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Increased risk of colon and pancreatic cancers in Egyptians subjects is associated with *Mycobacterium tuberculosis* infection

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The highly tuberculosis (TB) infectious nature urges the need to elevate the rapidity and efficiency of laboratory methods. Additionally, the relationship between TB and malignancy becomes even more challenging to be clarified. Thus we aimed to evaluate the association between TB and colon and pancreatic cancers using *Mycobacterium tuberculosis* antigen (55-KDa) based method other than conventional limited screening methods. A total of 110 individuals were included in this study; 24 healthy volunteers and 86 cancerous patients. The underlying malignant conditions were colon (n=44) and pancreatic (n=42) cancers. TB 55-kDa antigen was determined using ELISA. The detection rate of TB-antigen was found to be significantly high in cancerous patients. According to the site of malignancies, pancreatic cancer was associated with the highest TB risk (OR=15.7) *vs* colon cancer (OR=8.6). The mean TB-antigen level was significantly higher in pancreatic cancer (1.8±0.02) and colon cancer (1.3±0.04) cohort than healthy control (0.1±0.03). Interestingly, TB-antigen levels were significantly correlated with gastrointestinal tumor markers like CA19-9 and CEA (*r*=0.527 and 0.635, respectively). Additionally, old age (OR=3.1), male sex (OR=3.3), cancer treatment (OR=12.3), elevated CA19-9 and CEA levels (>200 U/mL) (OR=10.0 and 35.0, respectively) were identified as potential risk factors for TB in cancerous patients. In conclusion, *Mycobacterium tuberculosis* is an important pathogen in cancerous patients in particular those with pancreatic cancer and must be searched immediately if there are clinical doubts..

Keywords: Antigen, cancer, colon, CEA, and *Mycobacterium tuberculosis*.

INTRODUCTION

Tuberculosis (TB) and cancer are major public health problems which cause millions of deaths annually worldwide (Bray et al. 2018; Floyd et al. 2018). The relationship between TB and cancer is of dual nature; TB may sometimes be misinterpreted as cancer (El-Mahallawy et al. 2010). Alternatively, cancer can increase active tuberculosis risk but evidence is sparse (Simonsen et al. 2017). Hematologic malignancies had been identified to increase the risk of active TB disease. However, the association between solid-organ malignancies, that represent the vast majority of cancers and account for half of all new cancer diagnoses annually, and TB developing is less known with conflicting results (Jacobs et al. 2015). Also, the relative and absolute risk for different cancer types has not been well defined

(Cheng et al. 2016).

On the other hand, TB diagnosis remains a challenge because of its insidious nature, and the limitations of available diagnostic tools (Batz et al. 2012). It relies upon microscopic examination for the presence of acid-fast bacilli (AFB), and culture on solid and/or liquid media. Cultures which still the gold standard for TB diagnosis may take 2–8 weeks (Garg et al. 2003). Microscopic examination for AFB is rapid but has a very limited sensitivity ranged from 20 to 80% (Steingart et al. 2007). Moreover, nucleic acid amplification based tests such as polymerase chain reaction and Gene-Xpert are sensitive and rapid but are expensive (Singh et al. 2017). To overcome TB diagnostics limitations, serological tests have received attractiveness because of their suitability, rapidness and cost effectiveness (Perkins et al. 2006). Hence, a simple and rapid immunoassay in 2005 for the detection of TB 55-kDa circulating antigen for the clinical diagnosis of pulmonary and extra-pulmonary TB with a high degree of efficiency was previously developed (Attallah et al. 2005).

In this study we aimed primarily to assess the TB risk among cancerous patients according to the site of cancers by evaluating the circulating levels of TB 55-kDa antigen. Then, identify some risk factors that can be associated with TB development in these patients.

MATERIALS AND METHODS

Patients

This study was carried out on 110 patients with different malignant diseases clinically suspected of having *Mycobacterium tuberculosis* infection, collected from Oncology center, Mansoura University Hospitals, Mansoura, Egypt. According to the cancer anatomical sites, 44 cases were diagnosed with colon cancer and 42 with pancreatic cancer. Additionally, 24 healthy volunteers were included in this study as negative controls. The study was approved by the Ethics Committee of the Mansoura University Hospitals, Mansoura, Egypt and conformed to ethical guidelines of the Declaration of Helsinki. An informed written consent was obtained from all participants.

Identification of cancerous patients and laboratory assays

All patients were clinically examined in detail and investigated including radiological, imaging, and other laboratory tests to decide the cancer type. Colon cancer was investigated by measuring Carcinoembryonic Antigen (CEA) tumor marker (Locker et al. 2006), followed by colonic endoscope and confirmed by colonic biopsy. Pancreatic cancer was diagnosed by measuring of Carbohydrate Antigen 19.9 (CA19.9) tumor marker (Locker et al. 2006) and radiological studies (chest X-ray, abdominopelvic CT scans with contrast enhanced triple phase helicals and Doppler studies

Blood samples were withdrawn from all subjects and serum samples were obtained from one part of this blood. The remaining part of the blood within EDTA tubes was used for analysis of complete blood count on an automated hematology analyzer (Sysmes Corporation, Kobe, Japan). Tumor markers including CEA and CA19.9 were also detected according the manufacturer's instructions of commercial ELISA kit, (CanAg, Diagnostics AB, Gothenburg, Sweden).

Detection of TB 55- kDa antigen using ELISA

The determination of TB 55- kDa antigen serum levels was made by using ELISA technique. In brief, diluted serum samples (1:250 in coating buffer (50 mM carbonate/ bicarbonate buffer, pH 9.6) allowed binding overnight to wells of ELISA plates at 4°C. Then, 200 µl/well of 0.05% (v/v) PBS-T20 (pH 7.2) was added for blocking free active sites. Diluted monoclonal antibody to the TB 55- kDa antigen (1:50 in PBS) added separately 50 µl per well. The antigen-antibody binding allowed proceeding for 2 hours at 37°C. Then, diluted anti-mouse IgG alkaline phosphatase-conjugated (1:400 in 0.2% BSA in PBS-T20) was added. The wells were washed to remove any unbound material with PBS+0.5% Tween 20 (3 times) after each step. The amount of coupled conjugate determined by incubation with nitrophenyl phosphate substrate (50 µL/well). The enzyme converts a substrate (chromogen) to a colored product, indicating the presence of Ag-Ab binding. The absorbance was read at 450 nm after 10 minutes using a microtiter plate reader (Σ960, MetretechInc, Germany). Color intensity was proportional to the amount of bound conjugate and therefore is a function of the concentration of TB antigen present in the serum sample.

Statistical analysis

Data processing and analyses were performed using GraphPad Prism package; v.5.0 (GraphPad Software, San Diego, CA) and SPSS

software, version15.0 (SPSS Inc., Chicago, IL). Continuous variables were expressed as mean ±SD whereas categorical variables were expressed as numbers (percentages). Differences in continuous variables were determined using Student *t*- test and Chi-square (X^2) test for categorical data. The correlation was evaluated by Spearman's rank correlation coefficient. A value of $P<0.05$ was considered statistically significant. Odds ratios (OR) were calculated using logistic regression models to estimate the TB risk among patients with gastrointestinal malignancies.

RESULTS

Risk of TB in gastrointestinal cancerous patients

The demographic characteristics and the clinical background of all participants are summarized in Table 1. Overall cancerous patients are at increased risk of active TB. As a consequence the detection rate of TB antigen was found to be significantly ($P=0.002$) high in patients with pancreatic (40.5%) and colon (27.3%) cancers compared to healthy controls (4.2%) Figure 1A. Moreover, TB-55 level (OD) was higher in cancerous cohort; pancreatic (1.8±0.02), and colon (1.3±0.04) cancers patients than healthy control (0.1±0.03), Figure 1B. In addition, pancreatic cancer patients were associated with the highest TB risk (OR=15.7) *vs* colon cancer (OR=8.6), Figure 1C.

Risk of TB in malignant patients according to age, gender and cancer treatment

The TB risk elevated with the patients ages (≥40 years) (OR=3.1 and 2.1; for age of 40-59 years and ≥60 years; respectively). Although most of the study populations was female (34 males and 52 females), the detection rate of TB antigen was found to be significantly ($P=0.010$) higher in male (50.0%) than female (23.1%) with an estimated OR=3.3, Table 2.

Additionally, TB detection rate was higher in patients who received both radiotherapy and chemotherapy (63.6%, OR= 12.3) than patients who received each alone (53.3% (OR= 8.0) and 30.0% (OR=3.0); respectively).

Elevated TB antigen levels are associated with tumors markers

Interestingly, infected TB patients were found to be associated with significant high CA19-9 (290.9±27.6 U/mL) and CEA (200.1±26.3 U/mL) levels compared to those non-infected patients (89.9±33.0 and 63.5±18.8 U/mL; respectively) Figure 2A-B. Also, there was a significant correlation between TB antigen levels and CA19-9 that is an important indicator of gastrointestinal related tumors especially in pancreatic cancer ($r=0.527$, $P<0.0001$) and CEA ($r=0.635$, $P<0.0001$) that can be described as the most broad spectrum of indicators, as its rise can be seen in the colorectal cancer, stomach cancer, and pancreatic,.

Table 1: Demographic characteristics of the study populations

Variables*	Controls (n=24)	Colon cancer (n=44)	Pancreatic cancer (n=42)	*P* value†
Age (years)	50.3±15.8	53.2±12.5	51.5±10.5	0.893
Gender (No (%))				
Male	14 (58.3)	18 (49.9)	16 (38.1)	0.251
Female	10 (41.7)	26 (59.1)	26 (61.9)	
Treatment (No (%))				
Surgery	-----	6 (13.7)	2 (4.8)	0.032
Chemotherapy	-----	7 (15.9)	13 (30.9)	
Radiotherapy	-----	10 (22.7)	5 (11.9)	
Surgery & chemotherapy	-----	14 (31.8)	18 (42.9)	
Radio & chemotherapy	-----	7 (15.9)	4 (9.5)	
Hemoglobin (g/dL)	12.4±0.4	8.5±0.2	9.2±0.2	0.042
RBCs ($\times10^{12}$/L)	4.6±0.2	4.0±0.04	4.2±0.1	0.055
WBCs ($\times10^{9}$/L)	7.9±0.3	9.4±0.7	8.2±0.5	0.061
Platelets count($\times10^{9}$/L)	249.8±22.8	295.0±16.3	287.0±13.5	0.076
CA19.9 (U/mL)	15.2±4.3	90.9±17.6	200.7±98.7	<0.0001
CEA (U/mL)	3.1±0.7	109.8±33.3	39.8±13.7	0.001

*__RBCs__: red blood cells; __WBCs__: white blood cells; __CEA__: carcinoembryonic antigen and __CA__: carbohydrate antigen. Continuous variables were expressed as mean ±SD. †$P<0.05$ is considered significant
*References group. †CI: confidence interval. ‡$P<0.05$ is considered significant.

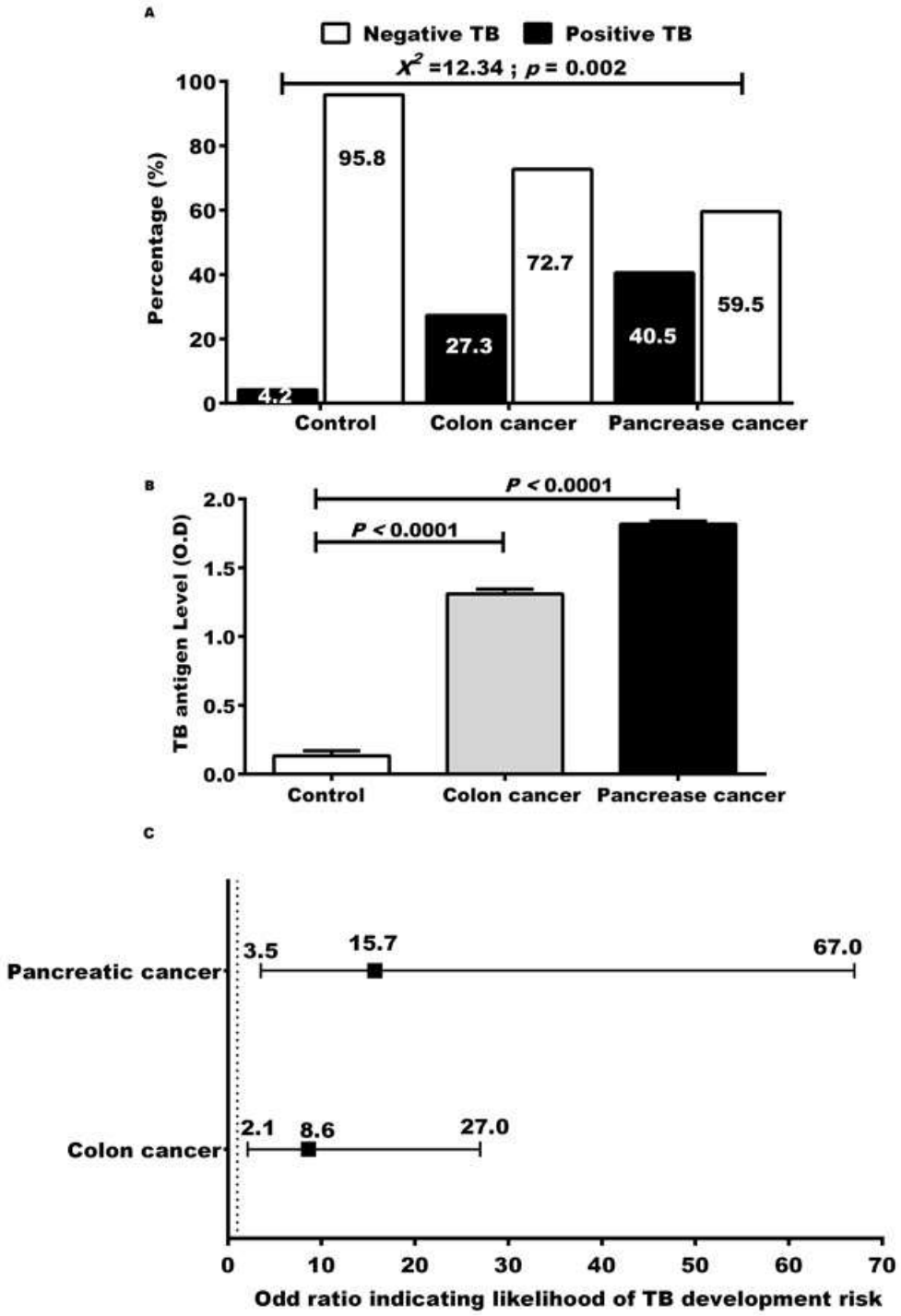

Figure 1: Tuberculosis risk in colon and pancreatic cancerous patients. (A): Detection rate of *Mycobacterium tuberculosis* infection according to tumor site. (B): Distribution of TB-antigen levels (OD) in cancerous patients. (C): Odds ratio showing TB risk according to cancer site.

Table 2: TB risk in terms of age, gender and cancer therapy

Variables	No	Positive TB; No (%)	Odd ratio (95 CI)†	X^2; *P* value‡
Age				
< 20 years*	5	1 (20.0%)		7.8; 0.050
20-39 years	19	3 (15.8%)	0.6 (0.1-9.3)	
40-59 years	39	17 (43.6%)	3.1 (0.3-30.2)	
≥ 60 years	23	8 (34.8%)	2.1 (0.2-22.4)	
Gender				
Male	34	17 (50.0%)	3.3 (1.3-8.5)	6.7; 0.010
Female*	52	12 (23.1%)		
Therapy for malignancy				
Surgery*	8	1 (12.5%)		10.7; 0.030
Chemotherapy	20	6 (30.0%)	3.0 (0.3-30.02)	
Radiotherapy	15	8 (53.3%)	8.0 (0.8-82.1)	
Surgery & chemotherapy	32	7 (21.9%)	2.0 (0.2-18.7)	
Radio & chemotherapy	11	7 (63.6%)	12.3 (1.1-138.9)	
Level of carbohydrate antigen 19.9				
> 100 U/mL*	48	8 (16.7%)		16.9;<0.0001
100- 200 U/mL	17	7 (41.2%)	3.5 (1.1-11.9)	
> 200 U/mL	21	14 (66.7%)	10.0 (3.1-32.6)	
Level of carcinoembryonic antigen				
> 100 U/mL*	43	3 (6.9%)		33.2; <0.0001
100- 200 U/mL	14	5 (35.7%)	7.4 (1.5-36.8)	
> 200 U/mL	29	21 (72.4%)	35.0 (8.4-145.9)	

*References group. †CI: confidence interval. ‡*P*<0.05 is considered significant.

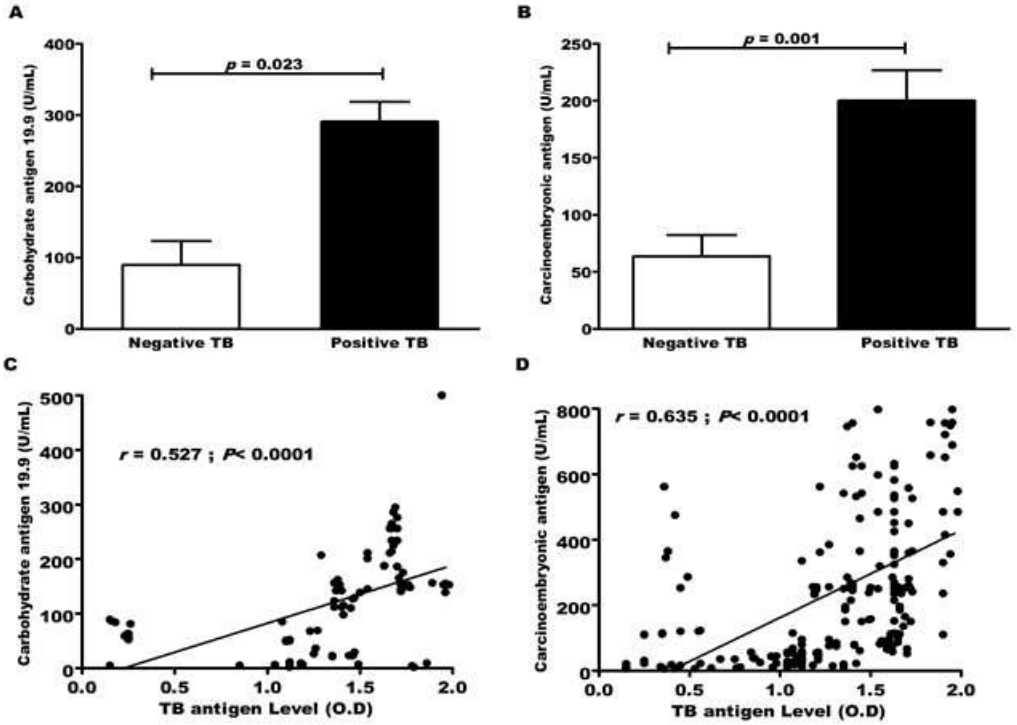

Figure 2: Elevated TB-antigen levels are associated with gastrointestinal tumors markers. Level of (A): CA19.9 and (B): CEA in TB-infected patients compared to non-infected cancerous patients. Correlation between TB-antigen level and (C): CA19.9 and (D): CEA. (CA=carbohydrate antigen; CEA=carcinoembryonic antigen)

Figure 2C-D. Additionally, elevated levels (>200 U/mL) of CA19-9 and CEA was found to be associated with increased TB risk in cancerous patients (OR=10.0 and 35.0, respectively) Table 2

DISCUSSION

Interestingly, patients with malignant diseases are at increased risk for mycobacterial diseases (Malone et al. 2004). On the other hand, more efforts are still directed toward the serological diagnosis of TB that could provide a considerable improvement over current limited TB screening methods (Wang et al. 2018). Therefore, in this study we aimed to evaluate the association between TB and two of gastrointestinal cancers using *Mycobacterium tuberculosis* antigen (TB-55 KDa) based method.

Overall, our results clearly demonstrated that TB risk is higher in cancerous patients and this risk differs according to the cancer site. TB detection rate was found to be significantly high in patients with pancreatic (40.5%) and colon (27.3%) cancers. These findings are in accordance with the previously few reports that reported the coexistence of cancer and TB infection in patients with gastrointestinal malignancies (Kim et al. 2008). Dobler et al indicated that patients with gastric, liver and colon cancers were associated with about 2.6, 2.02 and 2.0-fold increase in TB developing risk respectively in comparison to general population (Dobler et al. 2017). The overall incidence of new-onset TB per 100,000 person-years was 324 in gastric cancer patients, 204 among patients with liver cancer, 294 in colon cancer patients, 253 among patients with pancreatic cancer and 90 in breast cancer patients (Wu et al. 2011). Differences in the patient population may explain these discrepancies in the results.

The high TB risk after a cancer diagnosis is consistent with the hypothesis of malignancies and its therapies seem to create the appropriate environment for either the reactivation of a latent TB infection or make patients susceptible to a new TB infection (Falagas et al. 2010). Cancer may increases TB risk through a combined mechanism of decreasing infection barriers locally and generalized immunosuppression especially depression of the T-cell defense mechanisms (Falagas et al. 2010). Additionally, TB risk also associated with the polymorphism of multiple innate immunity and inflammatory response genes (Azad et al. 2012), that have been associated with cancer susceptibility (Kutikhin, 2011). Also, this association may be due to shared risks including tobacco smoking and malnutrition (Fang et al. 2015; Kuo et al. 2013).

In regards to the site of the disease, pancreatic cancer had the highest risk (OR=15.7) compared to colon cancer (OR=8.6). Similarly, Seo et al. found that, after the hematologic malignancy, the incidence of TB was the highest in patients with pancreatic cancer, followed by the patients with gastric and liver cancers (Seo et al. 2016). These findings may be explained, at least in part, by the fact that patients with pancreatic cancer had the highest rate of metastatic disease among the solid tumors (Seo et al. 2016).

Moreover, in relation to immunologic impairment due to the cancer treatment, we found that radiotherapy or/and chemotherapy treatments might be important influences to increase TB development in cancerous patients. As previously indicated, it seems reasonable that radiation could reduce infection barriers or/and lead to immunosuppression (Hubenak et al. 2014); making patients simultaneously susceptible to TB reactivation (Jacobs et al. 2015). Additionally, there is an additive effect when the systemic toxicity of the chemotherapy was combined with radiation therapy that elevates the risk of primary infections or reactivation of latent and chronic infections (Jacobs et al. 2015), which is consistent with our findings.

Furthermore, risk of TB developing was more prominent among male sex and older patients (OR=3.3 and 3.1; respectively). Similarly, old age and male sex were identified as independent TB risk factors in patients with gastric cancer (Fang et al. 2015; Huang et al. 2011). This is may be because the TB risk is generally higher among elderly individuals (Dye and Williams, 2010). Elderly patients often suffer from more comorbidities, worse immunity and malnutrition than younger patients, and may be more likely to TB development (Fang et al. 2015).

Indeed, CA 19-9 and CEA are well known tumor markers used that in cancers of the gastrointestinal tract including those of the colon and pancreas, with an upper limit of 37 U/mL and 5 U/mL; respectively. Among cancerous patients, TB infected individuals was found to be associated with high levels of CA 19.9 and CEA and there was a significant correlation between TB antigen levels and CA19-9 and CEA (r=0.527 and 0.635, respectively).Moreover, elevated levels (>200 U/mL) of CA19-9 and CEA was found to be associated with increased TB risk in cancerous patients (OR=10.0 and 35.0, respectively). Interestingly, many studies had been reported that

the serum levels of CA19.9 and CEA were higher in tuberculosis patients than normal population (Ma et al. 2016). Sekiya et al, found that the positivity rate for elevated CEA level in pulmonary tuberculosis is 16.9% (Sekiya et al. 2007).

CONCLUSION

In conclusion, TB risk is considerably higher in cancer patients. This risk is increased substantially in patients with pancreatic cancer. In addition, TB development risk is higher in male sex, older patients and patients receiving radiotherapy or/and chemotherapy treatments. Therefore TB should be recommended for systemic screening in malignant patients and clinicians should be aware of this risk. Further multicenter studies are needed to better define the risk and optimal management involving a greater number of patients.

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, Ghaly MF, Omran MM and Taha MA were chief investigators who conceptualized and designed the study. Khedr FM was investigator who collected data from the literature, collected samples and carried on with different experiments and techniques. Omran MM and Khedr FM acquired data and performed all data and statistical analysis. Attallah AM, Omran MM, Taha MA and Khedr FM interpreted data and wrote final manuscript. All authors read, reviewed and approved the final manuscript.

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