithm developed by Niramai) was compared to the sensitivity of mammography and to study how Thermalytix™ can complement the standard modalities in breast cancer screening in future.

10.3 SELECTION OF STUDY POPULATION

10.3.1 Inclusion Criteria

Subjects were eligible for participation in the study if they met the following criteria:

1. Female subjects equal to and above 18 years
2. Subjects who are willing to give written informed consent for study participation
3. Subjects who are ready to comply with the study related visits and procedures

10.3.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. Subjects who are pregnant
2. Subjects who are lactating
3. Subjects who have undergone either lumpectomy or mastectomy
4. Subjects who have undergone chemotherapy in the last 2 weeks at the time of study enrollment
5. Any active illness, psychological and/or pathological condition that would interfere with study participation in the opinion of the Investigator

10.3.3 Removal of Subjects from Assessment

Subjects were free to withdraw consent at any time during the study. Data was collected up to the time of withdrawal, but no additional information was collected after this time.

10.4 STUDY PROCEDURES

All the enrolled subjects underwent thermal imaging (to conduct Thermalytix™) by a technician trained by the sponsor in strict compliance to the procedure (Appendix I of the

study protocol) using thermal camera. The thermal images were interpreted by the AI-based algorithm 'Thermalytix™'. The Thermalytix™ report and the standard screening reports were collated by the study coordinator and entered in the CRFs.

The results of Thermalytix™ and mammography were correlated, and the correlation results were analyzed by PI. If the reports of the two tests correlated, a secondary test was not mandated. In case of inconclusive reports from standard modality, subjects underwent confirmatory breast USG/MRI/PET scan as determined by the PI. In case Thermalytix™ identified lesions suspicious of malignancy and mammography did not identify the lesion, to resolve the discrepancy between the two tests, the subject underwent confirmatory breast USG/MRI/PET scan as determined by the PI. USG-guided biopsy was the confirmatory test for cases reported as positive by imaging modalities. MRI-guided biopsy was performed in cases where results were positive for malignancy by MRI. When all the results were received and final diagnosis were confirmed, subjects were contacted telephonically to complete the end of trial procedure and their treatment course was decided by the treating doctor.

Study Coordinator transcribed all the data in the CRF. Data from individual CRFs were entered by the Study Coordinator in the excel spreadsheet and sent to the Data Management group for analysis.

10.4.1 Identity of Investigational Product

Thermalytix™: Machine Learning based Automated Thermographic Screening Algorithm 3.0 developed by Niramai.

10.4.2 Procedure Compliance

Any non-compliance was reported in CRF as deviation.

10.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF THE SAMPLE SIZE

10.7.1 Statistical and Analytical Plans

Analysis of the data was performed by the Biostatistics and Statistical Programming team of Statiza Statistical Services. All statistical analyses were performed using R Software version 3.5.0 and SAS® Version 9.4 or higher [SAS Institute Inc., USA].

The following standard descriptive summaries were presented:
The continuous data was summarized using the number of observations (n), arithmetic mean (mean), standard deviation (SD), median, minimum value (min), and maximum value (max). The categorical variables were summarized using the frequency count (n) and percentage (%) for each possible value. The frequencies were presented up to 0 decimal places and percentage up to 2 decimal places.

Thermalytix™ and standard modalities were evaluated using following measures of validity:

- Sensitivity – The probability of correctly identifying a true case of malignancy
- Specificity – The probability of correctly identifying a true case of non-malignancy (normal or benign)
- Positive Predictive Value (PPV) – The probability that a labelled positive was a true case of malignancy
- Negative Predictive Value (NPV) – The probability that a labelled negative was a true case of non-malignancy

All the methods of validity were computed for Thermalytix™ along with their 95% CI.

i. Subject Population

FAS Population: All enrolled subjects who underwent thermography (AI-based Thermalytix™) and mammography and satisfied inclusion criteria and rest of the protocol requirement, were considered in Full Analysis set (FAS). All statistical analyses were performed considering FAS population.

ii. Primary Effectiveness Analysis

Sensitivity, specificity, PPV and NPV of Thermalytix™ as compared to that of standard modalities were reported along with 95% confidence interval.

iii. Secondary Effectiveness Analyses

Influence of patient characteristics such as age group, menopause status and breast density on diagnostic accuracy (sensitivity, specificity, PPV and NPV) of Thermalytix™ as compared to that of standard modalities were reported along with 95% confidence interval.

iv. Safety Analysis

Thermography measures the skin temperature remotely and hence is virtually free of any adverse side effects. Thermography is a pain-free, non-invasive examination. Thermography does not require the use of sedatives, anesthesia, or any other medications. It is passive and measures the temperature on the skin, similar to a thermometer. However, in case of any adverse events (AEs), the AEs reporting followed regulations related to spontaneous Individual Case Safety Reports management, i.e., Investigators were asked to report only related AEs (non-serious and serious) to the sponsor.

10.7.2 Determination of Sample Size

The sample size was calculated with two-sided 95% confidence interval (CI) for a sample sensitivity and target width of 0.5 using Clopper-Pearson Interval (Exact) Method. The prevalence of breast cancer and other breast abnormalities in Indian women during their lifetime was assumed to be 4% (1 in 25 were expected to have a breast abnormality in their lifetime in India). A sample size of 300 screening patients allows estimating the true prevalence by means of an exact 95% confidence interval with width of 0.487 percent points when the sample sensitivity was assumed to be 80%. Considering exclusions from data analysis due to multiple visits needed, over 600 women were recruited. Eventually, data of 459 women could be used for statistical analysis as they satisfied inclusion criteria and adhered to the study protocol.

10.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

10.8.1 Changes in the Conduct of the Study

There were no changes in the planned study conduct.

10.8.2 Changes to the Analyses

There were no changes in the analyses before and after database lock.