

بسم الله الرحمن الرحيم

**Sudan University of Science & Technology**

**Collage of Graduate Studies and Scientific Research**

**Nucleolar Organizer Region Expression in  
Hepatocellular Carcinoma and Liver Cirrhosis Among  
Patients in Khartoum State.**

تعبير المنطقة المنظمة للنويات في حالات السرطان الخلوي الكبدي و  
تليف الكبد للمرضى في ولاية الخرطوم .

*A thesis submitted for partial fulfillment of master degree in histopathology and cytology.*

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## الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال الله تعالى :

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ  
(3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)

صدق الله العظيم

سورة العلق الآيات 1-5

## *Dedication*

*To my beloved mother and father*

*Who teach me to love others,*

*Not as I want them to be,*

*But as they are.*

## **Acknowledgement:**

First and foremost praise be to Allah, who give me the strength to carry this work.

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## **Abstract:**

This descriptive retrospective study was performed in Khartoum to assess the expression of NORs in hepatocellular carcinoma as compared to liver cirrhosis in Sudanese patients, to find out whether there is statistically significance differences in mAgNORs between the two cases as well as in different grades of hepatocellular carcinoma. 39 paraffin blocks representing the cases of hepatocellular carcinoma, were taken from hospital records in three different governmental centers in Khartoum, as well as 16 cases of liver cirrhosis. A minimum of three sections (3 $\mu$ ) were taken from each liver tissue, one section was stained routinely by haematoxylin and eosin to high-light tissue architecture and determine the tumor grade, other histological section was stained by silver-NOR method. Staining was performed on formalin-fixed, paraffin-embedded tissue sections NOR dots were counted in 100 randomly selected hepatocytes at  $\times 100$  oil immersion objective, and the mean count per cell was calculated for each case.

Statistical analysis was done by using the Student T-test for unpaired data and Spearman's correlation tests. AgNOR count results were later compared with the histologic diagnosis.

The mean AgNORs count was 2.94 and 6.54 dots per cell in liver cirrhosis and HCC respectively and the difference was statistically significant (P. value less than 0.01). The study revealed increasing mean AgNOR counts from cirrhosis to hepatocellular carcinoma.

The hepatocellular carcinomas were graded according to the Edmondson-Steiner histological grading system. Among the study population 23 of HCC cases was well differentiated hepatocellular carcinoma (constituting about 58.9% of HCC cases), 13 cases were

moderately differentiated carcinoma (constituting about 33.3% of HCC cases) and 3 cases were poorly differentiated hepatocellular carcinoma (constituting about 7.7% of HCC cases).

The mAgNOR in grade I, grade II and grade III HCC was  $4.36 \pm 1.5$ ,  $8.34 \pm 2.02$  and  $10.82 \pm 2.40$  respectively. There was statistically significant correlation between mAgNORS count and histological tumor grade (P.value less than 0.05). The AgNOR score increases with the increasing tumor grade.

## الخلاصة:

اجريت هذه الدراسة الوصفية ذات الأثر الرجعي في الخرطوم لتقييم تعبير المناطق المنظمة للنويات (NORs) في حالات السرطان الخلوي الكبدي بالمقارنة مع تليف الكبد للمرضى في ولاية الخرطوم لاكتشاف إذا كان هناك اختلاف ذو دلالة إحصائية في متوسط المناطق المنظمة للنويات (mAgNORs) بين الحالتين وكذلك في الدرجات المختلفة للسرطان الخلوي الكبدي .

39 عينة نسيجية اختيرت لتمثل السرطان الخلوي الكبدي بالإضافة إلى 16 عينة مختلفة من حالات تليف الكبد ( كل العينات أخذت من سجلات المستشفى في ثلاث مراكز حكومية مختلفة في الخرطوم). أخذت من كل عينة ثلاثة مقاطع نسيجية على الأقل (بسمك 3مايكرون). كل العينات مثبتة مسبقا "بالفورمالين 10% ومطمورة في شمع البرافين . لون قسم من المقاطع النسيجية بالهيماتوكسولين والأيوسين (H&E) لدراسة بنية النسيج وتقرير درجة الورم . القسم الآخر لطح بطريقة بولتن باستخدام أملاح الفضة . (AgNOR) ثم حسبت مواقع NORs في 100 خلية كبدية مختارة بشكل عشوائي لحساب متوسط المناطق المنظمة للنويات (mAgNORs) (لكل حالة . من ناحية أخرى , اجري التحليل الإحصائي باستخدام اختبار (Student T- test) للبيانات غير المزدوجة و اختبار (Sperman) لدراسة درجة الارتباط .

وجد أن متوسط المناطق المنظمة للنويات في الأنسجة المتليفة و السرطانية 2,94 و 6,54 على التوالي وأظهرت الدراسة ازدياد متوسط المناطق المنظمة للنويات (mAgNORs) بين حالات التليف والسرطان الخلوي الكبدي و كان الاختلاف ذو دلالة إحصائية. ( $P < 0.01$ )

من الناحية الأخرى صنفت درجات السرطان الخلوي الكبدي باستخدام نظام ادمنسون-ستينر (Edmondson-Steiner grading system) وكانت النتائج بين مجتمع الدراسة كالاتي : 23 حالة) حوالى (58,9% من السرطان الخلوي الكبدي واضح التمايز 13 حالة (33,3%) متوسط التمايز و 3 حالات (7,7%) ضعيف التمايز. وكان متوسط المناطق المنظمة للنويات في الحالات الثلاث  $4,36 \pm 1,5$ ,  $8,34 \pm 2,02$ ,  $10,82 \pm 2,4$  على التوالي . وجد أن هناك ارتباط هام إحصائيا في متوسط المناطق المنظمة للنويات (mAgNORs) بين درجات الورم الخلوي- الكبدي المختلفة (  $P < 0.05$  ) حيث يزيد متوسط عدد النقاط (mAgNORs) بزيادة درجة الورم .

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## List of abbreviations:

HCC:	Hepatocellular carcinoma.
ALT:	Alanine-aminotransferase.
AFP:	Alpha fetoprotein.
HBsAg:	Hepatitis B surface antigen.
HCV:	Hepatitis C virus
AgNOR:	Argyrophelic nucleolar organizer region.
MAGNORs:	Mean nucleolar organizer regions.
RNA:	Ribonucleic acid.
DNA:	Deoxy-ribonucleic acid
rDNA:	ribosomal Deoxy-ribonucleic acid(DNA that encodes ribosomal RNA synthesis)
GGT:	Gamma glutamyl transferase.
PT:	prothrombin time.
BrdU:	Bromodeoxyuracil.
CLIP:	Cancer of the Liver Italian Program (CLIP) score
LDH:	Lactate dehydrogenase
TNM:	Tumor,Node,Metastasis.
IUAC:	International Union Against Cancer.
AST:	Aspartate aminotransferase
LB:	Liver biopsy.
μ:	Micrometer.
BSG:	British Society of Gastroenterology.
N:C:	Nuclear,cytoplasmic ratio.
P. value:	Power of significance.
DPX:	Di-styrene Plasticizer and Xylene
SPSS:	Statistic Package for Social Sciences
OLT:	Orthoptic Liver Transplantation.