INTRODUCTION

Liver cancer is a leading cause of cancer deaths worldwide, accounting for more than 700,000 deaths each year. The American Cancer Society's estimates of primary liver cancer and intrahepatic bile duct cancer in the United States for 2018 are about 42.220 of newly diagnosed cases and 30.200 people will die of these cancers ***(American Cancer Society, 2018).***

In Egypt, HCC represents 70.48% of liver malignancies; its occurrence is due to cirrhosis which in turn is a complication of chronic viral hepatitis infection. Approximately 4.7% of chronic liver disease patients develop HCC ***(Holah et al., 2015).***

It has been well known that tumor development is a multistage process to accumulate alterations at the genetic and/or epigenetic levels which ultimately reprogram a cell to undergo uncontrolled proliferation and metastasis ***(Gao et al., 2014)***. The expression of initial mutations depends not only on the internal interaction between oncogenes but also on extracellular factors such as exosome, which could change the patterns of specific gene expression temporarily ***(Melo et al., 2014).***

Exosomes are cell-derived vesicles that convey key elements in modulating intercellular communication. They are known to be secreted from all types of cells, and are crucial messengers that can regulate cellular processes by 'trafficking' molecules from cells of one tissue to another. As an intercellular communicative vector, exosomes facilitate the tumor genesis by transferring oncogene and oncogenic factors ***(Fevrier* & Raposo *2004)***.

Exosomes secretion and delivery to their correct destinations are regulated by RAB (Ras-related in brain) GTPases (Guanosine Triphosphatase.) pathways ***(Stenmark, 2009; Ostrowski et al., 2010)***. Additionally, endosomal sorting complexes required for transport (ESCRTs), intra- and intercellular pH, also $Ca2^{+}$ channels and H^{+} pumps are also known to play critical roles in exosome secretion and delivery to the recipient cells ***(Savina et al., 2003; Ramachandran & Palanisamy 2012***; ***Zhang et al., 2015)***. The uptake of the exosomes by recipient cells is accomplished by endocytosis, receptor-ligand interaction or fusion (***Zhang et al., 2015)*** and is dependent on microenvironmental pH. At a low pH, the exosomes show increased release and uptake of recipient cells via fusion ***(Parolini et al., 2009)***.

Based on their functions, exosomes are considered molecular cargo (***Kalluri 2016)*** and signalosomes ***(Syn et al., 2016)***. According to the ExoCarta database (http://www.exocarta.org/), exosomes carry a variety of contents, including various proteins, DNA, mRNA, microRNA (miRNA), long non-coding RNA (lncRNA), and even viruses ***(Keerthikumar et al., 2016).*** These exosomal genetic materials could be considered as suitable biomarkers for diagnosis and prognosis of cancer via a minimal invasive procedure. Exosomes promote invasive phenotypes, angiogenesis and metastatic growth to the distant organs and as a result, aid in therapeutics by acting as biomarkers and vehicles for genetic therapy; they can be potentially beneficial since exosomes are non-invasive bioavailable vehicles as they could be detected in serum and body fluid. The dynamic role of exosomes in cancer initiation, progression and metastasis offers a fertile ground to determine the cause, effect and treatment of various cancers ***(Tickner et al. 2014).***

The destruction/inactivation of cancer-derived exosomes may lead to inhibition of angiogenesis and metastasis, thus controlling the tumor progression. Ultimately, as research continues to decode the regulatory languages of exosomes'

signals, this will pave the way for new strategies for cancer therapies ***(Subramanian et al., 2016; Pan et al., 2018)***.

Acidification and hypoxia are whole mark characteristics of tumor microenvironment. Proton-pump inhibitors (PPIs) are a family of drugs whose main effect is a reduction of gastric acid secretion by blocking the gastric proton pump in the gastric parietal cells (***Sakai et al., 2016)***. Recently, similarity is strongly noticed between the neoplastic vacuolar H^+ATPase (V-ATPase) over expressed by cancer cells, and gastric H^+ proton pump, thus PPIs could have benefits in blocking V-ATPase activity in cancer cells (***Han et al., 2014***). PPIs are different from traditional cytotoxic agents as they exert anti-tumor effects by altering the tumor microenvironment (***Vander Heiden et al., 2009)***.

AIM OF THE WORK

1- To validate the expression of HCC-specific exosomal coding and non-coding RNAs after retrieving them from public microarray databases, this was carried out on serum samples of HCC patients versus control groups.

2- To characterize the efficacy of Proton Pump Inhibitors PPI on modulating exosomal production and exosomal RNA expression using HCC animal models.

Chapter 1

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is the most common liver cancer type in adults (***Bertuccio et al., 2017).*** It is considered as one of the most challenging tumors with high incidence, prevalence and mortality rates ***(Elbaz et al., 2013)***.

I- Epidemiology

Having an international glance about HCC, More than 700,000 people are diagnosed with this cancer each year throughout the world. For example in United States of America, it is expected that about 42,220 new cases will be diagnosed in 2018 (30,610 in men and 11,610 in women) and About 30,200 (20,540 men and 9,660 women) people will die due to HCC (***American Cancer Society; 2018).***

Historically; malignancy occurrence has noticeably increased since 1980. Liver cancer death rates have increased approximately 3% per year since 2000 (***American Cancer***

Society; 2018). It represents the second most common cause of death from cancer worldwide (***Ferlay et al ., 2015).***

Geographically; HCC is more common in sub-Saharan Africa and Southeast Asia (***Ferlay et al., 2015).*** It is more diagnosed in areas were chronic hepatitis B, C viral infection and co-infection with HBV and Hepatitis C virus (HCV) are widely spread, this drew attention to them as promoting agents in HCC development ***(El-Serag, 2012).***

In Egypt HCC represents an important public health problem, the percentage of HCC in males about 33.6 %, it is the most common cancer in males and in females, it's the second common cancer after breast cancer and it's percentage about 18.5% based upon results of National Cancer Registry Program ***(Ibrahim et al., 2014)***. Liver cancer forms 11.75% of the malignancies of all digestive organs and 1.68% of the total malignancies. HCC constitutes 70.48% of all liver tumors among Egyptians ***(Mokhtar et al., 2007)***. [*f*]The rising rates of HCC in Egypt are due to the high prevalence of hepatitis B virus (HBV) and hepatitis C virus infection (HCV) among Egyptian population. Therefore; we need effective strategies for early detection and better management of HCC which will be of great value in developing countries with limited resources, such as Egypt ***(Abdelgawad et al., 2013).***

During the period 2013–2050, population of Egypt is expected to increase to approximately 160% the 2013 population size. The estimated number of liver cancer cases in Egypt 2013, **27,991** for both sexes (19,646 for men and 8,345 for women) and the expected number of liver cancer cases in Egypt 2050**, 85,471** for both sexes (59,047 for men and 26,425 for women). This increase reflected both population growth and demographic change mainly due to ageing of population ***(Amal et al., 2014***).

II- Risk Factors

HCC is not a single cause disease but it results from the synergetic effect of multiple risk factors.

i- Liver cirrhosis

HCC risk factors lead to liver injury through inflammation, fibrosis and disordered liver architecture which is the main characteristic of liver cirrhosis. About 30%-35% of all cirrhotic patients will develop HCC in the course of their disease that's why Cirrhosis is considered the main risk factor for the development of HCC, which may be due to chronic viral hepatitis, alcohol, hereditary metabolic diseases, or autoimmune and non-alcoholic fatty liver disease ***(Gao et al., 2012***). Studies suggest that the annual risk of developing HCC in the cirrhotic patients is 1%-8% according to the etiology ***(Waghray et al., 2015).***

ii- Hepatic viral infection

- **Chronic hepatitis B virus infection**

HBV is double-stranded DNA-containing virus that is able to integrate its DNA into the hepatic cells, act as a mutagenic agent and cause secondary chromosomal rearrangement and increasing genomic instability ***(Szabó et al., 2004)***. This is the reason why the risk of HCC development is 100-fold higher for patients who are infected with HBV in comparison with those who are not infected ***(Alan et al., 2012)***.

- **Chronic hepatitis C virus infection**

HCV-infected patients have 17 fold higher risk of developing HCC than uninfected ***(Alan et al., 2012).*** High viral loads and HCV genotype 1b infection have been associated with higher risk of HCC occurrence ***(Raimondi et al., 2009).*** The levels of inflammatory markers of oxidative stress are higher in patients infected with HCV and HCC (***Maki et al., 2007)***. Egypt has possibly the highest HCV prevalence worldwide ***(Mohamoud et al., 2013)***, estimated among the general population to be around 14% (***El-Zanaty* & Way *2009).***

iii- Other risk factors

There are many other risk factors that have also been linked to HCC. For example personal habits like smoking, heavy alcohol drinking, and anabolic asteroids used by some athletes are considered as HCC risk factors. Chemical compounds like vinyl chloride, thorium oxide, aflatoxins and arsenic increase the risk of HCC. Certain diseases might increase the risk of liver cancer such as Glycogen storage disease, Alpha1-antitrypsin deficiency, and Wilson disease ***(American Cancer Society's 2018; Scianclepore et al., 2018).***

III- Pathogenesis of HCC:

Pathogenesis of HCC helps us to have a deeper understanding about changes in cell molecular environment that might lead to malignancy development ***(Ho et al., 2016).***

i- <u>Disturbed cell cycle regulation</u>

There are many cycle regulators contributing in cell cycle including multiple cell cyclins, Cyclin-dependent kinases (Cdks) and Cyclin-dependent kinases inhibitors. These cyclins regulate cell division in eukaryotic cells. Disruption of G1/s and G2/M checkpoints leads to uncontrolled cell growth and so development and progression of HCC. Disturbed cell

regulations might happen due to genetic or epigenetic mutations ***(Braicu et al., 2009).***

Hepatocellular carcinogenesis supposed to be due to accumulation of genetic alterations in proto-oncogenes and tumor suppressor genes. Proto-oncogene cause HCC with bad prognosis after its activation to oncogene by mutations or increased expression. The activation of the MYC transcription signature was related to the malignant conversion of preneoplastic liver lesions ***(Croce, 2008; Kaposi-Novak et al., 2009)***

Tumor suppressor genes are genes that have negative effect on cell growth. Low levels of expression of these genes are associated with malignancy cases. For example, low p53 levels or mutations in p53 are found in multiple cancers including HCC, it acts as tumor suppressor through promoting apoptosis and cell cycle arrest ***(Hussain et al., 2007).***

Epigenetic alterations result in changes in chromatin organization and gene expression without change in DNA sequence. In HCC carcinogenesis epigenetic mutation affects gene transcription, chromosomal stability, and cell differentiation, through changes in the methylation or acetylation of histones or DNA as well as mechanisms of gene regulation by non-coding RNAs ***(Wang et al., 2015).***

ii- Disturbed apoptosis

Apoptosis is a mechanism causing cell death. Disturbed apoptosis contributes in development of HCC. This leads to inability of regeneration in a normal architecture and so development of liver fibrosis and cirrhosis ***(Calado et al., 2011; Hartmann et al., 2011).***

iii- Increased angiogenesis:

Angiogenesis is one of the vital biological processes in which new blood vessels are formed from preexisting vasculature during liver regeneration. Hepatocellular carcinoma is typically a highly vascular tumor characterized by neovascularization. The development of neovasculature in the tumor is essential for the growth and metastasis of a cancer ***(Yang et al., 2008).***

iv- Role of Non-coding RNAs

Noncoding RNAs are categorized into two main types based on length: small/short noncoding RNAs that are <200 nucleotides and long noncoding RNAs with length >200 nucleotides. They can have oncogenic or tumor suppressor role in HCC ***(Rong et al., 2013).*** For example, Oncogenic miRNA-221 target cell cycle inhibitors (CDKN1C/p57, CDKN1B/p27) resulting in increased proliferation of HCC cells ***(Fornari et al.,***

2008). However, miRNA-214 has an tumor-suppressor activity of HCC by inhibiting CDK6, CDK3, and E2F2 ***(Wang et al., 2016)***. Also the LncRNA "Metastasis associated lung adenocarcinoma transcript" (MALAT-1) affects cell proliferation and apoptosis. MALAT-1 over expression is associated with poor prognosis and tumor recurrence in HCC patients (***Konishi et al., 2016***). On the other hand LncRNA PTENP1 repressed the tumorigenic properties of HCC cells. Injection of the PTENP1-expressing vector into mice bearing HCC tumors effectively decrease the tumor growth, suppressed intratumoral cell proliferation and inhibited angiogenesis ***(Chen[1] et al., 2015)***.

IV- Histopathological types of HCC

According to the degree of cellular anaplasia, HCC is divided into well differentiated, moderately and poorly differentiated tumor; namely grade 1, 2 and 3, respectively (***Ishak et al., 2001***).

V- Staging systems of HCC

Staging systems of HCC are systems that describe the degree of spread of the HCC. The current stage of HCC in patients is crucial in choosing the treatment method and predicting patient's prognosis. That's why staging systems must be simple, reliable, and accurately describing patient's case (***Subramaniam et al., 2013).***

i- TNM system

The American Joint Committee on Cancer (AJCC) developed the TNM system. This system uses combination of letters and numbers to describe cancer stages (***Ishak K et al,. 1995).***

1- "T" describes number and size of primary tumor.
2- "N" describes the spread of tumor to lymph nodes.
3- "M" describes the distant metastasis.
4- "G" histologic grades