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ORIGINAL ARTICLE

Establishment of reference range of Human Epidermal Growth factors receptor (HER2) by chemiluminescence method in healthy females.

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ABSTRACT… Objective: To establish reference range for serum HER2 levels in normal Asian females and provide a comprehensive overview of its current understanding to step towards improving the accuracy and utility of serum HER2 as biomarker. **Study Design:** Cross Sectional study. **Setting:** Farooq Hospital Westwood, College of Allied Health Sciences, Akhtar Saeed Medical & Dental College Lahore. **Period:** October 2024 to December 2024. **Methods:** The female participants visited aged 30-70 years, free of symptoms and signs suggestive of any breast abnormality were included. The 120 female participants were enrolled for establishing reference value of serum HER2 levels. The venous blood sample was drawn for HER2 levels. The auto analyzer was used for HER2 estimation. The data was analyzed through the IBM SPSS V.27.0. **Results:** A total 120 females were enrolled. The mean + SD was 45.0 + 9.879. The weighted average test was used to determine 25th and 95th percentile value in order to establish lower reference limit and upper reference limit of HER2 assay. The established reference range was 9.41 to 14.03 ng/ml. The results revealed that 25% data was below 9.41ng/ml and 95 % of females HER2 levels were under 14.03ng/ml. **Conclusion:** The present study established a preliminary reference range for serum HER2 in healthy females, contributing to improved breast cancer diagnostics and monitoring in our region.

Key-words: Asian Females, Human Epidermal Growth Factor Receptor 2, Reference Range.

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INTRODUCTION

In Pakistan, breast cancer is a serious health issue that has become more common in recent years. In Pakistan, about 38.5% of females have breast cancers, making it the most prevalent cancer among women.[1] An estimated 90,000 new cases of breast cancer are diagnosed each year, and the incidence has been continuously increasing.[2] However, breast cancer is frequently discovered in Pakistan at an advanced stage, which lowers the likelihood of a successful course of treatment and raises the death rate.[3] As the breast cancer is a heterogeneous disease, distinct tumor regions may exhibit different genetic changes or traits.[4] There are various subtypes of this cancer, each with unique clinical characteristics and reactions to treatment.[5]

Human Epidermal Growth Factor Receptor 2, or HER2, is a protein that is essential for cell division, growth, and proliferation. It belongs to the protein family known as the epidermal growth factor receptor (EGFR). Many cancer types are frequently linked to HER2 overexpression or amplification, most notably breast cancer (20-30%).[6] HER2-positive tumors in breast cancer have excessive HER2 protein on their cell surfaces, which causes the cells to grow out of control and makes the cancer more aggressive. Compared to other types of breast cancer, HER2-positive breast cancer is more aggressive and may have a worse perspective. This protein has recently become a very important biomarker and target for diagnosis, prognosis, and treatment in breast cancer patients. The HER2 protein has three parts: the transmembrane, extracellular, and intracellular tyrosine kinase domains. The extracellular domain can be released into the blood after cleavage and shedding from the tumor cell surface by metalloproteases. Therefore, it can be detected in the serum. In 18% of primary breast cancers and 46% of metastatic breast cancers, serum HER2 levels are higher.[7]

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There are several evidence regarding the correlation of serum HER2 levels and tissue HER2 protein overexpression as well as poor prognosis in a metastatic type of breast cancer.[8,9] HER2 status is a critical factor in breast cancer treatment decision-making. Patients with HER2-positive breast cancer are typically recommended to receive HER2 targeted therapy in addition to standard treatments like chemotherapy and surgery. This personalized approach to treatment has led to better outcomes for patients with HER2-positive breast cancer.[10]

Despite the growing interest in serum HER2 level as a biomarker, there is lack of data on reference ranges for serum HER2, particularly in specific demographic groups such as Asian females. Biomarker levels can be influenced by genetic and ethnic variations, and extrapolating reference ranges from Western populations to Asian populations might not be suitable. Serum HER2 levels may be impacted by the fact that Asian women, for example, typically have lower body mass indices (BMIs) and distinct hormonal profiles than Western women. Serum HER2 levels may be impacted by environmental and lifestyle factors in addition to demographic ones.

For instance, it has been demonstrated that HER2 expression are modulated by diet, exercise, and exposure to environmental toxins. Asian populations frequently follow unique dietary habits, such as consuming large amounts of green tea and soy products, which contain bioactive compounds that may affect HER2 signaling pathways. Moreover, obesity and metabolic syndrome, which are linked to changed HER2 expression, are more common in Asia as a result of urbanization and changing lifestyles. These results underline the necessity of more investigation to develop population-specific reference ranges. For serum HER2 testing to be accurate and reliable in clinical practice, a population-specific reference range must be established.[11,12]

The most popular methods for measuring serum HER2 levels are chemiluminescence immunoassays (CLIAs) and enzyme-linked immunosorbent assays (ELISA), which can identify the extracellular domain of HER2 that has been released into the blood. However, it can be difficult to interpret results from various labs and studies due to the absence of standardized assays and reference ranges. Assay sensitivity, specificity, and reproducibility variations can produce contradictory results, highlighting the necessity of standardized procedures and clearly defined reference intervals.[13,14]

Given the increasing prevalence of HER2-positive breast cancer in Asia, it is especially important to establish a reference range for serum HER2 levels in healthy Asian females. In order to improve the precision and usefulness of serum HER2 as a biomarker, the present study set a reference range for serum HER2 levels in healthy Asian females and provides understanding to step towards improving the accuracy and utility of serum HER2 as biomarker. The present study can improve outcomes for patients with HER2 positive breast cancer and increase the clinical relevance of HER2 testing by filling these gaps.

METHODS

The study is cross-sectional and was conducted at the Farooq Hospital Westwood Branch, College of Allied Health Sciences, Akhtar Saeed Medical & Dental College Lahore. The intuitional review board authorized this study (Letter No: CAHS-08/2024-MLT-61). The non-probability convenient sampling technique was followed. The female participants visited during the month of October 2024 to December 2024, aged 30-70 years, free of symptoms and signs suggestive of any breast abnormality were included. Females with any breast related abnormality, and used or using any medications were excluded. The 120 female participants were enrolled for establishing reference value of serum HER2 levels.

After verbal informed consent, the demographic details of each participant were recorded. Under aseptic conditions, venous blood sample was drawn for HER2 levels in yellow top vaccutainer, labeled with individual's name and identification number. The sample was allowed to clot and serum was separated through centrifugation at 3000rpm. The serum was stored in labeled eppendorf cups at -80 till further analysis. Chemiluminescence-based auto analyzer Maglumi X8 by SNIBE was used for analysis. It uses nano-magnetic microbeads separation technology, the luminescence substrate

being N-(aminobutil)-N-(ethyl)-isoluminol (ABEI). The performance characteristics of the assay are given (Table-I). The samples were run in a batch. The internal quality control provided in the reagent kit was run with each batch analysis.

TABLE-I

Performance characteristics of HER2 assay

Precision (Intra-assay CV%)	Analytical Sensitivity	Calibration Traceability	Interfering Substances	Hook Effect
2.84%	2.0 ng/mL 0.5-350ng/mL	Snibe Internal Reference Material	Substances upto the following concentrations did not interfere with the assay ▪ Bilirubin 30mg/dL ▪ Hemoglobin 1000mg/Dl ▪ Triglycerides 1000mg/dL	None

The Statistical Package for Social Sciences (IBM SPSS V.27.0) was employed to analyze the data. The Kolmogorov-Smirnov (K-S) test was employed to evaluate the normality of the data. The age was represented as the mean ± (standard deviation). The reference values were calculated using non-parametric methods due to the non-normal distribution of the data. The 25th and 95th percentile values were determined using the weighted average test to establish the lower and upper reference limits of the HER2 assay.

RESULTS

From total 120 females, the minimum to maximum age was 30-70 years. The mean + SD was 45.0 + 9.879. Normality of data was analyzed by using K-S test which showed that data was not normally distributed as p value was < 0.05 (p=0.005). The present study was conducted to establish upper reference limit and lower reference limit of HER2 assay, which is used as diagnostic and prognostic biomarker of HER2 positive breast cancer. The estimated value of HER2 assay is given (Table-II).

The data was not normally distributed so the weighted average test was used to determine 25th and 95th percentile value in order to establish lower reference limit and upper reference limit of HER2 assay (Table-III). The established reference range was 9.41 to 14.03 ng/ml. The results revealed that 25% data was below 9.41ng/ml and 95 % of females HER2 levels were under 14.03ng/ml.

TABLE-II

Descriptive statistical analysis for estimated value of HER2 assay

Descriptive Analysis	Serum HER2 Levels
Median + Inter quartile range	11.3800 + 1.855
Standard error of mean	0.16866
Kit literature cut-off value	15.0 ng/ml
Observed minimum value	7.75 ng/ml
Observed maximum value	14.70 ng/ml

TABLE-III

25th and 95th Percentile values of HER2 assay

	Percentiles	
Weighted average	25th	95th
	9.41 ng/ml	14.03 ng/ml

DISCUSSION

HER2 overexpression is most commonly detected in breast Cancer.[13] HER2 is an integral membrane protein that weighs 185 kD. The extracellular domain of HER2 is separated from other components of HER2 and enters the bloodstream after its breakdown by metalloproteases. Currently, the most frequently employed methods for evaluating the expression of HER2 are immunohistochemistry, which involves the immunostaining of the HER2 ECD, and Fluorescence In Situ Hybridization (FISH), which involves the fluorescent labelling of amplified HER2 DNA. In breast cancer patients, serum HER2 levels have been reported to be correlated with tissue HER2 status.[15] Since determining the tissue HER2 status requires biopsy which is an invasive and time taking procedure, therefore serum HER2 levels are being studied as biomarker of HER2 positive breast cancer.[16]

The establishment of reference range for serum HER2 levels is essential for accurate clinical interpretation in breast cancer diagnostics and monitoring.[13] In present study, the reference range for serum HER2 in healthy females was determined to be 9.41-14.03 ng/mL, based on a sample size of 120 healthy female participants. This range

provides a critical baseline for distinguishing normal physiological levels from pathological elevations seen in HER2-positive breast cancer patients. The present study findings are in consistent with previous studies conducted in Asian populations but show slight variations when compared to Western data. For instance, a study conducted in Nepal determined the optimal cutoff value of serum HER2 levels to be 16.02 ng/ml which is close to the upper reference limit established in present study.[17] Whereas, in different studies conducted in Western countries, the cut off has been found to be between ranges 8.83-21ng/mL.[13,18]

Several other factors may contribute to variations in serum HER2 levels, including age, hormonal status, and BMI. Studies demonstrated that postmenopausal women tend to have slightly elevated HER2 levels compared to premenopausal women, possibly due to hormonal fluctuations.[11] Although the present study did not stratify participants based on menopausal status, future research should explore this aspect to refine reference ranges further. Additionally, there is impact of assay methodologies on HER2 measurements, underscoring the need for standardized testing protocols to ensure consistency across laboratories.[14] In present study, the state-of-the-art random access fully automated system (Maglumi X8) for the analysis of serum HER2 level was used. This system is the ultimate development of conventional ELISA plate readers that required immense time and manpower resources with many potential sources of errors.[19] Naoki et al. conducted a study and concluded that CLIA was more sensitive method than ELISA for measuring serum HER2 levels.[20]

The establishing a reference range for serum HER2 in healthy Asian women has important clinical ramifications, especially for the treatment of breast cancer. HER2-positive breast cancer is a more aggressive subtype that necessitates targeted treatments like trastuzumab, is linked to elevated serum HER2 levels. Clinicians can decrease false-positive diagnosis and improve their interpretation of borderline cases by establishing a normal range. Furthermore, since decreasing HER2 levels frequently correspond with therapeutic efficacy, this reference range can help with tracking treatment response.[21] In order to validate these reference ranges across an array of Asian subpopulations, future research should incorporate larger, multi-center cohorts. Furthermore, longitudinal research evaluating HER2 variations over time in healthy individuals may offer more profound understanding of its biological variability.

CONCLUSION

The present study improved breast cancer monitoring and diagnosis in our region by establishing a preliminary reference range for serum HER2 in healthy females. The clinical utility of these findings will be improved by additional studies with larger cohorts and in-depth subgroup analysis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION	
1	**Maham Shakoor:** Concept of project, data collection.
2	**Asim Mumtaz:** Review.
3	**Atika Masood:** Drafting.
4	**Atiqa Arshad:** Literature search.
5	**Zainab Yousaf:** Writeup manuscript, data collection.
6	**Zaniab Akram:** Statistical analysis.