RECOMMENDATIONS

The results of the present study may have several future clinical implications:

- A better understanding of the cross talk between mTOR pathway and autophagy during chronic HCV infection is needed particularly with the presence of several autophagy inducers in the liver microenvironment.

- Future research is needed to evaluate the possible role of anti-viral therapy in modulating mTOR and autophagy dysregulation in patients with chronic HCV infection in relation to the improvement in HCV-induced liver histology.

- Studies with larger numbers of patients are needed to verify the value of serum mTOR level as a potential diagnostic biomarker for the development of HCC in patients with HCV-related liver cirrhosis.

- Clinical trials are required for studying the effects of mTOR inhibitors in limiting liver inflammation, fibrosis and steatosis in patients with chronic HCV infection and the role of targeting mTOR as a potential anti-cancer therapy for HCC.

- Further studies are needed to clarify the role of autophagy in the process of liver fibrosis and hepatocarcinogenesis before a therapeutic approach targeting autophagy can be used to treat patients with liver fibrosis and HCC.

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TARGET OF RAPAMYCIN (TOR) AND AUTOPHAGY IN CHRONIC HEPATITIS C VIRUS INFECTION: RELATION TO DISEASE ACTIVITY

هدف الراپاميسين (تور) والإلتقام الذاتى في الإصابة المزمنة بفيروس الإلتهاب الكبدي سي: العلاقة بنشاط المرض

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دكتور في الأمراض الباطنة

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INTRODUCTION

Hepatitis C virus (HCV), a member of the *Flaviviridae* family, of the genus *Hepacivirus*, is a hepatotropic, single stranded, positive-sense RNA virus.[1] Persistent infection with HCV is a major cause of chronic liver disease worldwide leading to chronic hepatitis and long-term progression to cirrhosis and hepatocellular carcinoma.[2,3] Although both host and virus factors are considered to affect the outcome of HCV infection, yet, the potential mechanism(s) underlying viral persistence and disease chronicity are not fully understood.[4] Chronic hepatitis C is characterized by hepatocyte necrosis, chronic inflammation, steatosis and progressive liver fibrosis of variable degrees, which eventually result in irreversible cirrhosis.[5] Several HCV proteins have been shown to dysregulate several hepatocyte signaling pathways and to interfere with different cellular processes.[5,6] Intensive research to unravel the multifunctional molecular pathway(s) implicated in the progression of chronic HCV infection, could identify novel therapeutic targets to tackle this devastating disease.[7]

The target of rapamycin (TOR) is an evolutionarily conserved serine/threonine protein kinase encoded by FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) gene.[8] Recently, the mammalian TOR (mTOR) has been shown to function as a central regulator of a wide array of cellular processes such as protein translation, cell metabolism, growth, differentiation, survival and cell cycle progression by sensing nutritional status and allowing progression from G1 to S phase in the cell cycle.[9] Activation of mTOR is regulated by the kinase cascade consisting of phosphatidyl inositide 3 kinase (PI3K)/AKT.[10] The phosphorylation of mTOR promotes downstream targets such as the ribosomal p70-S6 kinase

(p70S6K) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), which promote protein translation.[11,12] Inhibitors of mTOR such as rapamycin, potently inhibit the downstream signaling of mTOR.[13] In addition, mTOR is a key player in the immune response promoting T-cell differentiation and dendritic cell development and function.[14] Also, the Akt/mTOR/p70S6K stimulates type I procollagen expression in fibroblasts and enhances hepatic stellate cell proliferation.[15] Several viruses have been discovered to manipulate the mTOR pathway for viral replication and survival.[16-18] Of specific importance, is the nonstructural NS5A protein of HCV, which can activate mTOR via disruption of the mTOR-FKBP38 complex and interaction with p85 subunit of PI3K leading to its activation.[19]

In recent years, increasing evidence demonstrates that mTOR tightly regulates the intracellular process of autophagy in response to cellular physiological conditions and environmental stress.[20] Autophagy or 'self-eating' involves the sequestration of cellular constituents within double-membrane vesicles (autophagosomes) and delivering them to the lysosomes for degradation and recycling (autolysosomes).[21] This allows the cell to recycle nutrients and remove unwanted cytosolic components, such as damaged organelles and protein aggregates from the cytoplasm.[22] Autophagy can be induced by a variety of stimuli (*eg*, nutrient deprivation, hypoxia, cytokines, hormones, viruses and DNA damage).[23] Beyond maintaining homeostasis, autophagy is involved in multiple biological processes and aberrant regulation of autophagy induces many diseases including inflammatory disorders [24] and viral infections.[25]

At present, there are more than 30 autophagy-related genes that have been found to be involved in the different stages of autophagy.[21] The protein products of these genes (ATG or autophagy-related proteins)

organize into functional complexes that mediate the following steps in the autophagic process: nucleation of the limiting membrane, elongation of the membrane, sealing to form a vesicle and fusion of the double membrane vesicle with lysosomes.[26] Among the ATG proteins, ATG5 is essential in proceeding of autophagy.[27] Conjugation of ATG5 with ATG12 and formation of a complex with ATG16, is required for elongation of the isolation membrane to form a complete spherical autophagosome.[28] Apoptotic stimuli cleave ATG5, which is subsequently translocated to mitochondria and triggers caspase activation.[29] mTOR, a well-characterized negative regulator of autophagy, prevents the recruitment and interaction of specific ATGs of the nucleation complex to the site of autophagosome formation.[30] Also, mTOR inhibits p73, a member of p53 family, which regulates the expression of several ATG genes including ATG5 in response to different cellular stresses.[31] It is likely that dysregulation of the mTOR-autophagy pathway may contribute to many human disorders and could be an attractive avenue for future therapeutic approaches.[32]

AIM OF THE WORK

The aim of the present work is to study the role of mTOR and autophagy in the progression of chronic HCV infection.

MATERIAL

Subjects:

The present study will include 30 treatment-naïve patients with chronic HCV infection who will be referred to the Hepatobiliary Unit, Department of Internal Medicine, Faculty of Medicine, University of Alexandria. The diagnosis of chronic HCV infection will be based on the following criteria: (1) positive test for HCV antibody; (2) detectable serum HCV RNA: and (3) histopathological findings in liver biopsy. The severity of chronic HCV infection will be scored according to the METAVIR histological activity grade (A0-A3) and fibrosis stage (F0-F4).[33]

Also, 15 age- and sex-matched healthy subjects with no evidence of liver disease will be included as control group to obtain the normal values of biochemical assays.

Exclusion criteria:

Patients with CHC will be excluded from the study if they have seropositivity for hepatitis B virus (HBV) infection; history of alcohol consumption; other known causes of chronic liver disease; concomitant schistosomiasis; hepatic decompensation; bleeding diathesis; chronic diseases such as diabetes mellitus, connective tissue diseases or other autoimmune diseases; other infections; any kind of malignancy; and cardiac, respiratory or renal disease. Also, patients who have received previous antiviral or immunomodulatory therapy will not be included in the study.

The study will be conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines. An informed consent will be obtained from all subjects included in the study.

METHODS

All patients included in the study will be evaluated as regards:

1. Clinical Evaluation focusing on:

The apparent duration and possible risk factors of HCV infection, symptoms and signs of chronic liver disease (Right hypochondrial pain, jaundice, ascites, hepatic encephalopathy, previous gastrointestinal bleeding, bleeding diathesis) and liver and spleen sizes.

2. Laboratory Investigations including:

a. ***Complete blood picture.***[34]

b. ***Liver Test Profile:*** Serum aspartate and alanine aminotransferases (AST and ALT), serum albumin, serum bilirubin, serum gamma glutamyl transpeptidase[35] and prothrombin activity.[34]

c. ***Viral Testing including:***

i. HCV antibodies, hepatitis B surface antigen and hepatitis B core antibody using enzyme-linked immunosorbant assay (ELISA).[36]

ii. HCV RNA levels in serum using real time polymerase chain reaction assay.[37]

3. Measurement of serum levels of mTOR using commercially-available ELISA kit.[38]

4. Ultrasonographic examination for assessment of liver echopattern and size and presence of cirrhosis, ascites and splenomegaly[39]

5. Histopathological Evaluation:

Core liver biopsies will be obtained from all patients with chronic HCV infection. Tissue specimens will be fixed in 10% solution of formalin, embedded in paraffin and subsequently stained with:

- Hematoxylin-eosin and trichrome stains for assessment of histological activity grade and fibrosis stage according to the METAVIR scoring system [33] and the grade of steatosis.[40]
- Immunohistochemical staining using anti-human antibodies against mTOR [41] and ATG5 (a marker of autophagy).[42]

Ethics of Research

Research on human or human products:

Prospective study :informed consent will be taken from patient. In case of incompetent patient, the informed consent will be taken from the guardians.

Retrospective study: confidentiality of record will be considered.

DNA/genomic material: informed consent for DNA/genomic test and for research will be taken from patients. No further tests will be carried out except with further approval of committee and patients. If the samples will travel outside Egypt, the researcher will be responsible for transportation and security approval.

All drugs used in the research is approved by the Egyptian ministry of health.

Research on animal:

The animal species is appropriate to the test.

After test, if the animal will suffer, it will be euthanized and properly disposed.

After operation, it will have a proper postoperative care.

RESULTS

The results of this study will be tabulated and analyzed with the use of appropriate statistical methods and appropriate figures and diagrams.

DISCUSSION

The results will be discussed in view of achievement of the aim, their significance and their comparison with previous related researches.

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الملخص العربى

هدف الراپاميسين (تور) والإلتقام الذاتى في الإصابة المزمنة بفيروس الإلتهاب الكبدي سي: العلاقة بنشاط المرض

مقدمة البحث:

تعد الإصابة بفيروس الالتهاب الكبدي سي من أهم الأسباب المؤدية إلي أمراض الكبد المزمنة وعادة ما تكتشف الاصابة في مرحلة الالتهاب الكبدي المزمن الذى يتطور الي التليف الكبدى انتهاءا بالسرطان الكبدى الخلوى. وبالرغم من أن تحديد مصير الأصابة بفيروس الالتهاب الكبدى سي يعتمد على العوامل المتعلقة بالفيروس والخلايا الكبدية والمناعية الا ان المسارات البيولوجية التى تسهم فى استمرار الفيروس وتقدم مراحل الأصابة لم يتم بعد تحديدها بوضوح.

هدف الراپاميسين "تور" ينتمي الي مجموعة سيرين/ثريونين بروتين كيناز ويمثل نقطة تلاقي ودمج بين العديد من الاشارات من داخل وخارج الخلية ، ويعمل ايضا كمنظم مركزي ومحوري للعديد من العمليات الحيوية الخلوية مثل تكاثر ونمو وبقاء الخلية وتقدم دورة الخلية والتمثيل الغذائي للبروتينات والجلوكوز والدهون وكذلك يلعب "تور" دورا مهما فى الاستجابات المناعية الفطرية والتكيفية. وبالإضافة إلى ذلك فأن "تور" يثبط عملية الالتقام الذاتي داخل الخلايا فى الظروف الفسيولوجية الخلوية والإجهاد البيئي.

الالتقام الذاتي هو عملية خلوية يتم من خلالها تشكيل حويصلات مزدوجة الغشاء وعزل المكونات الخلوية وتسليمها إلى الجسيمات الحالة مما يساعد على اعادة تدويرالبروتينات لتوفير المواد البيولوجية للخلية والتخلص من المكونات الخلوية التالفة وذلك للحفاظ على التوازن الخلوي، كما أن الالتقام الذاتي يلعب دورا هاما في مراقبة الجودة وإنتاج الطاقة فى الخلية وحماية الخلية من المؤثرات الخارجية والميكروبات.

وقد وجد أن الخلل فى مسار "تور" والالتقام الذاتي يمكن أن يؤدى الى العديد من الأمراض مثل الأصابات الفيروسية والأمراض الالتهابية والسرطانية.

الهدف من البحث:

الهدف من هذا البحث هو دراسة دور "تور" والإلتقام الذاتى في تقدم مرض الكبد المرتبط بالاصابة المزمنة لفيروس الالتهاب الكبدى سى وتكون السرطان الكبدى الخلوى.

حالات البحث:

اشتملت الدراسة علي ٥٤ مريضا من المصابين بالاصابة المزمنة بفيروس الالتهاب الكبدى سى الذين لم يسبق علاجهم بعد منهم ٢٧ مريضا مصابا بالالتهاب الكبدي المزمن و١٣ مريضا مصابا بالتليف الكبدى و١٤ مريضا بالسرطان الكبدى الخلوى الذى تم استئصاله جراحيا. وقد استند تشخيص الالتهاب الكبدى الفيروسى سى المزمن على وجود الأجسام المضادة والحمض النووى للفيروس في الدم والتغييرات بأنسجة الكبد نتيجة الاصابة المزمنة بالفيروس. كما اعتمد تشخيص السرطان الكبدى الخلوى علي مستوى الألفا فيتوبروتين في مصل الدم واستخدام الفحص بالموجات فوق الصوتية والأشعة المقطعية ثلاثية المراحل وعند اللزوم باستخدام أشعة الرنين المغناطيسى وكذلك بالفحص الهيستوباثولوچى لعينة من الورم المستأصل جراحيا. كما تم إختيار ١٥ من الأشخاص الأصحاء كعينة ضابطة.

وقد استثنى من البحث المرضى الذين يعانون من الاصابة بفيروس الالتهاب الكبدى بى أوتناول الكحوليات أوأسباب أخرى لأمراض الكبد المزمنة أو لديهم قصورشديد بوظائف الكبد أو التهابات حادة أوأورام سرطانية اخري أو أمراض قلبية أو صدرية أو كلوية أو أمراض مزمنة أخري مثل مرض السكري أو امراض النسيج الضام. كما استثنى المرضى الذين سبق لهم علاج الإلتهاب الكبدى القيروسي سى المزمن او من سبق لهم علاج السرطان الكبدى الخلوى اما موضعيا او باستخدام العلاج الدوائي.

خطة البحث و طرق الفحص:

اشتملت خطة البحث و طرق الفحص علي تقييم جميع المرضى كالاتى:

- الفحص الإكلينيكى الشامل مع التركيزعلي مدة الأصابة الظاهرية بفيروس الالتهاب الكبدى سى والأسباب المحتملة للأصابة والأعراض والمظاهر الاكلينيكية لمرض الكبد المزمن وحجم الكبد والطحال ووجود ورم محسوس بالكبد.
- الفحص باستخدام الموجات فوق الصوتية والأشعة المقطعية ثلاثية المراحل لتشخيص التليف الكبدي واستسقاء البطن وتضخم الطحال و لتحديد مواصفات ورم الكبد من حيث قطر الورم الأقصى وعدد ومكان وانتشارالورم داخل الكبد.
- الفحوصات المعملية و قد اشتملت علي:
  - صورة الدم الكاملة.
  - قياس مستوى الكرياتنين في مصل الدم.
  - إختبارات وظائف الكبد [قياس مستوى انزيمات الكبد (انزيم ناقل أَمين الاسبارتات وانزيم ناقل أمين الأَلانين وانزيم ناقل جاما جلُوتاميل) والالبومين والصفراء في مصل الدم ونشاط البروثرومبين].
  - اختبارات وجود الأجسام المضادة لڤيروس الإلتهاب الكبدى سى والأنتيجين السطحى لڤيروس الإلتهاب الكبدى بى باستخدام طريقة الانزيم المرتبط بالامتصاص المناعى (ايليزا) ومستوي الحمض النووي لفيروس الالتهاب الكبدي سي فى مصل الدم بإستخدام التفاعل التسلسلى بأنزيم البوليميريز.
  - قياس مستوى الألفا فيتوبروتين في مصل الدم.
  - قياس مستوي بروتين هدف الرابامايسين "تور" في مصل الدم للمرضي والأشخاص الأصحاء باستخدام طريقة ايليزا.
- تحديد درجة إصابة الكبد بإستخدام تقسيم " تشايلد- بيو" وتقسيم نموذج المرحلة النهائية لمرض الكبد "ميلد".
- تحديد مرحلة الورم بإستخدام تقسيم عيادة برشلونة لسرطان الكبد وتقسيم البرنامج الايطالى لسرطان الكبد "كليب".
- الفحص الهيستوباثولوچى لعينات أنسجة الكبد من ٣٠ مريضا بالاصابة المزمنة بفيروس الالتهاب الكبدى سى وكذلك لعينات السرطان الكبدى الخلوى وأنسجة الكبد المحيطة به باستخدام الصبغات التالية:
  - صبغة هيماتوكسلين وإيوسين لتحديد درجة النشاط الهيستولوجى ومرحلة التليف الكبدى بإستخدام تقسيم "ميتاڤير" ودرجة التدهن الكبدى فى أنسجة الكبد من مرضي الاصابة المزمنة لفيروس الالتهاب الكبدى سى وأنسجة الكبد المحيطة بالورم وكذلك لتحديد درجة الورم الهيستولوجية بإستخدام تقسيم إدمونسن وإشتينر.
  - الصبغة المناعية الهيستولوچية الكيميائية بإستخدام الأجسام المضادة نحو "تور" ونحو البروتين المرتبط بالالتقام الذاتي ٥ " آ تى جى- ٥" كدلالة للالتقام الذاتي. وقد تم تقسيم درجة الصبغة المناعية كالأنى: "صفر" = عدم وجود صبغة بالخلايا، "١ " = اقل من ١٠٪ من الخلايا موجبة للصبغة، "٢ " = من ١٠-٥٠٪ من الخلايا موجبة للصبغة، "٣ " = أكثر من ٥٠٪ من الخلايا موجبة للصبغة.

نتائج البحث:

أظهر التحليل الإحصائى للبيانات التى تم الحصول عليها من الدراسة الحالية النتائج التالية:

- تراوحت المدة الظاهرية للإصابة بفيروس الالتهاب الكبدى سي بين ٣- ١١ شهرا (متوسط ٦ ± ٢,٢٧ شهراً) فى مرضى الالتهاب الكبدى المزمن وبين ٧-١٥ عاما (متوسط ١١,٠٨ ± ٢,٦٣ عاماً) فى مرضى التليف الكبدي وبين ٣- ١٤ عاماً (متوسط ٩,٥٨ ± ٣,٣٢ عاماً) فى مرضى السرطان الكبدى الخلوى.

- تراوح مستوي الحمض النووي لفيروس الالتهاب الكبدي سي فى مصل الدم بين ١٩–٦٥٠٠ x ١٠^{٣}وحدة دولية/ ملليليتر (متوسط ٩٢٣,٢٧ ± ١٥٠٤,٤٤ x ١٠^{٣} وحدة دولية/ملليليتر) في مرضي الالتهاب الكبدى المزمن وبين ٥٢,٦- ٦٢٣١ x ١٠^{٣} وحدة دولية/ملليليتر (متوسط ١٥٤٣,٥٣ ± ٢٢٠٨,٢١ x ١٠^{٣}وحدة دولية/ملليليتر) في مرضي التليف الكبدي ، و بين ١١٠ – ٢٣٠٠ x ١٠^{٣}وحدة دولية/ملليليتر (متوسط ٦٧٥ ± ٦٢٦,٤٢ x ١٠^{٣}وحدة دولية/ملليليتر) فى مرضى السرطان الكبدى الخلوى.

- اظهر تقييم درجة أصابة الكبد فى المرضى النتائج الاتية:

- بالنسبة لمرضي التليف الكبدي وجد أن ١٠ من المرضى (٧٦,٩٪) كانوا من الفصيلة (أ) و٣ من المرضي(٢٣,١٪) من الفصيلة (ب) حسب تقسيم "تشايلد- بيو". وتراوح معامل "ميلد" بين ٦ – ٩ (متوسط ٧,٦٢ ± ٠,٨٧).
- بالنسبة لمرضي السرطان الكبدى الخلوى وجد أن ١٢ من المرضى (٨٥,٧٪) كانوا من الفصيلة (أ) و٢ من المرضي (١٤,٣٪) من الفصيلة (ب) وتراوح معامل "ميلد" بين ٦ – ١٠ (متوسط ٨,١٤ ± ١,٤٦).

- أظهرالفحص باستخدام الموجات فوق الصوتية والأشعة المقطعية ثلاثية المراحل فى مرضى السرطان الكبدى الخلوى أن القطر الأقصى للورم تراوح بين ٢,٧ - ١١,٦ سم (متوسط ٦,١٩ ± ٢,٩٣ سم). ووجدت بؤرة واحدة للورم في كل المرضي وكان الورم فى الفص الايمن من الكبد في ٥ من المرضي (٣٥,٧٪) و الفص الايسر في ٩ من المرضي (٦٤,٣٪) وكان امتداد الورم داخل الكبد في كل المرضى اقل من ٥٠٪ من حجم الكبد. وقد تراوح مستوى الألفا فيتوبروتين في مصل الدم فى مرضي السرطان الكبدى الخلوى بين ١١ – ٢٠٩٧٣ نانوجرام/ملليليتر.

- بإستخدام تقسيم عيادة برشلونة لسرطان الكبد لتحديد مرحلة السرطان الكبدى الخلوى وجد أن الورم فى المرحلة "أ٢" فى ٧ من المرضى (٥٠٪) وفى المرحلة "أ٣" فى ٢ من المرضى (١٤,٣٪) وفى المرحلة "ب" فى ٥ من المرضى (٣٥,٧٪). أما باستخدام تقسيم "كليب" فقد وجد أن الورم كان فى مرحلة كليب "صفر" فى ٥ من المرضى (٣٥,٧٪) وفى مرحلة كليب "١" فى ٧ من المرضى (٥٠٪) وفى مرحلة كليب "٢" فى ٢ من المرضى (١٤,٣٪).

- وجدت زيادة ذات دلالة احصائية فى متوسط مستوي "تور" في مصل الدم فى مرضي الالتهاب الكبدي المزمن ومرضي التليف الكبدي ومرضي السرطان الكبدى الخلوى (٢,٢٢ ± ١,٢ نانوجرام/ ملليليتر ، ٣,٤٩ ± ٠,٧٩ نانوجرام/ ملليليتر ، ٦,١٩ ± ١,٦٣ نانوجرام/ ملليليتر على التوالى) مقارنة بمستواه لدي الأشخاص الأصحاء (١,١٣ ± ٠,٣ نانوجرام/ ملليليتر). وكذلك وجدت زيادة ذات دلالة احصائية فى متوسط مستوي "تور" في مصل الدم فى مرضي السرطان الكبدى الخلوى ومرضي التليف الكبدي مقارنة بمرضي الالتهاب الكبدي المزمن وفى مرضي السرطان الكبدى الخلوى مقارنة بمرضي التليف الكبدي.

- برسم منحني خصائص تشغيل المتلقى (روك) وجد أن حساسية وخصوصية مستوي "تور" في مصل الدم فى التمييز بين مرضي التليف الكبدي الذين لديهم السرطان الكبدى الخلوى والذين ليس لديهم الورم هما ٩٢,٢ ٪ و١٠٠ ٪ علي التوالي عند مستوي الحد الفاصل ٤,٥٥ نانوجرام/ملليليتر.

- أظهر الفحص الهيستوباثولوچى لأنسجة الكبد النتائج الأتية:

- طبقا لتقسيم "ميتافير" في مرضي الاصابة المزمنة بفيروس الالتهاب الكبدى سى وجد أن درجة النشاط الهستولوجي كانت من الدرجة "أ١" في ١٠ من المرضي (٣٣,٣٪) ومن الدرجة "أ٢" في ١٣ مريضا (٤٣,٣٪) ومن الدرجة "أ٣" في٧ من المرضي (٢٣,٣٪) ووجد أن التليف الكبدي كان فى المرحلة "أف١" في٧ من المرضي (٢٣,٣٪) والمرحلة "أف٢" في ١٥مريضاً (٥٠٪) و المرحلة "أف٣" في ٥ من المرضي (١٦,٧٪) و المرحلة "أف٤" في ٣ من المرضي (١٠٪). ووجد التدهن الكبدي فى هؤلاء المرضى بدرجة بسيطة في ١٠ من المرضي (٣٣,٣٪) و بدرجة متوسطة في ٨ من المرضي (٢٦,٧٪) وبدرجة شديدة في ٧ من المرضي (٢٣,٣٪) وكان غير موجودا فى ٥ من المرضى (١٦,٧٪).

- كانت درجة الورم الهستولوجية في مرضي السرطان الكبدى الخلوي من الدرجة الثانية فى ٧ من المرضى (٥٠٪) ومن الدرجة الثالثة فى ٢ من المرضى (١٤,٣٪) ومن الدرجة الرابعة فى ٥ من المرضى (٣٥,٧٪) طبقا لتقسيم إدمونسن وإشتينر.
- وجد في أنسجة الكبد المحيطة بالورم أن درجة النشاط الهستولوجي كان من الدرجة "أ٢" في ٤ من المرضى (٢٨,٦٪) والدرجة "أ٣" في ١٠ من المرضي (٧١,٤٪) ودرجة التليف كانت في المرحلة "أف٤" في جميع المرضي (١٠٠٪) بينما كانت درجة التدهن بسيطة في ٢من المرضي (١٤,٣٪) ودرجة متوسطة في ١١ مريضا (٧٨,٦٪) وبدرجة شديدة في مريض واحد (٧,١٪).

- أظهرالفحص باستخدام الصبغة المناعية الهستولوجية الكيميائية لأنسجة الكبد النتائج الأتية:

- كان إظهار "تور" في سيتوبلازم أو نواة خلايا الكبد موجبا في ١٨ مريضا (٦٠٪) من مرضي الاصابة المزمنة بفيروس الالتهاب الكبدى سى وكانت درجة الصبغة المناعية ضعيفة في ٤ من المرضي (١٣,٣٪) و متوسطة في ٦ من المرضي (٢٠٪) وشديدة في ٨ من المرضي (٢٦,٧٪).
- وجد ان إظهار "تور" في سيتوبلازم او نواة خلايا السرطان الكبدى الخلوى كان موجبا في ١٢ مريضا (٨٥,٧٪) وكانت درجة الصبغة المناعية ضعيفة في مريضا واحدا (١,٧٪) ومتوسطة في مريضا واحدا (١,٧٪) وشديدة في ١٠ من المرضي (٧١,٤٪). أما فى أنسجة الكبد المحيطة بالورم فقد وجد ان إظهار "تور" كان موجبا في ١٢ مريضا (٨٥,٧٪) وكانت درجة الصبغة المناعية ضعيفة في ٣ من المرضي (٢١,٤٪) و متوسطة في ٦ من المرضي (٤٢,٩٪) وشديدة في ٣ من المرضي (٢١,٤٪).
- وجدت زيادة ذات دلالة احصائية في إظهار "تور" في أنسجة السرطان الكبدى الخلوي بالمقارنة بأنسجة الكبد المحيطة بالورم وأنسجة الكيد من مرضي الاصابة المزمنة بفيروس الالتهاب الكبدى سى بينما لم يوجد فارق ذو دلالة احصائية بين المجموعتين الاخيرتين.
- كان إظهار"آ تى جى- ٥" في سيتوبلازم خلايا الكبد موجبا في ٢٨ مريضا (٩٣,٣٪) من مرضي الاصابة المزمنة بفيروس الالتهاب الكبدى سى وكانت درجة الصبغة المناعية ضعيفة في مريضا واحدا (٣,٣٪) ومتوسطة في ١٠ من المرضي (٣٣,٣٪) وشديدة في ١٧ من المرضي (٥٦,٧٪).
- وجد ان إظهار " آ تى جى- ٥" كان موجبا في خلايا الورم فى كل مرضى السرطان الكبدى الخلوى وكانت درجة الصبغة المناعية ضعيفة في ٥ من المرضي (٣٥,٧٪) ومتوسطة في ٥ من المرضي (٣٥,٧٪) وشديدة في ٤ من المرضي (٢٨,٦٪). أما فى أنسجة الكبد المحيطة بالورم فقد وجد ان إظهار " آ تى جى- ٥" كان موجبا في كل المرضى وكانت درجة الصبغة المناعية متوسطة في ٦ من المرضي (٤٢,٩٪) وشديدة في ٨ من المرضي (٥٧,١٪).
- كان هناك نقص ذو دلالة احصائية في اظهار" آ تى جى- ٥" في أنسجة السرطان الكبدى الخلوي بالمقارنة بأنسجة الكبد المحيطة بالورم وأنسجة الكيد من مرضي الاصابة المزمنة بفيروس الالتهاب الكبدى سى بينما لم يوجد فارق ذو دلالة احصائية بين المجموعتين الاخيرتين.
- لوحظ أن إظهار كلا من "تور" و" آ تى جى- ٥" كان عاليا (من الدرجة ٢ أو ٣) فى نفس الخلية فى ١١ مريضا (٣٦,٧٪) من مرضي الاصابة المزمنة بفيروس الالتهاب الكبدى سى وفى ٦ مريضا (٤٢,٩٪) من مرضي السرطان الكبدى الخلوي.

- أظهرت العلاقات الاحصائية بين مستوى "تور" في مصل الدم وإظهار "تور" و" آ تى جى- ٥" فى أنسجة الكبد مع العوامل الأخرى فى مرضي الاصابة المزمنة بفيروس الالتهاب الكبدى سى النتائج الأتية:

- لم توجد علاقة ذات دلالة احصائية بين مستوى "تور" في مصل الدم وإظهار "تور" و" آ تى جى- ٥" فى أنسجة الكبد من ناحية وعمر المريض ، ومستوى انزيم ناقل جاما جلُوتاميل ومستوى الحمض النووي لفيروس الالتهاب الكبدي سي فى مصل الدم من ناحية أخري.

- كانت هناك علاقة طردية ذات دلالة احصائية بين من مستوى "تور" في مصل الدم وإظهار "تور" فى أنسجة الكبد من ناحية ومستوى انزيم ناقل أَمين الاسبارتات وانزيم ناقل أمين الأَلانين في مصل الدم ودرجة النشاط الهستولوجي ودرجة التليف الكبدى ودرجة التدهن الكبدى من ناحية أخري.

- وجدت علاقة عكسية ذات دلالة احصائية بين إظهار " آ تى جى- ٥" فى أنسجة الكبد من ناحية ومستوى انزيم ناقل أَمين الاسبارتات وانزيم ناقل أمين الأَلانين في مصل الدم ودرجة النشاط الهستولوجي ودرجة التليف الكبدى من ناحية أخري، بينما لم توجد علاقة ذات دلالة احصائية بين إظهار " آ تى جى- ٥" فى أنسجة الكبد ودرجة التدهن الكبدى.

- كانت هناك علاقة طردية ذات دلالة احصائية بين مستوى "تور" في مصل الدم وإظهار "تور" فى أنسجة الكبد ووجدت علاقة عكسية ذات دلالة احصائية لكليهما مع إظهار" آ تى جى- ٥" فى أنسجة الكبد.

- أظهرت العلاقات الاحصائية بين مستوي "تور" في مصل الدم وإظهار "تور" و" آ تى جى- ٥" فى أنسجة الورم مع العوامل الأخرى فى مرضي السرطان الكبدى الخلوي النتائج الأتية:

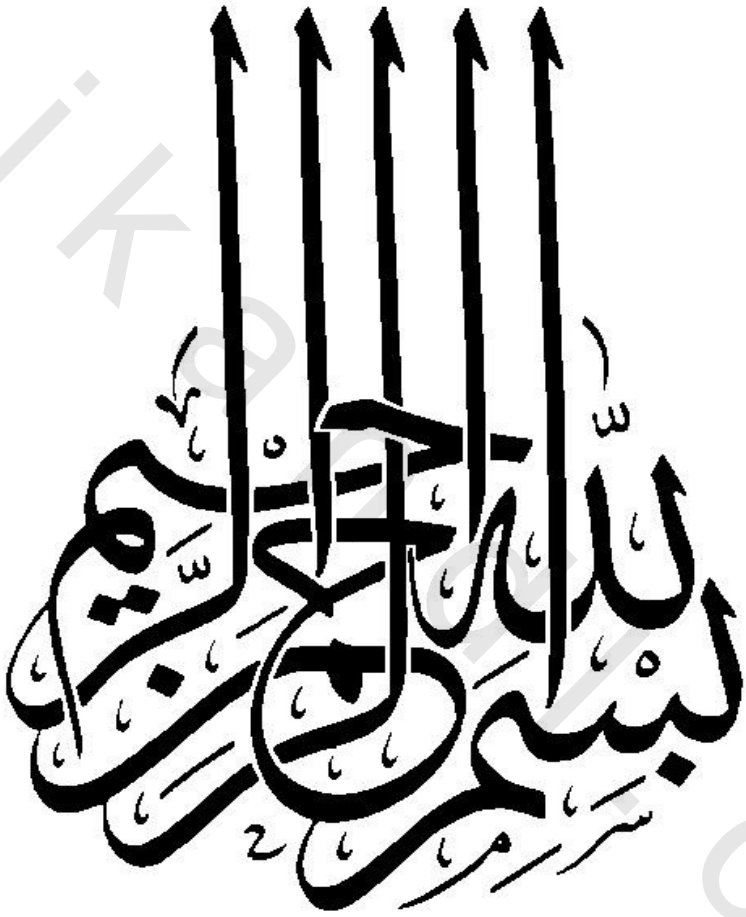
- لم توجد علاقة ذات دلالة احصائية بين مستوي "تور" في مصل الدم وإظهار "تور" و" آ تى جى- ٥" فى أنسجة الورم من ناحية وتقسيم " تشايلد- بيو" وتقسيم "ميلد"، و إظهار "تور" و" آ تى جى- ٥" فى أنسجة الكبد المحيطة بالورم من ناحية اخري.

- كانت هناك علاقة طردية ذات دلالة احصائية بين مستوي "تور" في مصل الدم وإظهار "تور" فى أنسجة الورم من ناحية ومستوي الألفا فيتوبروتين في مصل الدم والقطر الأقصي للورم ومرحلة الورم بتقسيم "كليب" ودرجة الورم الهستولوجية من ناحية أخري.

- وجدت علاقة عكسية ذات دلالة احصائية بين إظهار " آ تى جى- ٥" فى أنسجة الورم من ناحية ومستوي الألفا فيتوبروتين في مصل الدم والقطر الأقصي للورم ومرحلة الورم بتقسيم "كليب" وتقسيم عيادة برشلونة لسرطان الكبد ودرجة الورم الهستولوجية من ناحية أخري

- وجدت علاقة طردية ذات دلالة احصائية بين مستوي "تور" في مصل الدم وإظهار "تور" فى أنسجة الورم. ووجدت علاقة عكسية ذات دلالة احصائية لكليهما مع إظهار" آ تى جى- ٥" فى أنسجة الورم.

من هذه الدراسة يمكن استخلاص أن الاصابة المزمنة بفيروس الالتهاب الكبدى سى تؤدى الى تنشيط "تور" مما قد يؤدى الى تثبيط عملية الالتقام الذاتي وهذا يساعد على بقاء الفيروس وتقدم المرض مع حدوث الالتهاب المزمن والتدهن والتليف الكبدى وبالتالى نمو السرطان الكبدى الخلوى. لذا فأن هناك حاجة لاجراء المزيد من الابحاث لدراسة التوازن بين "تور" كمثبط لعملية الالتقام الذاتي والعوامل الأخرى المنشطة للالتقام الذاتي فى الاصابة المزمنة بفيروس الالتهاب الكبدى سى وكذلك دراسة دور "تور" والالتقام الذاتي فى التنبؤ بالأستجابة للأدوية المضادة لفيروس الالتهاب الكبدى سى وأثرهذه العقاقير فى تعديل الخلل فى نشاط "تور" والالتقام الذاتي .كما أن هناك حاجة ماسة لاجراء الدراسات لتحديد مدى فاعلية استخدام مضادات "تور" مع/أو منشطات الالتقام الذاتي كعلاج لمرضى الاصابة المزمنة بفيروس الالتهاب الكبدى سى ومدى تأثيرهما على الالتهاب المزمن والتدهن والتليف الكبدى وكذلك السرطان الكبدى الخلوى.



السادة المشرفون

الاستاذ الدكتور/ هدى عبد المجيد العجان .....

أستاذ الأمراض الباطنة

وحدة أمراض الكبد و المرارة

كلية الطب

جامعة الإسكندرية

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أستاذ الباثولوجيا الاكلينيكية والكيميائية

كلية الطب

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الأستاذ الدكتور/ ليلى كمال يونس .....

أستاذ علم الأمراض

كلية الطب

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المشرف المشارك

الدكتور/ ايهاب مصطفى حسونة .....

مدرس الأمراض الباطنة

وحدة أمراض الكبد و المرارة

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هدف الراپاميسين (تور) والإلتقام الذاتى في الإصابة المزمنة بفيروس الإلتهاب الكبدي سي: العلاقة بنشاط المرض

مقدمة من

سـامـح الدسوقي أنور لاشـين

بكالوريوس الطب والجراحة – ماجستير أمراض الباطنة

كلية الطب – جامعة الإسكندرية

للحصول على درجة

الدكتوراة فى الأمراض الباطنة

لجنة المناقشة والحكم على الرسالة **موافقون**

الاستاذ الدكتور/ هدى عبد المجيد العجان .....

أستاذ الأمراض الباطنة

وحدة امراض الكبد والمرارة

كلية الطب

جامعة الإسكندرية

الاستاذ الدكتور/ جمال السيد محمد شيحة .....

أستاذ أمراض الكبد و الجهاز الهضمي

رئيس وحدة الكبد

كلية الطب

جامعة المنصورة

الاستاذ الدكتور/ فتح الله صدقي محمد اسماعيل .....

أستاذ الأمراض الباطنة

رئيس وحدة امراض الكبد والمرارة

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جامعة الإسكندرية

التاريخ : ١٩/ ٢ /٢٠١٥

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رسالة علمية

مقدمة لكلية الطب – جامعة الإسكندرية
إيفاءاً جزئياً لشروط الحصول على درجة

الدكتوراة فى الأمراض الباطنة

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