The dysregulation of Cyclin Dependent Kinase Regulators Role in SV40 Related renal cell carcinoma

Abstract:

The purpose of this study was to explore the possible involvement of SV40 polyomavirus in the development of renal cell carcinoma (RCC) in patients from the province of Al-Najaf. The study analyzed 75 paraffin-embedded block tissues of RCC, collected from archives of AL-Sader Medical City, and some private histopathology laboratories in Najaf governorate. The patients included 45 males and 30 females, aged between 22 and 70 years. The study used advanced scientific techniques, including Polymerase Chain Reaction (PCR) and immunohistochemistry (IHC), to detect the presence of SV40 and evaluate the expression state of Cyclin-Dependent Kinase Regulators (KAP or cyclin-dependent kinase inhibitor 3 (CDKN3) & Cyclin E1 markers). Hematoxylin and Eosin staining was used for diagnosing RCC. The study found that RCC is associated with the dysregulation of Cyclin-Dependent Kinase regulators (CDK), caused by the SV40 polyomavirus. The results of the IHC analysis showed an increased positive percentage for KAP or CDKN3 marker and a decreased positive percentage of Cyclin E1 marker. Additionally, the clear cell type was found to be the most common, accounting for 56% of the cases, while grade I was the most prevalent, representing 41.3% of the cases. Tumor stage type I was found to be higher, with 25 cases. PCR detected the presence of SV40 in 20 cases, accounting for 26.7% of the total cases studied. The study concluded that the Simian Virus 40 (SV40), particularly its Large T Antigen (Tag), affects CDK regulators and disrupts the delicate equilibrium of cell cycle regulation systems. Therefore, the study suggests a possible link between the development of renal cell carcinoma and the SV40 polyomavirus. The study recommends routine testing for the detection of RCC using PCR and IHC methods.

Key wards: SV40, Renal cell carcinoma, Immunohistochemistry, PCR

Introduction:

Polyomaviruses (PyV) is recognized as a small, non-enveloped, double-stranded deoxyribonucleic acid, icosahedral symmetry with 5 kbp genomes, belonging to *polyomaviridae* family. The term polyomavirus (PyV) comes from Greek origin, where poly- indicatenumerous and -oma which denote tumors, was belong to *Papovaviridae*family, an abbreviationsuggestedvia Melnick,as well asgainedviacombing the names of the following virusesrepresented by'*Pa*pilloma', '*Po*lyoma', and '*Va*cuolating' (Dalianis and Hirsch, 2013).

The detection of Simian virus 40 SV40, was reported within 1960 when millions of populations in Africa, Europe, Canada, Asiaand North and South America were

inoculatedfrom both in-activated and a live polio-vaccines, initiate to be infected by Simian virus 40 (Butel, 2012).

SV40genome is circular ds DNA,which encodes for 6 proteins: threestructural proteins (including VP-1; VP-2 and VP-3, which are structural proteins allowgenetic material to be accumulated in SV40 virion (Kawano *et al*., 2015), 2 proteinsimportant for the life cycle, thatinducereplicationofSV40, gene-expression, in addition toentry of S phase and DNA synthesis, by this meansinducing cycle development(large "T" antigen plus small "t" antigen oncoproteins)(Qi *et al*., 2011) and 2 small proteins of unidentified function (the agnoprotein, which rule the perinuclear localize of "VP-1"throughout virion construction, after thatinduceassemblage ofvirion (Saribas *et al*., 2018), and17kT, which participatethe majority of amino acid sequence withN terminal domain ofT-ag, encourageprogression ofcell cycle in existence of t-Ag, as well astumorigenic formation(Comerford *et al*., 2012).

Simian virus 40 return to Polyomaviridae, genus Betapolyomavirus, which is stronglycorrelatedto other types of polyomavirusesincluding JCPyV and BKPyV(Calvignac-Spencer et al.,2016). SV40 be capable of transmitted bydiversewayslike sexual course and faecal-oral waysthat are accountable for horizontal virusinfection in peoples (Vanchiere*et al*., 2005).

The infection of cell beginning by attachment capsid ofSV40 to the cell surface by binding among VP-1, cell surface receptor ganglioside GM1 and the major histocompatibility complex class-I(MHC-I), whichfunction as coreceptors and allow viral DNA to be contained within the SV40 virion, which is formed by 360 VP 1 molecules, comprising 72 pentamers (Kawano*et al*., 2015).

This virus in nature infects specific species of Asian macaques, especially rhesus monkey. Sequences of SV40 were detected in samples of urine and stool as well as in both children and adults, this representing that the sexual and oro-fecal ways of spreadthatpossible to accountable for horizontalSV40 infection in individuals (Academies, 2003; Vanchiere*et al*., 2005).

On the other hand, the liberate of SV40 with noexhibit a cytopathic effect (CPE) found in particulartypesof cells, for instance humanepithelial, fibroblasts, mesothelial and embryonic renal cell which points that kidney tissue can function as reservoir for SV40 in humans (Cacciotti *et al*., 2001).

Expression of bothT-Ag and t-Ag can cause elevated cell transformation professionally. In reality, Tag prohibit the actions of numerousdiverse cellular factors concerned in differentiation, cell growth and the cell cycle, for instance p130, p300 and p400. As well as, T-Ag and t-Ag wasprohibitingthe activity of pRb and p53. These interconnections are obligatoryso as toaccomplishcomplete cell transformation in human (Khalili*et al*., 2008).

The oncogenic role ofpolyomavirus was formerlyrelated with a widearray of tumor types for instance malignant pleural mesothelioma (MPM) and bone (Thanh*etal*., 2016), brain (Wang*et al*., 2017), lung (Ramael and Nagels, 1999), thyroid (Vivaldi *et al*., 2003), pituitary (Woloschak*et al*., 1995), and urothelial (Loghavi and Bose, 2011) tumors, pleomorphic adenomas of parotid glands (Martinelli*et al*., 2002), ependymomas choroid and plexus tumors in youth (Bergsagel*et al*., 1992). Additionally, footprints from DNA of SV40 have been reported in breast (Hachana*et al*., 2009) and colon carcinoma (Campello*et al*., 2010).

Also,Tag of SV40 possibly causes transformation by stimulating mutations to the genome of cellular or numerical/structural variation of chromosomes, like gaps, breaks, ring and dicentric chromosomes, chromatid exchanges, translocations, duplicationsand deletions (Tognon*et al*., 1996). The majorfunction of t-Ag in transformation is to linkboth

subunits,catalytic (36 kDa) and regulatory (63 kDa) of protein phosphatase 2A (PP2A), in-activating role (Garcea and Imperiale, 2003).

Grading Renal Cell Carcinoma:

Patients distributing according grading of The World Health Organization (WHO)/InternationalSociety of Urological Pathology.

Table 1: The world health organization/International society of urological pathology grading system for clear cell and papillary renal carcinoma (WHO/ISUP, 2021)

Grade 1	Tumour cell nucleoli absent or inconspicuous and basophilic at 400× magnification
Grade 2	Tumour cell nucleoli conspicuous and eosinophilic at 400× magnification and visible but not prominent at 100× magnification
Grade 3	Tumour cell nucleoli conspicuous and eosinophilic at 100× magnification
Grade 4	Tumuors showing extreme nuclear pleomorphism, tumour giant cells and/or the presence of any proportion of tumour showing sarcomatoid and/or rhabdoid dedifferentiation

Method:

This present study was planned as cross-section study to detect SV40 with renal cell carcinoma and includes 75 (45 males and 30 females, whose ages ranged from 22 to 70 years) paraffin impeding block tissues of renal cell carcinoma from archives of AL-Sader Medical City and some archives of private histopathology laboratories in Najaf governorate. The data are from January 2016 to the December of the same year by using Polymers Chain Reactionfor detection of DNA SV40 and immunohistochemistry technique for detect expression state of Cyclin Dependent Kinase Regulators (KAP or cyclin-dependent kinase inhibitor 3 (CDKN3) & Cyclin E1 markers), using Hematoxylin and Eosin stain for diagnosis of RCC.

Result:

Increased positive percentage for KAP or CDKN3 marker and decreased positive percentage of Cyclin E1 marker were seen in the results of the Immunohistochemistry technique (IHC).As well, found that clear cell type was higher with **42 (56%),** grade I was higher with 31 (41.3%) and tumor stage type I was higher (25). The positive results by PCR techniques in RCC patient showed that 20 (26.7 % out of 75 cases) of block tissues.

-1-Clinicopathological analysis:

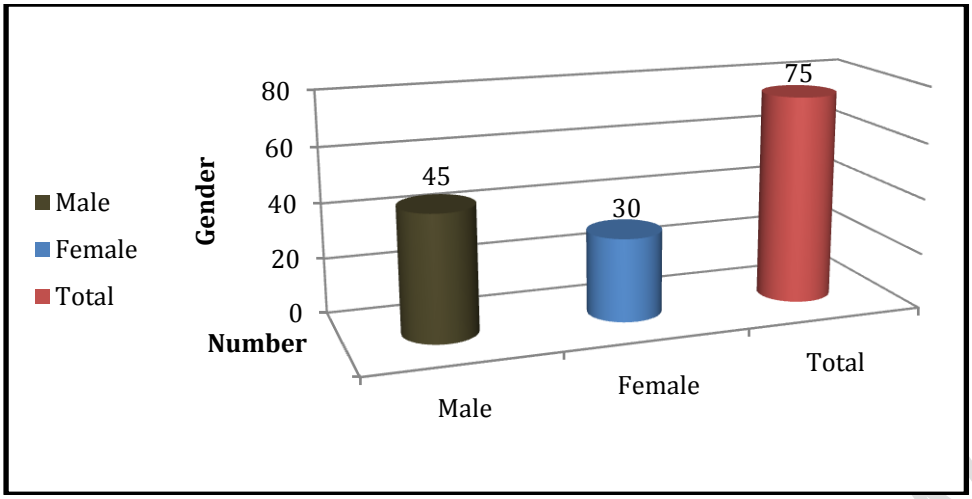

Fig 1: Distribution of RCC Patients according to Gender

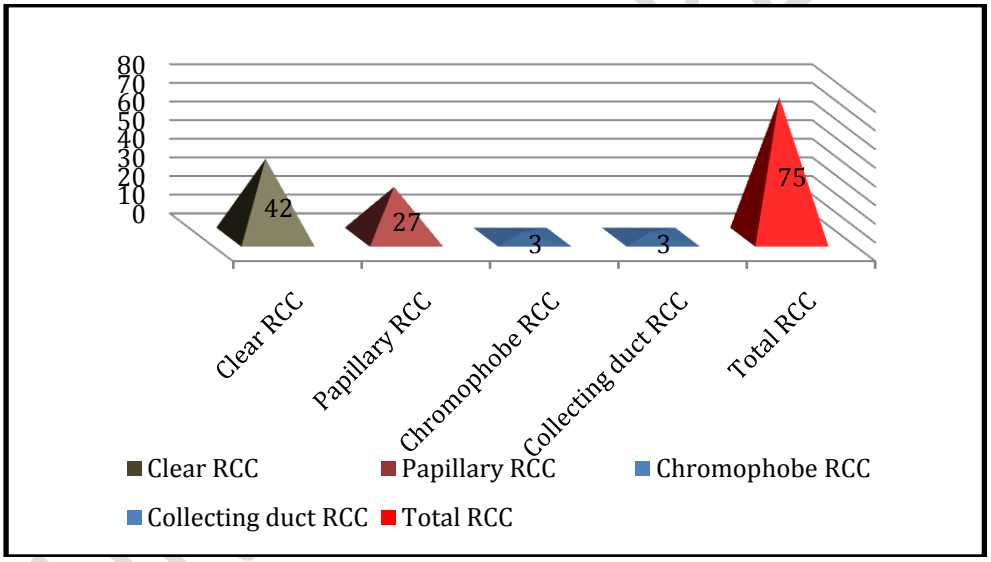

Fig 2: Distribution of RCC Patients according to histological types.

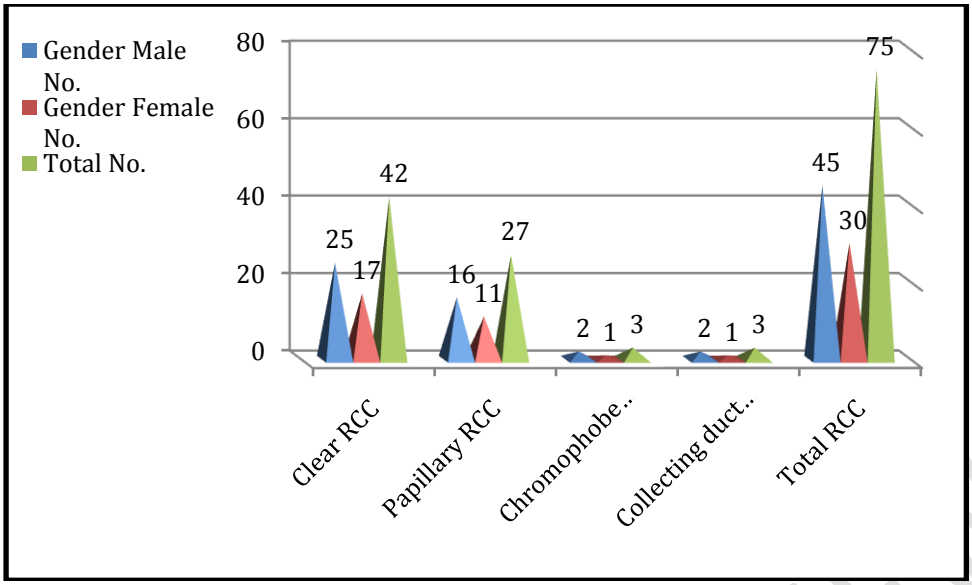

Fig 3: Distribution of RCC patients according to their Histopathological types and Gender

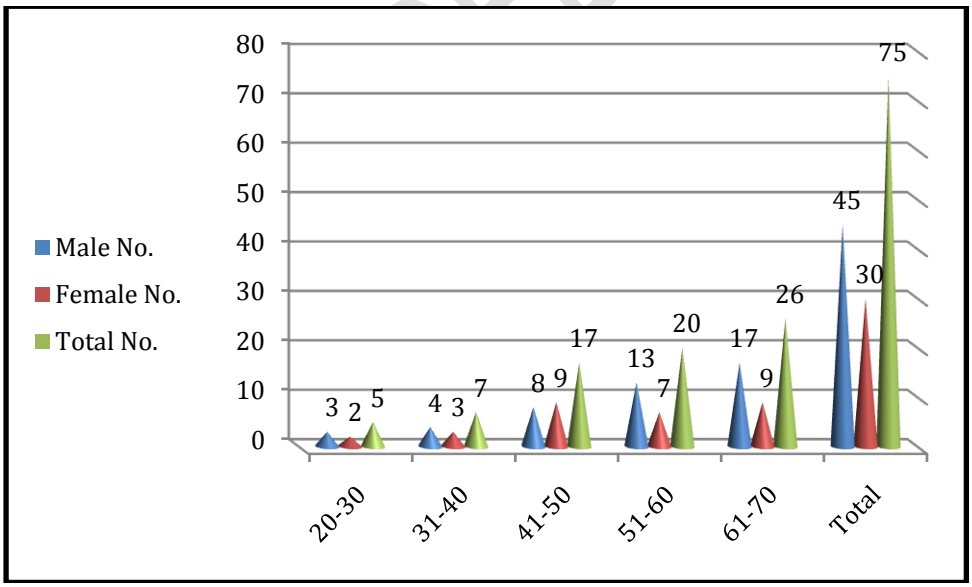

Fig 4: Distribution of RCC patients according to their Gender and Age

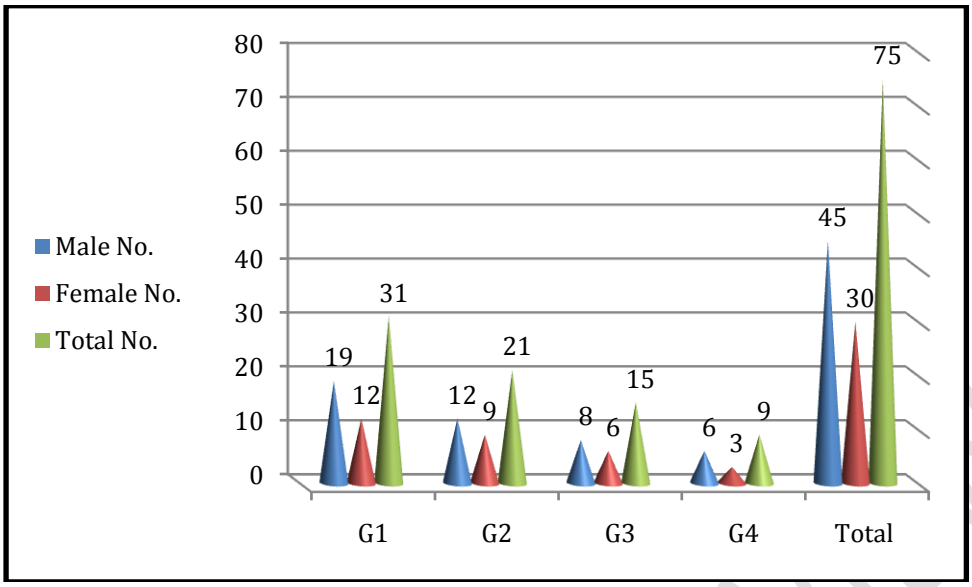

Fig 5: Distribution of RCC patients according to their Gender and Grading Systems

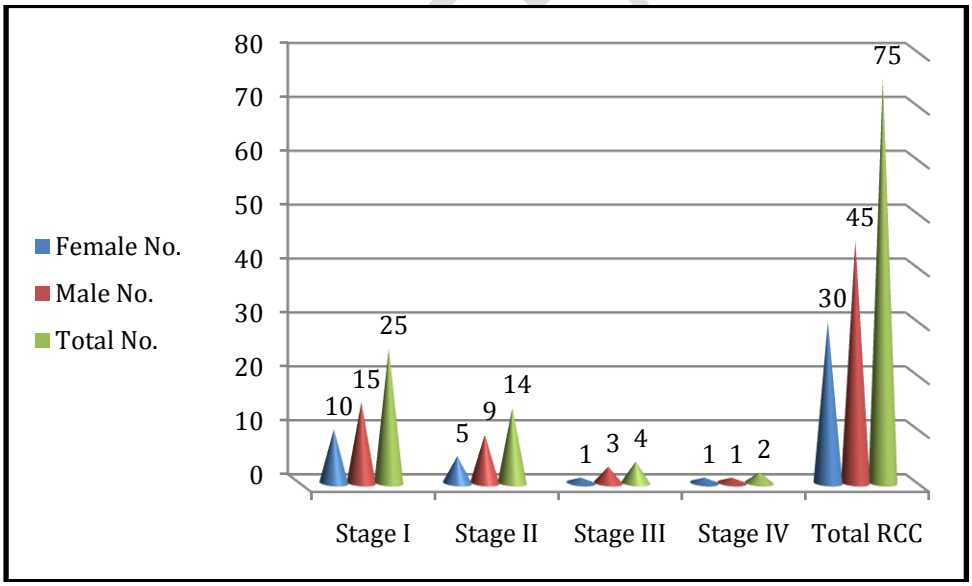

Fig 6: Distribution of RCC patients according to their Gender and Pathological Tumor Stage

-2- Immunohistochemical Analysis (Cyclin E1 & CDKN3)

In the this study, the results of IHC by utilizing EnVisionTMFLEX stain revealed a brownish discoloration of nucleus or nucleoplasm for Cyclin E1 whereas in CDKN3 was staining the cytosol or cytoplasm, as showed in figures (7&8&9).

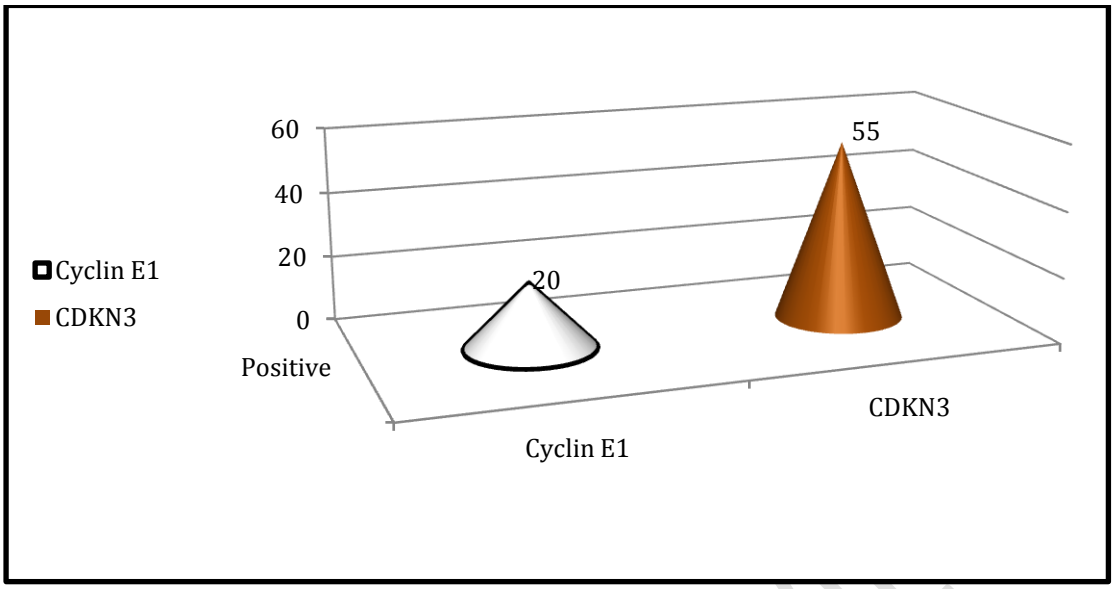

Fig 7:Circulation of Cyclin E1 and CDKN3 by using IHC.

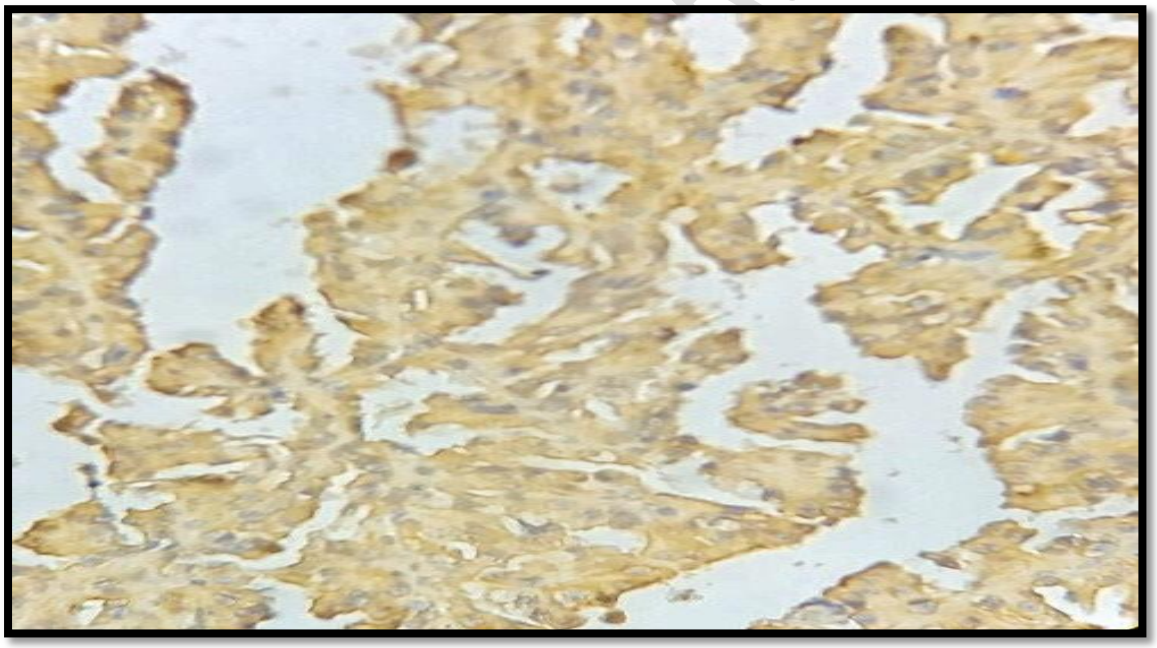

Fig8: Negative cyclineE1 stain of papillarytype of RCCpatients

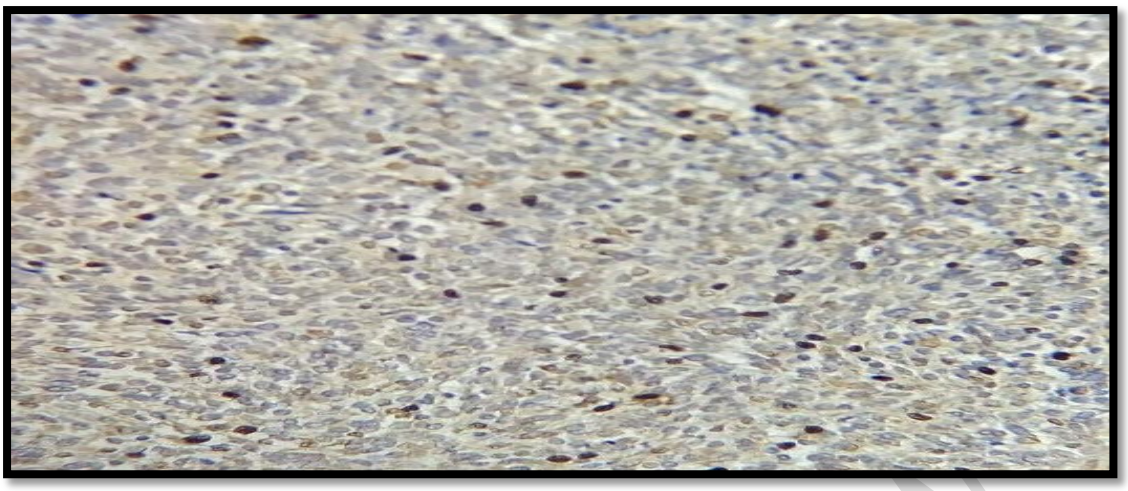

Fig9: Sarcomatoid carcinoma positive strong cyclineE1 stain score 2 (10 X40)

Discussion:

In this study, the existence of SV40 in blockedtissue takingfrom 75 patients suffering from RCC, it uses molecular technique involving PCR technique for detection of SV40 DNA united with immunohistochemistry technique (IHC)which are significant to verify the existence of Simian virus 40.

Simian virus 40 (SV40) define as a monkey virus whichby accidententered toman, in 1955-1963years,throughoutpolluted polio-virus vaccines that found the transforming and oncogencityactionsof T-Ag and t-Ag of this virus, whichprovokedexaminationof SV40 in humans'cancer.Generally, it is thought that contamination of polio vaccines was consider the major cause of infection with SV40 in humans, nearly all researches have defined exposure of SV40 founded on vaccination (Engels *et al*., 2003).

Most studiesdemonstrate that the kidney can function as a reservoir for SV40 in individuals. The sequence of this virus was reported in renal tissue and cells of urine sediments suffering from RCC (Li *et al*., 2002) like Garcea and Imperiale, (2003)who found that SV40 causes infection in renal cells somewheremight possiblyreactivation by immunosuppression. Also, Vanchiere*et al*., (2005) reported that discovery of SV40 in renaltissue of human which indicatesthat kidney represented a position of viral latency, similar to in the usualsimian host.

Bofill-Mas *et al*., (2000)does notdiscoverSV40 sequences in any tissue of RCC combine in diverse geographic regions of Europe and South Africa, while other types of polyomavirus's sequences were detected from the majority of these tissues. In contrast to Manfredi *et al*., (2005) who have failed to discover the sequences of SV40 in these tumors.

In molecular technique involving PCR, it was found that only 20 of 75 paraffin-embedded block tissue yielded SV40 for the reason that only extremelylittle amounts of these tissue block were offered for investigation, it was inspiring that DNA of SV40

recognized from 75renal block tissue.The likelihood of occasional laboratory pollution of tissue block was excludeddue togenetic material (DNA)linkedwith cancer and DNA ofSV40 from laboratory progenydiverge sequences both within the viral regulatory area and at the carboxy terminus of T-Ag(Stewart *et al*., 1998).

Some reports have lacking proof that SV40 was causationsignificant in the progression of human cancer but,Butel and Lednicky, (1999) reported that the presence of the DNA of SV40 will suggests that the opportunity of these virus in the genesis of some RCC in human.

Bergsagel*et al*. (1992) have revealed that negative SV40 outcome in renal tumor possibly because ofutilize of few technical approaches.Also similarly, Leithner*et al*., (2002)and Priftakis*et al.,* (2002) have recorded that never detected the sequences of SV40 in bothAustria andTurkey, as in Sweden. While the predominance ofSV40 DNA that are revealed in thesecancers was diversecountry for instance in Germany and Hungary (Heinsohn*et al*., 2009).

Various repots recorded by Lopez-Rios *et al*., (2004) showing that positive sequences of SV40 DNA by PCR technique as well as Mayall*et al*., (2003) and Aoe *et al*., (2006) reported that negative results by using quantitative PCR assay.

In general Iraq is considered as one of variouscountries in the Middle East regions that have special excitingto renal cell carcinoma and which regarded as the second mainlyfrequent urological malignancy (Ibrahim, 2013). As aresult, it is found the elevated proportion of males than females have in agreement with many studies finished by Vikram *et al*., (2016) and Mahasin *et al*., (2018).

Renal cell carcinoma is the majorityfrequent malignancy of kidney, as well as canclassified into five types including ccRCC, pRCC, chRCC, cd RCC and unclassified types. It is found in the presented study the most frequent type was clear cell RCC (42 of 75) whichconcordance with repotsaccomplished by Aiman *et al*., (2013) and Mahasin *et al*., (2018).

By using TNM classificationof malignant tumors of RCC rely on the American Joint Committee on Cancer (AJCC), Stafford *et al*., (2008)recorded that males' patientshave higher stage tumors while females'patients havelower stage cancers, thisis in concordance with ourstudy.When in examination of the Fuhrman nuclear grade,Mukhopadhyay *et al.,* (2015) have discoveryhigherfrequency of Grade 1 and lower frequency of Grade 3 and Grade 4.

The most common age group in their study is 61-70 years followed by 51-60 years. These results are in conformity with numerousreportssuch Noroozinia*et al*., (2014) Khafaja*et al.,* (2015)and Hassan *et al*., (2017) whileunlikeness with Latif *et al.,* (2011) and Takure*et al*., (2013).

In immunohistochemical technique, the immunohistochemical indicators are significant in identifying RCC patients that are investigated by the EnVision System, this is agreement with the report done by Lai *et al*., (2017) who have recorded that a elevated expression of CDKN3 in renal tissues whilstBisteau*et al*., (2014) have found that tough expression of cyclin E1 which is related with poor prognosis of patients.

Also, Brousset*et al*., (2005) have unsuccessful to discover Tag of SV40 in these tumors by using immunohistochemistry technique with a extremely sensitive technique in spite of actuality that recorded in experienced tissues have DNA sequences of SV40.

The results of analysisof DNASV40 polyomavirus by PCR in patient with RCC as; the total number of positive results of PCR is 20 (26.7%) whilst the negative results of PCRare 55 (73.3%).

Conclusion:

Renal cell carcinoma (RCC) is a complex and heterogeneous disease that is associated with a dysregulation of cyclin-dependent kinase (CDK) regulators. The CDK regulators play a crucial role in the regulation of the cell cycle, and any dysregulation can lead to the uncontrolled proliferation of cancer cells. Recent studies show that the Simian virus 40 (SV40) is responsible for the dysregulation of CDK regulators in RCC. SV40 has been identified as a potential oncogenic virus in humans, and its association with RCC has been established. The dysregulation of CDK regulators in RCC associated with SV40 is a complex molecular pathway that involves the interaction between the viral proteins and host cell pathways. The findings of this study have expanded our knowledge of the condition. It has been suggested that the creation of tailored treatments meant to counteract SV40-related RCC by reestablishing the equilibrium of cell cycle regulation is possible. These treatments could target the CDK regulators and the mechanisms by which the virus interacts with the host cell pathways, thus leading to the restoration of normal cellular function. In conclusion, this study has shed light on the complex molecular pathways involved in the etiology of RCC associated with SV40. The findings have opened the door for the creation of targeted treatments meant to restore the equilibrium of cell cycle regulation and counteract the dysregulation caused by the virus. This has the potential tosignificantly improve the prognosis of patients with SV40-related RCC.

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