

DEDICATION

To parents (Mansur and Fareeda) whose prayers, love and never ending support inspired me to achieve my goals.

To my brothers and sisters, they offer a limitless giving.

To the victims of Breast Cancer who are waiting for real help through research.

ACKNOWLEDGEMENTS

First and foremost, I raise my sincerest thanks to Allah (Subhanahu Wa Ta'ala) by saying: (Alhamdulellah) for all and everything.

Then,

This research work would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

Foremost, I offer my sincere gratitude to my supervisor, Prof. Hussain Gad Elkarim Ahmed, Professor of Histopathology and Cytology, who has supported me throughout this work with his patience, wisdom and extraordinary knowledge whilst allowing me a place to work in my own way. I attribute my advancement to his encouragement and effort and without him this thesis, too, would not have been completed or written. One simply could not wish for a better supervisor.

I also want to express my gratitude to my Co-supervisor, Prof. Amel Omer Bakhet, Professor of Pathology; she inspires me to be better in the way of study and research.

I must also acknowledge Mr. Basheer Abdulmalik Mohammed, for his cooperation in sample collection and cutting. Thanks also go to Mr. Ahmed Mohammed Khairy and Mr. Ibrahim Idris Ibrahim for their help in sample collection. I would like to thank Mr. Sharafeldeen Elradi, for his help in tissue processing.

ABSTRACT

This is a retrospective descriptive study conducted in Khartoum State to find out (if any) association between ER receptors and Carbohydrates or Connective tissue fibres in specimens obtained from 60 women presented with breast lesions (35 were with malignant lesions and 25 were benign lesions). All specimens were diagnosed at the histopathology department of Laboratory Administration in Khartoum State, Sudan. Malignant tumors include, DCI, IDC and ILC representing 2/35(5.7%), 23/35(65.7%) and 10/35 (28.6%), per capita. Benign tumors include, Fibroadenoma, Fibrocystic changes and others representing 18/25(72%), 4/25(16%) and 3/25(12%) respectively.

All specimens were then re-examined for ER status by immunohistochemistry, Carbohydrates by (Alcian blue, Alcian blue-methylation, PAS and PAS-diastrase), Connective tissue fibres by (Vangieson, Masson Trichrome, Silver stain and Verhoeff) and Nucleolar Organizing regions by AgNOR.

Of the 60 breast lesions, 35/60 (58.3%) were with malignant tumors. The remaining 25 samples were with benign breast lesions. Of the 35 malignant samples immunohistochemical staining for ER revealed positive expression in 12/35(34.3%) and negative in 23/35(65.7%) specimens. Regarding benign samples there were 8/25(32%) ER positive and 17/25(68%) ER negative. Out of the total study subjects there were 20/60(33.3%) ER positive.

Regarding malignant samples, DCI, IDC and ILC ER positive expressions were identified among 1/2(50%), 7/23(30%) and 4/10(40%), respectively. Regarding benign samples, Fibroadenoma, Fibrocystic changes, and others

ER positive expression were detected among 6/18(33.3%), 1/4 (25%) and 1/3(33.3%), in this order.

To the best of our knowledge, there is no study investigated the association between ER, carbohydrate and connective tissue fibres. Thereafter, and according to present study there is a strong relationship between ER positivity and presence of acid mucin ($P = 0.000$), but not with sulphated mucin. Furthermore, a strong relationship was found between ER positivity status and PAS positive materials (P value = 0.000), but not with glycogen. Since, there is a significant correlation between ER and Carbohydrates, these positive PAS materials might be PAS positive acid mucin. Regarding connective tissue fibres there is a significant correlation between ER+ and presence of collagenic fibres (P value = 0.000). Furthermore, Elastic fibres were significantly associated with ER+ (P value = 0.039), as well as, Reticulin fibres (P value = 0.020). The mean number of AgNORs was relatively similar in malignant and benign breast lesions.

The significant positive association between ER expression and carbohydrate and also between ER expression and connective tissue fibres suggest that the ER expression could be predicted in histopathology laboratory using histochemical demonstration of carbohydrates and connective tissue fibres of female breast tissue. The histochemical prediction of ER receptor status may be helpful, cost effective and time saving more than that of more sophisticated investigations. Further studies with large sample size and more sophisticated techniques are required to strength the findings of this study.

الخلاصة

هذه الدراسة هي دراسة وصفية ارتجاعية أجريت في ولاية الخرطوم لمعرفة الارتباط (إن كان هناك أي ارتباط) بين مستقبلات الإستروجين (ER) والكربوهيدرات أو ألياف النسيج الضام في العينات التي تم الحصول عليها من 60 امرأة حضرت وهي مصابة بآفات الثدي (35 آفات خبيثة ، و 25 آفات حميدة). تم تشخيص جميع العينات في قسم التشريح المرضي في إدارة المعامل ولاية الخرطوم، السودان. الأورام الخبيثة تشمل (DCI) و (IDC) و (ILC) وتمثل 2/35 (5.7٪)، و 23/35 (65.7٪) و 10/35 (28.6٪)، على التوالي. أما الأورام الحميدة تشمل ، الورم الغدي الليفي، والتغيرات الليفية التكيسية، وحالات أخرى وتمثل 18/25 (72٪)، 4/25 (16٪) و 3/25 (12٪) على التوالي. ثم أعيد فحص جميع العينات لحالة (ER) بواسطة الكيمائية المناعية للأنسجة، وتم فحص الكربوهيدرات بواسطة (زرقة الألسيان، وزرقة الألسيان-الميثيلية، وطريقة (PAS)، وطريقة (PAS) دياستان)، وتم فحص ألياف النسيج الضام بواسطة طريقة (Vangieson) وطريقة ثلاثي الألوان ماسون، وطريقة (Verhoeff)، وطريقة (Silver stain)، وتم فحص مناطق التنظيم النوبي.

من عينات آفات الثدي الـ 60 هذه، كان 35/60 (58.3٪) أورام خبيثة. وكان ما تبقى وهو 25 عينة من آفات الثدي الحميدة. من العينات الـ 35 الخبيثة والتي تم صبغها بالطريقة الكيمائية المناعية النسيجية لمستقبلات الإستروجين (ER) كان هناك 12/35 (34.3٪) أظهرت التعبير الإيجابي و 23/35 (65.7٪) كانت سلبية. فيما يتعلق بالعينات الحميدة كان هناك 8/25 (32٪) (ER) إيجابي و 17/25 (68٪) (ER) سلبية. من مجموع الخاضعين للدراسة هناك 20/60 (33.3٪) (ER) إيجابي.

فيما يتعلق بالعينات الخبيثة (ILC)، (IDC)، (DCI) وجد أن التعبير الإيجابي لمستقبلات الإستروجين (ER) هو 1/2 (50٪)، 7/23 (30٪) و 4/10 (40٪) على التوالي. فيما يتعلق بالعينات الحميدة، الورم الغدي الليفي، والتغيرات الليفية التكيسية، والحالات الأخرى وجد أن التعبير الإيجابي لمستقبلات الإستروجين (ER) هو 6/18 (33.3٪)، 1/4 (25٪) و 1/3 (33.3٪)، بهذا الترتيب.

إلى حد علمنا، ليس هناك أية دراسة قامت بالتحقيق في العلاقة بين مستقبلات الإستروجين (ER)، والكربوهيدرات وألياف النسيج الضام. بعد ذلك، ووفقاً لهذه الدراسة تم إيجاد علاقة قوية بين الحالة الإيجابية لـ(ER) وحامض الميوسين ($P = 0.000$)، ولكن ليس مع الميوسين المكبر. وعلاوة على ذلك، تم العثور على علاقة قوية بين الحالة الإيجابية لـ(ER) والمواد الإيجابية بطريقة ($P = 0.000$) (PAS)، ولكن ليس مع الجليكوجين. لأن هناك ارتباط كبير بين (ER)، والكربوهيدرات، فإن هذه المواد الإيجابية بطريقة (PAS) قد تكون هي حامض الميوسين الإيجابي لطريقة (PAS). فيما يتعلق بألياف النسيج الضام هناك علاقة كبيرة بين (ER) ووجود الألياف الكولاجينية ($P = 0.000$). وعلاوة على ذلك، الألياف المرنة ترتبط بشكل كبير مع ($P = 0.039$) (ER)، وكذلك بالألياف الشبكية ($P = 0.020$). وكان متوسط عدد مناطق التنظيم النووي متساوٍ نسبياً في كل من الآفات الخبيثة والآفات الحميدة في الثدي.

الارتباط الإيجابي الكبير بين التعبير عن (ER) والكربوهيدرات، وكذلك بين (ER) وألياف النسيج الضام تشير إلى أنه من الممكن توقع التعبير عن (ER) في مختبر أمراض الأنسجة باستخدام الدراسة الكيميائية النسيجية للكربوهيدرات وألياف النسيج الضام من نسيج الثدي للإناث. التنبؤ الكيميائي النسيجي لحالة مستقبلات الإستروجين (ER) قد يكون مفيداً، وذو تكلفة فعالة، وموفرًا للوقت أكثر من تلك الفحوصات الأكثر تطوراً. يلزم إجراء المزيد من الدراسات بحجم عينة أكبر وتقنيات أكثر تطوراً من أجل تقوية نتائج هذه الدراسة.

List of contents

| Contents | Page No. |
|---|----------|
| Dedication | vii |
| Acknowledgement | viivii |
| Abstract (English) | viiivii |
| الخلاصة | vii |
| List of Contents | vii |
| List of Tables | vii |
| List of Photomicrographs | vii |
| List of Figures | vii |
| Chapter One | |
| Introduction | 1 |
| Study Objectives | 10 |
| Chapter Two | |
| Review of Literature | 11 |
| Scientific background | 11 |
| Normal histology of the breast | 11 |
| Inflammatory conditions of the breast | 12 |
| Non inflammatory conditions of the breast | 12 |
| Benign non-neoplastic lesions of the breast | 13 |
| Benign neoplastic breast lesions | 14 |
| Breast cancer | 14 |
| Epidemiology of breast cancer | 16 |
| Aetiology of breast cancer | 18 |
| Diagnosis of breast cancer | 26 |
| Breast self examination | 27 |
| Clinical Breast Examination | 27 |
| Imaging techniques | 28 |
| Conventional histopathology | 30 |
| Fine needle aspiration cytology | 30 |
| Core needle biopsy | 31 |
| Open excision biopsy | 32 |
| Sub-typing of breast carcinoma | 32 |

| | |
|---|-----|
| <i>In Situ</i> carcinomas | 32 |
| Invasive carcinoma | 33 |
| Special histologic type of invasive carcinoma | 35 |
| Tumor characteristics | 35 |
| Staging of invasive breast cancer | 35 |
| Immunohistochemistry | 37 |
| General aspects of immunohistochemistry | 37 |
| Immunohistochemistry in breast cancer | 41 |
| Estrogen Hormone | 42 |
| Estrogen Receptor | 43 |
| Triple negative (TN) breast cancer | 45 |
| Clinical importance for evaluation of ER in breast cancer | 45 |
| Argyrophilic nucleolar organizer regions | 46 |
| Molecular diagnosis and prognosis of breast cancer | 50 |
| Chapter Three | |
| Materials and methods | 52 |
| Chapter Four | |
| Results | 58 |
| Chapter Five | |
| Discussion | 90 |
| Conclusion and Recommendations | 95 |
| References | 96 |
| Appendices | 137 |

LIST OF TABLES

| Contents | | Page No. |
|------------------|---|----------|
| Table.1. | Distribution of the study population by Age | 64 |
| Table.2. | Distribution of study population by Pathology | 65 |
| Table.3. | Distribution of ER status by Tumor type | 66 |
| Table.4. | Distribution of ER status by Carbohydrates | 68 |
| Table.5. | Distribution of ER status by Connective Tissue Fibres | 69 |
| Table.6. | Distribution of Carbohydrate (Alcian Blue) by Tumor type | 71 |
| Table.7. | Distribution of Sulphated Mucin (Alcian Blue Methylation) by Tumor type | 72 |
| Table.8. | Distribution of PAS +ve Carbohydrates by Tumor type | 73 |
| Table.9. | Distribution of Glycogen (PAS Diastase) by Tumor type | 74 |
| Table.10. | Distribution of Collagen (Vangieson) by Tumor type | 75 |
| Table.11. | Distribution of Collagen (Masson) by Tumor type | 76 |
| Table.12. | Distribution of Elastic fibres (Verhoeff stain) by Tumor type | 77 |
| Table.13. | Distribution of Reticulin (Silver stain) by Tumor type | 78 |

LIST OF Photomicrographs

| Contents | | Page No. |
|-----------|---|----------|
| Photo.1. | H&E as a stain for general picture. ×10. | 79 |
| Photo.2. | Alcian Blue for Acid mucin (blue colour areas). ×10. | 80 |
| Photo.3. | Alcian Blue Methylation for sulphated acid mucin (blue colour areas). ×10. | 81 |
| Photo.4 | PAS for carbohydrates and other PAS+ structures (magenta colour). ×20. | 82 |
| Photo.5: | PAS Diastase PAS for carbohydrates and other PAS+ structures other than glycogen (magenta colour). ×20. | 83 |
| Photo.6. | Van Gieson for collagen fibres (red colour). ×10. | 84 |
| Photo.7. | Masson trichrome for collagen fibres (blue colour). ×10. | 85 |
| Photo.8. | Verhoeff for elastic fibres (black colour). ×10. | 86 |
| Photo.9. | Silver Stain for reticulin fibres (black colour). ×10. | 87 |
| Photo.10. | ER +ve sample by Immunohistochemistry. ×20. | 88 |
| Photo.11. | ER –ve sample by Immunohistochemistry. ×20. | 89 |

LIST OF Figures

| Contents | | Page No. |
|-----------|--|----------|
| Figure.1. | Description of ER + by Pathology | 67 |
| Figure.2. | Description of ER+ status by Histochemical Identification of carbohydrates and fibres | 69 |