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Potential association between Hepatitis B infection and colorectal cancer progression

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Establishing a link between hepatitis B virus (HBV) infection and colorectal cancer (CRC) can potentially prevent future CRC progression. HBV extrahepatic manifestations are related to immune complex deposition and are driven by viral antigens. We aimed to evaluate the relation between hepatitis B infection and CRC progression by evaluating hepatitis B surface antigen (HBsAg) serum levels in CRC in comparison with colon benign diseases and healthy controls. Moreover, we studied the relation between elevated HBsAg levels and some cancer aggressiveness features. A total of 110 CRC patients, 50 patients with benign colon diseases and 35 healthy individuals were included. HBsAg was detected using western blot and ELISA. In contrast to healthy individuals, sharp band corresponding to HBsAg (24-kDa) was obtained in sera of patients with colon diseases who infected with HBV. HBsAg detection rates increased in detected CRC patients (34.5%) than patients with benign colon conditions (14%). Also, HBsAg serum level (OD) in colon cancer patients (2.8±0.2) was significantly (P=0.034) higher than patients with benign conditions (1.7±0.1). Regarding tumor aggressiveness features, both HBsAg detection rate and serum levels were significantly (P<0.05) higher in patients with late tumor stages, positive lymph node and distant organ metastasis and high tumor grades compared to patients with early stage, negative lymph node and organ metastasis and low tumor grades. In conclusion, patients who under CRC treatment should consider HBV diagnosis to prevent future disease progression as virus infections were shown to be associated with tumor progression.

Keywords: Hepatitis B surface antigen, Hepatitis B virus, Colorectal cancer, Progression

INTRODUCTION

CRC is nowadays the world's 3rd most commonly diagnosed tumor and the 4th most deadly malignancy with almost annually 900 000 deaths (Dekker et al., 2019; Keum and Giovannucci, 2019). In Egypt, it is the 7th common tumor, representing 3% of female cancers and 3.47% of male cancers (Metwally et al., 2018). Sporadic and heterogenetic nature of CRC has led to several epidemiological associations with causes of the disease development and progression (Collins et al., 2011). As important pathogenic elements, infectious agents are acquiring relevance in human cancer (Coelho et al., 2010). Although several studies have reported viral DNA in CRC tissues, whether viral infections contribute to tumor progression risk is still under debate (Chen et al., 2015).

HBV is double-stranded DNA virus that belongs to *Orthohepadnavirus* genus and the

Hepadnaviridae family and causing both acute and chronic infections (Al-Sadeq et al., 2019). It has been implicated in the cause of up to 80% of hepatocellular carcinoma cases (Song et al., 2019). HBV chronic infection persisting in hepatic tissue is associated with elevated immune-mediated liver inflammation, chronic hepatocytes oxidative damage and tumor development (Levrero and Zucman-Rossi, 2016). Some prospective population-based studies have reported the associations between various non-liver tumors and chronic HBV infection, but these results were discordant (Andersen et al., 2015; Kamiza et al., 2016). Also, in various types of non-liver tissues, few clinical case studies detected HBV suggesting HBV potential role in non-liver tumors oncogenesis (Dejean et al., 1984; Mason et al., 1993). Establishing a link between HBV infection and colorectal tumor can potentially prevent future CRC progression (Patel et al., 2015).

It is difficult to establish human viruses as direct cause of different tumors (Moore and Chang, 2010). Therefore, some guidelines for establishing the link between human cancers and viruses recommended assessment of viral markers concentrations in cancer patients in comparison to non-malignant controls, and evaluated these levels as tumor progression risk (Attallah et al. 2018). In this study, we aimed to evaluate the relation between hepatitis B infection and CRC progression by evaluating HBsAg serum levels in CRC in comparison with colon benign diseases and healthy controls. Moreover, we assessed the relation between its levels and some aggressiveness cancer features such as lymph node and distant organ metastasis, high tumor grades and late stages.

MATERIALS AND METHODS

Subjects

This study included 160 Egyptian patients who were diagnosed with colon diseases in Mansoura University Oncology Center, Mansoura, Egypt between June 2016 and October 2018. They were classified into two groups, group 1 included 110 (63 male and 47 females) patients with colon cancer aged from 27-75 years with mean age ± SD= 50.4 ± 11.3 years and group 2 included 50 (27 male and 23 females) patients with non-cancerous (benign growth) colon conditions aged from 22-65 years with mean age ± SD= 46.0 ± 12.5. Diagnosis of colon diseases was undergone using colonoscopy and the tumor features were registered according to TNM staging system (Greene, 2003). Moreover, a group of 35 healthy individuals (20 male and 15 females); mean age ± SD was 48.8±10.2 years were included as negative controls group. Exclusion criteria were included any patients infected with hepatitis viral (A and C) and HIV. Any patients with malignancy were excluded. An informed consent was getting from each individual according to the guidelines of Mansoura University Hospitals and Helsinki declaration Ethics and Scientific Committees.

Identification of HBsAg in serum samples

According to Laemmli (1970) method, proteins of the serum samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Following electrophoretic separation, protein bands were transferred by Western blotting, according to Towbin et al. (1979), onto nitrocellulose membrane (Sigma, USA). They were then immunostained using monoclonal antibody specific for HBsAg (ABC Diagnostics, New Damietta, Egypt) and anti-mouse IgG alkaline phosphatase conjugate (Sigma).

Quantitation of HBsAg serum levels

HBsAg serum levels were quantified using ELISA. Diluted serum samples (1:250) in coating buffer (pH 9.6, carbonate/bicarbonate buffer (50mM)) coated a microtiter plate at 4°C for overnight. After blocking with BSA (0.5% in coating buffer), 50 µL/well of HBsAg specific monoclonal antibody (1:200 in PBS) was added and incubated at 37 °C for two hours. Then, 50 µL/well of anti-mouse IgG alkaline phosphatase-conjugated (1:500 in PBS) was added. An enzyme detection system composed of nitrophenyl phosphate substrate (50 µL/well) was added. Colour intensity at 450 nm was a function of HBsAg concentration.

Statistical analysis

HBsAg serum levels were expressed as mean ± standard deviation (SD), whereas categorical variables were expressed as percentages or numbers. To compare HBV infection proportions, Pearson's chi-squared (X^2) test was used. Differences in HBsAg serum levels among the included subjects groups and between different tumor features were assessed by ANOVA or *t*-test as appropriate. *P* values <0.05 were considered statistically significant. GraphPad

Prism (San Diego, CA) and SPSS (Chicago, IL) programs were used for all statistical analyses.

RESULTS

Colorectal cancer was associated with HBV infection and elevated HBsAg serum levels

At 24-kDa, sharp band was observed in serum samples of patients with colon diseases who infected with HBV. There was no reaction with serum samples of healthy individuals (Figure 1A). HBsAg was detected in 34.5% (38/110) of CRC patients and in 14% (7/50) of patients with benign colon conditions. No case of 35 healthy controls was positive for HBsAg (P<0.0001) (Figure 1B). Among HBV-infected patients, mean HBsAg serum level (OD) in colon cancer patients (2.8±0.2) was significantly (P=0.034) higher than patients with benign conditions (1.7±0.1) (Figure 1C).

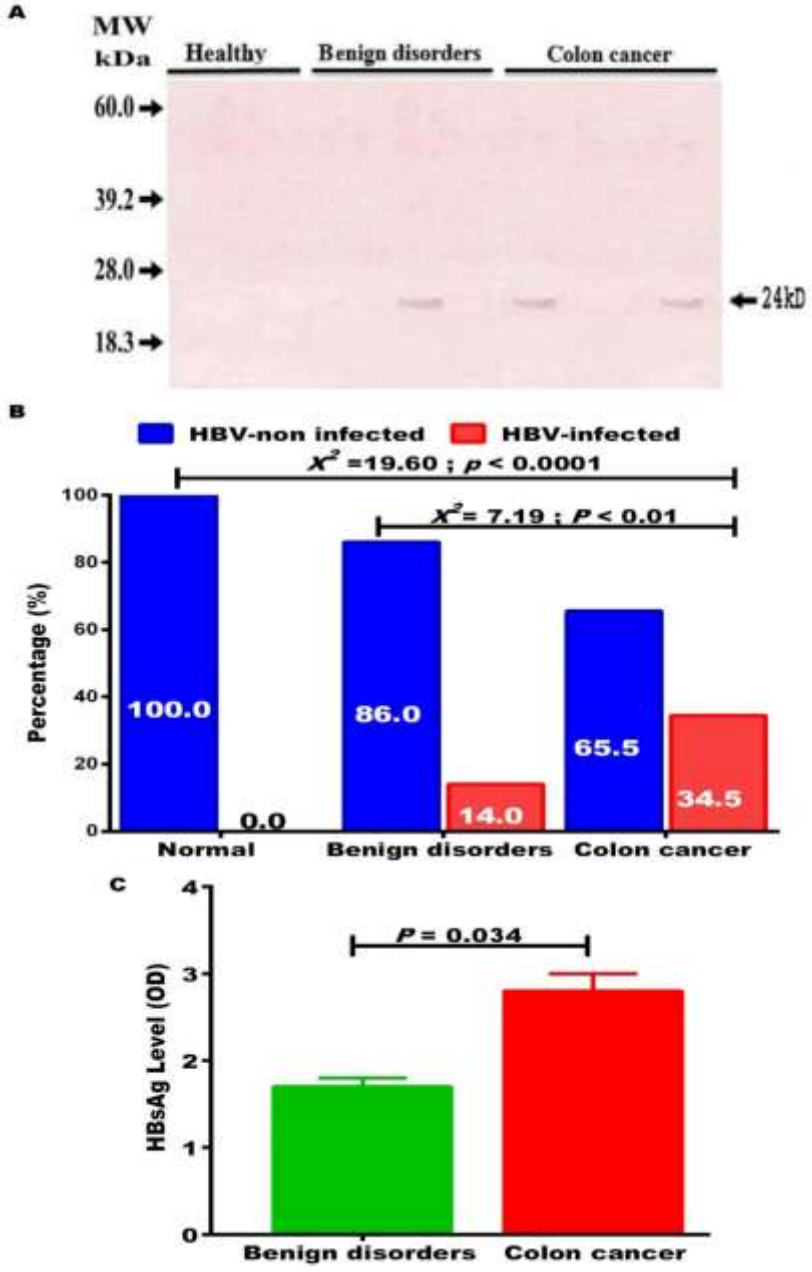

Figure 1: HBV infection and colon cancer. (A) Western immunoblot analysis for HBsAg. HBsAg (B)

detection rates and (C) serum levels were increased in CRC compared to controls.

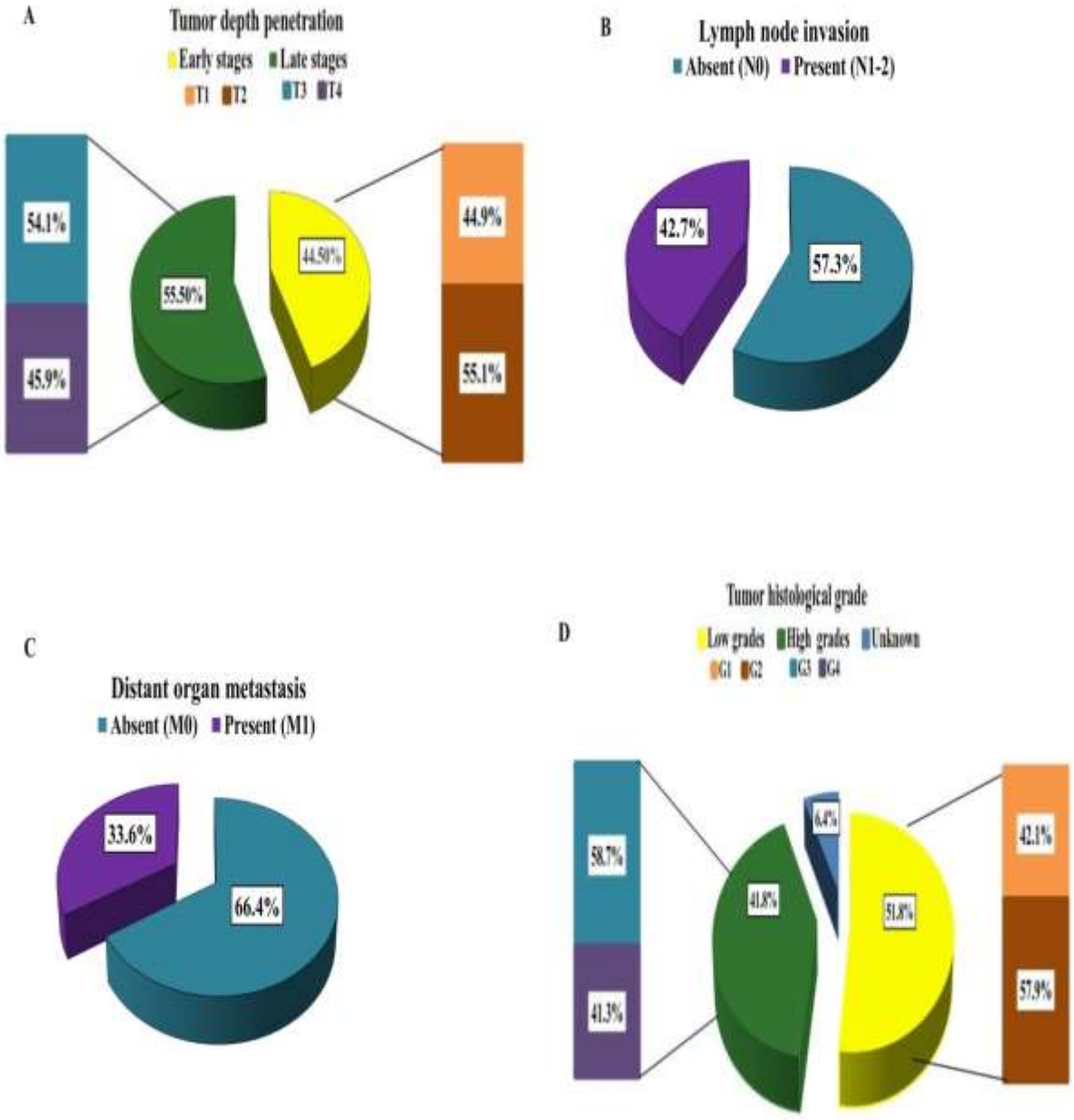

Figure 2: Classification CRC patients according to some tumor features.

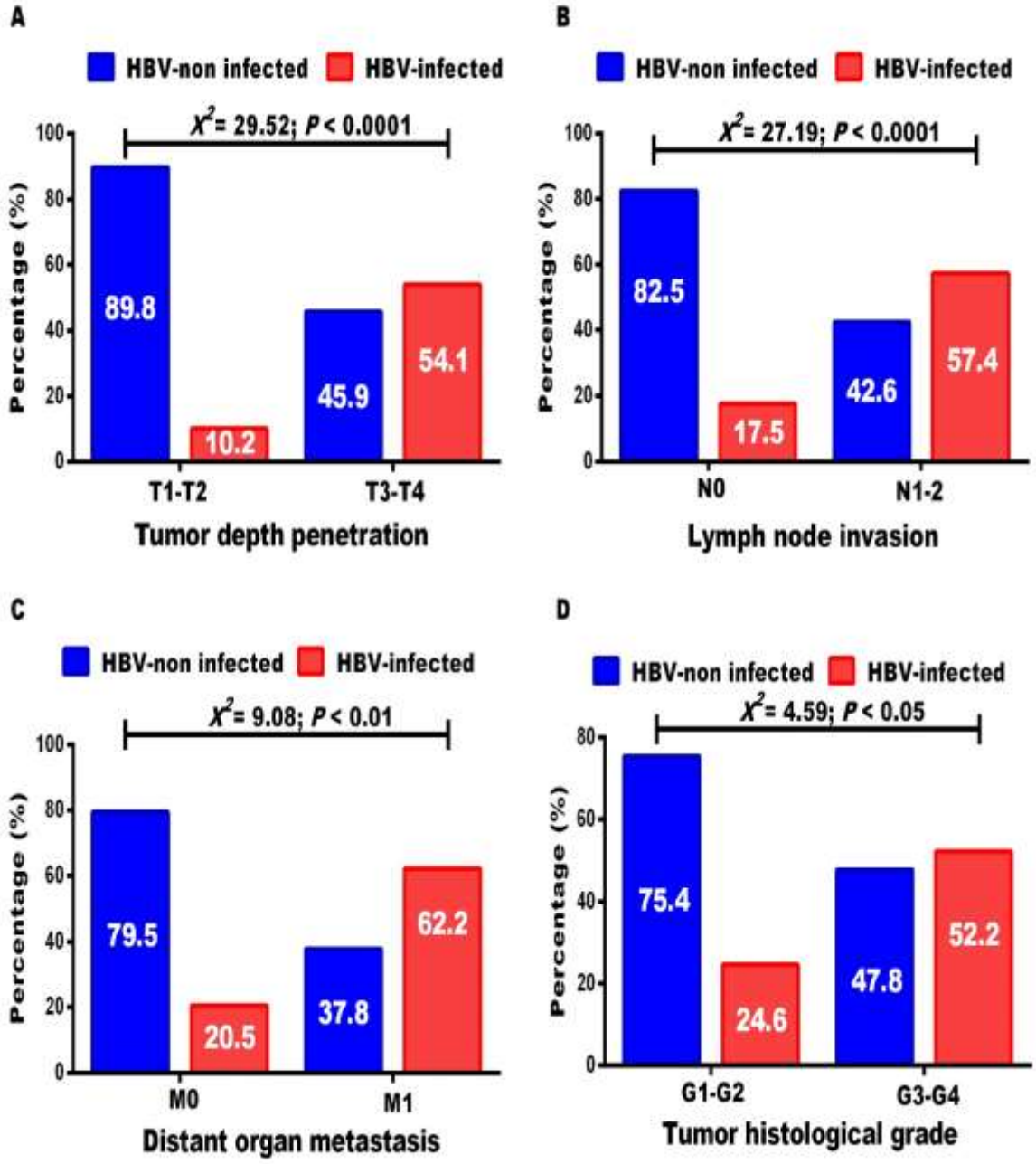

Figure 3. Detection rate of HBsAg according to (A) tumor stage, (B) Lymph node invasion, (C) distant organ metastasis and (D) tumor grade.

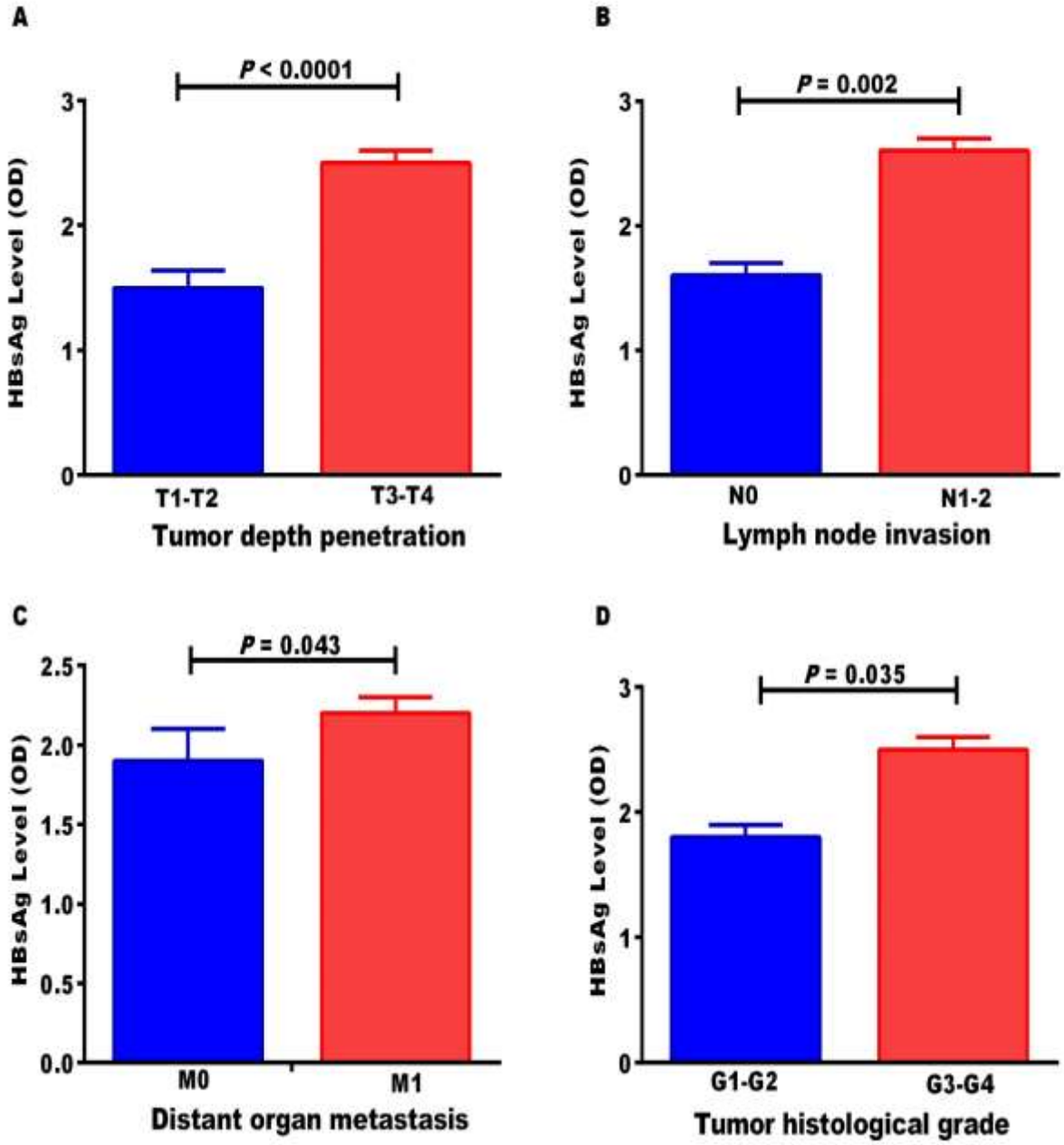

Figure 4: HBsAg serum levels (O.D.) according to (A) tumor stage, (B) Lymph node invasion, (C) distant organ metastasis and (D) tumor grade.

Elevated HBsAg levels were associated with CRC progression

As shown in Figure 2, CRC patients were classified according to tumor stage, lymph node and distant organ metastasis and tumor histological grade. The detection rate of HBsAg significantly (P<0.05) increase in patients with late tumor stages, positive lymph node and distant organ metastasis and high tumor grades (Figure 3). Also, its quantitative level was elevated in these patients compared to patients with early stage, negative lymph node and organ metastasis and low tumor grades (Figure 4).

Discussion

Besides hepatic disorder, HBV chronic infection is associated with wide spectrum of extrahepatic manifestations. Most extrahepatic manifestations are related to immune complex deposition and are driven by viral antigens. In antigen excess, the soluble immune complexes often deposit in specific body sites leading to large

extrahepatic manifestations panel (Virlogeux and Trépo, 2018). The potential association between HBV infection and CRC development is unclear (Patel et al. 2015). There are few studies that debate colonoscopy screening in HBV infected patients (Patel et al., 2015), but, therefore, none of them considered colon benign diseases as controls.

To identify proteins of interest in complex samples, the use of western blot has advantage of antibodies exquisite sensitivity (Mishra et al., 2017). In this study for the first time, we identified HBsAg using western blotting in colon diseases at 24-KDa. Similar HBsAg molecular weights were previously reported (Sunil Kumar et al., 2003; Abolhassani et al., 2013; Attallah et al., 2018). Here, CRC patients also tended to have significantly (P<0.0001) higher HBV infection rates compared to patients with benign colon diseases.

Some guidelines for establishing the link between human cancers and viruses recommended comparison between viral proteins levels in cancer and non-cancer patients (Attallah et al., 2018). Here, HBsAg serum levels were also significantly (P=0.034) higher in CRC patients compared with benign controls. Among patients with CRC and HBV coexistence, none of the previous studies regards hepatitis B effect on the disease progression (Patel et al., 2015; Song et al., 2019). In this study, elevated HBsAg detection rates and serum levels were related to cancer aggressiveness features like late tumor stages, positive lymph node and distant organ metastasis and high tumor histological grades.

HBV carcinogenic features are likely multi-factorial and remain a great research area of interest Patel et al., 2015. One suggested hypothesis highlights HBV carcinogenesis process is through Ras signaling pathways, PB Wnt/β-catenin, the chromosomal segments including P53 and transforming growth factor β (TGF-β). Also, the X protein of HBV (HBx) can bind to p53 tumor-suppressor protein, interfering its role in DNA damage repairing (Cho and Vogelstein, 1992). In CRC progression, p53 role was reported in the adenoma-carcinoma time sequence during colorectal adenomas transformation into carcinomas (Cho and Vogelstein, 1992), and may serve as a link between CRC and HBV.

Few prospective studies have examined HBV association with non-liver tumors (Song et al., 2019). Generally, most previous studies reported that hepatitis B was related to lymphoma (Ulcickas Yood et al. 2007; Sundquist et al., 2014; Kamiza et al. 2016) and pancreatic tumor (Iloeje et al. 2010; Kamiza et al., 2016). HBsAg detection rates were reported previously in gastrointestinal tract tumors [colonic (8%), rectal (10%), gastric (12%) and hepatocellular (25%) carcinoma] (fzz et al., 1995).

CONCLUSION

This study results urge oncologists, treating CRC patients, to consider diagnosis of HBV infection for these patients to prevent disease progression. More studies are required looking specifically at mechanisms of hepatitis B inducing carcinogenesis, specifically colorectal carcinoma.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Attallah AM, El-Far M and Omran MM were chief investigators who conceptualised and designed the study. Gamal H was investigator who collected data from the literature, collected samples and carried on with different experiments and techniques. Omran MM and Gamal H aquired data and performed all data and statistical analysis. Attallah AM, Omran MM and Gamal H interpreted data and wrote final manuscript. All authors read, reviewed and approved the final manuscript.

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