

ABSTRACT : Background: Thyroid malignancies account for most common endocrine cancers world-wide with incidence increased annually. Cytology-based diagnosis has limitation in accurate delineation of thyroid malignancies. Hence, the study aims to develop an affordable molecular- cytology tool to improve the diagnoses. **Objectives:** i) to identify type of aberrant glycosylation in benign and malignant thyroid nodules by lectin histo-chemistry ii) to determine the efficacy of lectins in delineating thyroid malignancy by cytology. **Methodology:** Lectins, Wheat germ agglutinin (WGA) and Maackia amurensis Agglutinin (MAA) were selected for lectin immunohistochemistry based on preliminary data from oral cancer. Lectin histochemistry was performed on histologically annotated benign and malignant thyroid tissue and the marker expression was compared with histology diagnosis. Statistical significance was assessed using ANNOVA/ t test (P value<0.05) and Receiver Operating Characteristic (ROC) curve analysis. **Results:** WGA and MAA expression were significantly higher in malignant as compared to benign thyroid tissue (p<0.05). WGA and MAA showed a sensitivity of 89.5% and 84.2% respectively and specificity of 92.3% and 88.5% respectively in delineating benign and malignant thyroid nodules. Area under the curve (AUC) of both markers were 0.89. **Conclusion:** The study aims to address the clinical unmet need in diagnosis of thyroid nodules by improving current standard of care. The outcome will be a panel of lectins that can differentiate benign and malignant thyroid nodules; they can be developed into a lectin-based cytology assay in the future

BACKGROUND

Thyroid malignancies account for most common endocrine cancers world-wide with incidence increased annually.(1)
Current diagnostic methodology: USG guided FNAC followed by Bethesda reporting.

- Diagnostic accuracy high in Category II (benign) and VI (malignant).
- Categories III/IV/V(indeterminate) : diagnostic hemi-thyroidectomies to obtain a definite histological diagnosis (2,3)

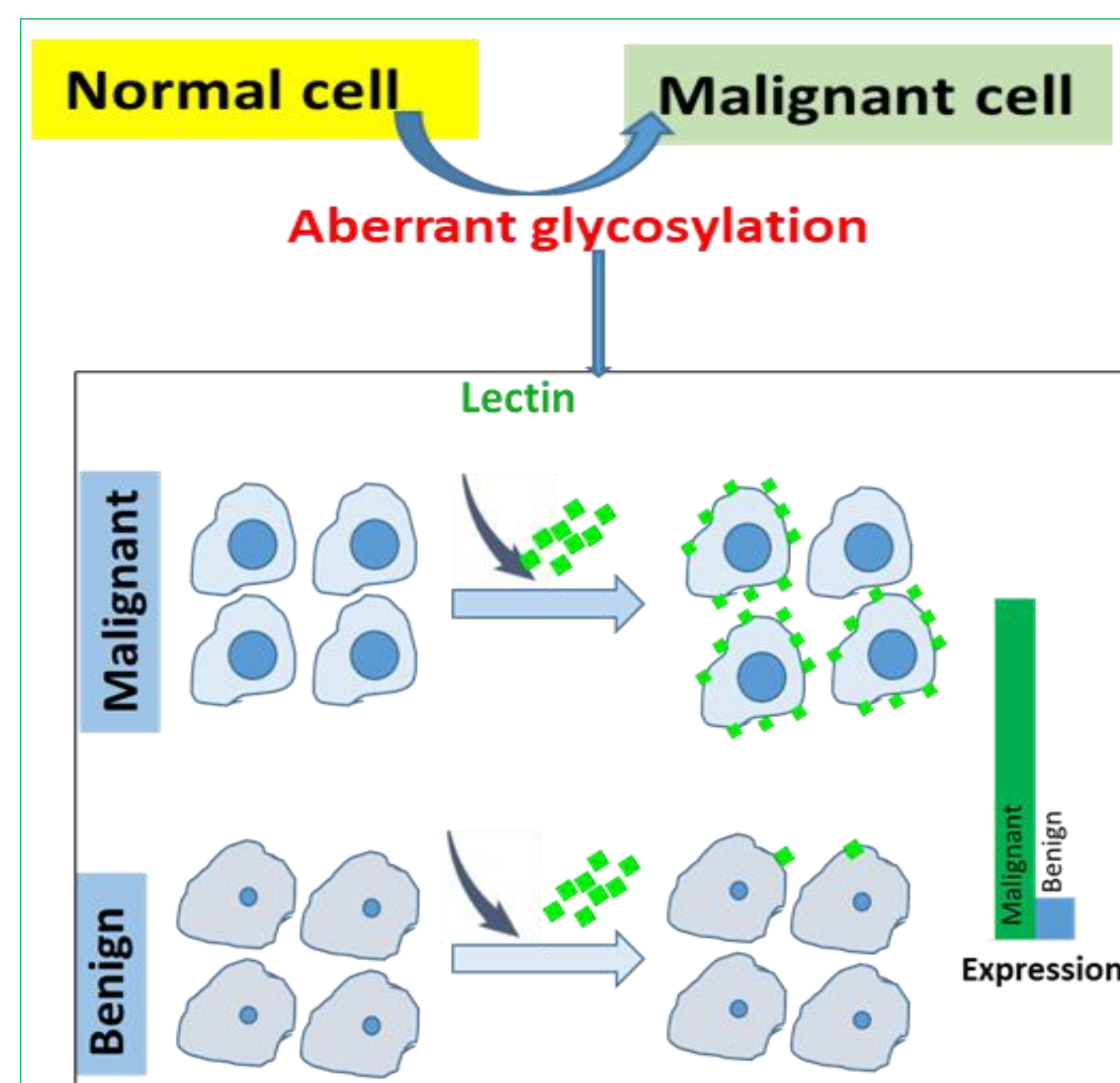
Primary challenges

- Indeterminate category of nodules comprise 30-45% of nodules
- Diagnostic hemi-thyroidectomies : over-treatment for benign nodules
- Under-treatment for malignant nodules needing revision second surgery (4)

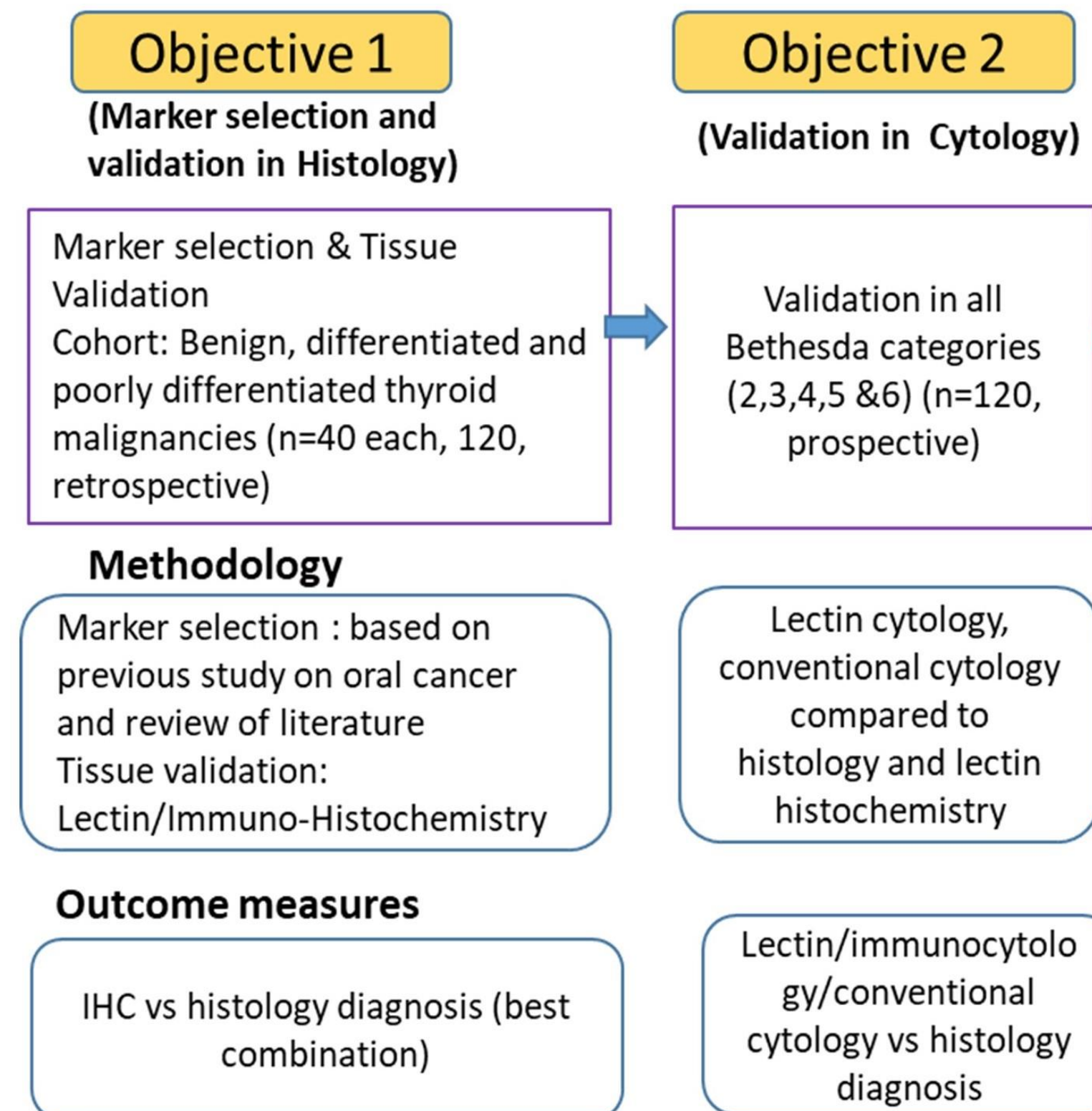
OBJECTIVES

- To identify type of aberrant glycosylation in benign and malignant thyroid nodules by lectin histo-chemistry
- To determine the efficacy of lectins in delineating thyroid malignancy by cytology

HYPOTHESIS



METHODOLOGY

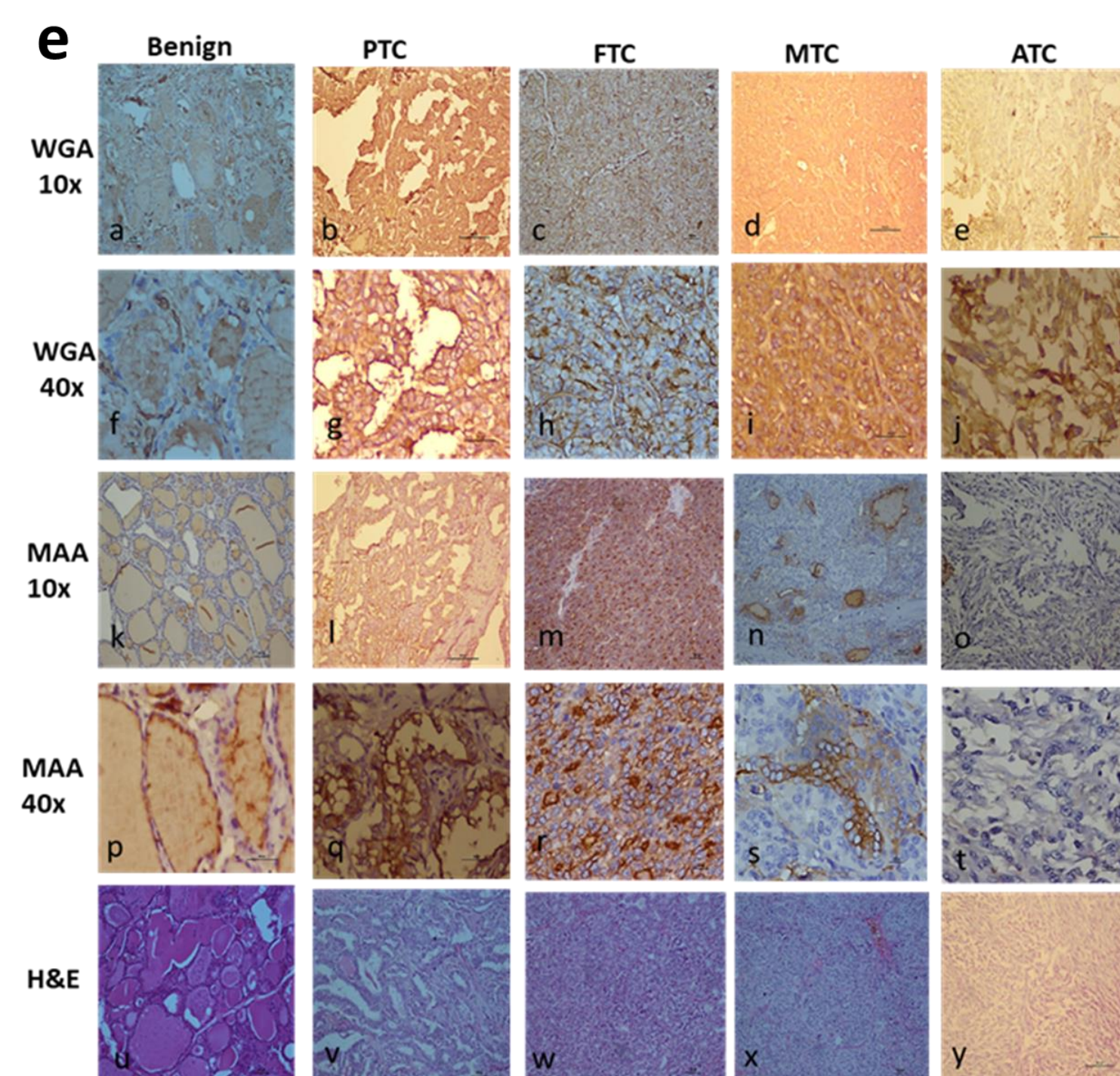


RESULTS

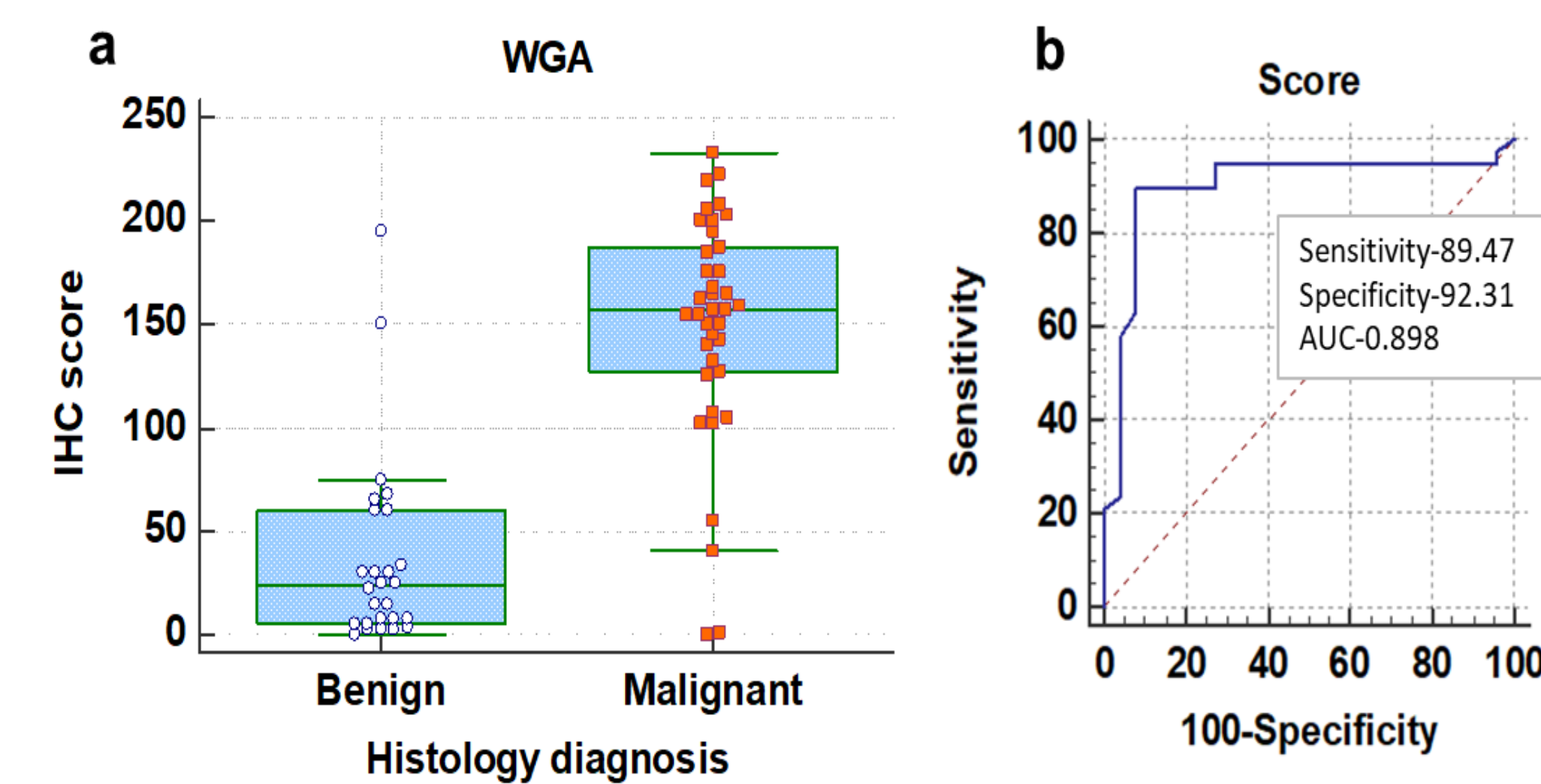
- Marker selection-** Lectins WGA (Wheat-Germ-Agglutinin): and MAA (Maackia-Amurensis-Agglutinin) selected based on preliminary data available from lectin profiling in oral cancer done by our group.
- WGA, MAA expression validated in the first set of histologically annotated benign (n=26) and malignant (n=38) FFPE tissue sections
- Malignant thyroid-follicular cells exhibit diffuse membranous expression of the marker. Benign-follicular cells are negative for the markers

Thyroid IHC : Staining pattern with WGA and MAA

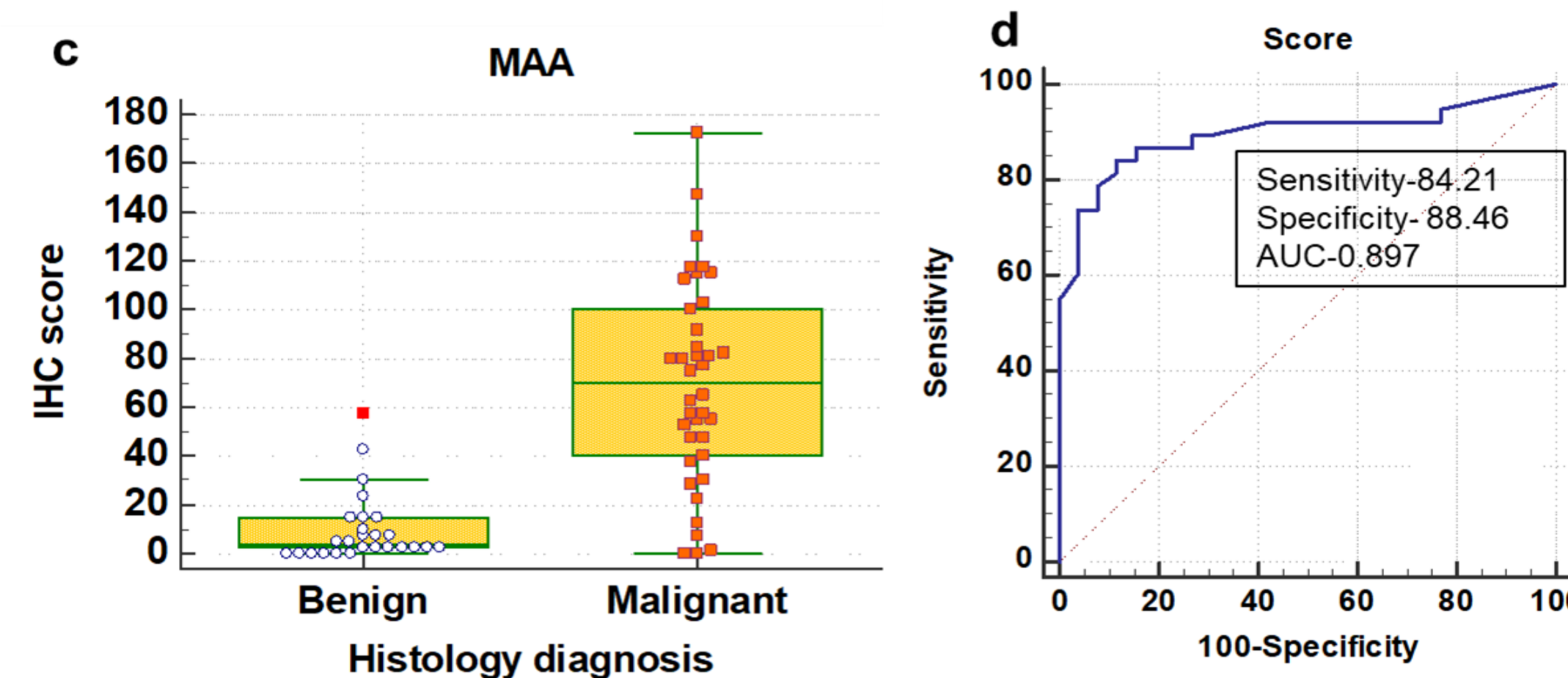
PTC-Papillary thyroid carcinoma, FTC-follicular thyroid carcinoma, ATC- Anaplastic thyroid carcinoma, MTC- Medullary thyroid carcinoma



Profile of WGA and MAA expression: (a,c) Box and whisker plot : Malignant thyroid tissue show significantly (P<0.0001) high expression of WGA and MAA .(b,d) ROC curve analysis of WGA and MAA. (Malignant-38, Benign-26)



WGA Expression Profile WGA – higher expression in malignant (149.37±8.9) thyroid samples as compared to benign (36.25 ± 9.13).(p<0.0001) (a). WGA: Sensitivity- 89.5% , Specificity – 92.3, AUC- 0.89 (b)



MAA Expression Profile: Higher expression in malignant(69.57±6.81) as compared to benign (9.95±2.78), (p<0.05) © ROC analysis indicated that MAA had high sensitivity- 84.2% and Specificity- 88.5%, AUC – 0.89 (d)

The conventional cytology diagnosis using Bethesda system (cut off; ≤3 is taken as benign and >3 as malignant) of the above cases showed sensitivity of 67% (n= 20/30) and specificity of 90% (18/20) respectively in delineating benign and malignant cases

CONCLUSION

- Given the increasing incidence of thyroid malignancies and urgent need for accurate diagnosis and appropriate disease management, this study proposes the usage of the aberrant glycosylation profile to develop a novel, molecular marker-based FNAC assay for delineation of thyroid malignancies.
- Hence, this will be a step towards development of a Point of Care (PoC), affordable diagnostic assay, which will refine and improve the current cytology based delineation of thyroid nodules.

FUTURE PERSPECTIVES

- Incorporation of automated image analysis to improve the amenability & remove subjectivity.
- A point of care assay platform development (PoC)

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