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# Circulating Long Intergenic Non-Coding RNA LINC01538 as Potential Novel Biomarker for Acute Myocardial Infarction: Prospective Cohort Study

## Akut Myokard Enfarktüsü için Potansiyel Yeni Bir Biyobelirteç Olarak Dolaşan Uzun İntergenik Kodlamayan RNA LINC01538: Prospektif Kohort Çalışma

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**ABSTRACT Objective:** Non-coding RNAs are the RNAs with no protein coding function, long non-coding RNAs (LncRNAs) are the non-coding RNAs with more than 200 nucleotides. During the journey of discovering novel biomarkers for diagnosis of myocardial infarction (MI), the principle of studying the LncRNA as a potential would be always attractive, as it needs easy sample collection, has high detection sensitivity and high myocardial tissue specificity. Our main objective was to investigate the potential of LINC01538 as a novel diagnostic biomarker for MI. **Material and Methods:** Quantitative real-time polymerase chain reaction (RT-qPCR) was used to assess the expression of the serum LINC01538 in 50 ST Elevation MI (STEMI) patients and 48 controls. **Results:** The study showed a significant increase in serum level expression of LINC01538 in MI patients compared to controls [12 (6.6-21.8) vs. 0.07 (0.01-0.2), p<0.001]. LINC01538 expression level in MI group was positively correlated with creatine kinase MB and high sensitive cardiac troponin I (hs-cTnI) (r=0.39, p=0.006 and r=0.22, p=0.007, respectively). Hs-cTnI found to have diagnostic value for MI with an area under curve (AUC) 0.917 [95% confidence interval (CI): 0.855-0.979, p<0.001] at an optimal cutoff point of 1.45 ng/L, 90% sensitivity and 91% specificity. However, LINC01538 showed the highest diagnostic value with an AUC 0.980 (95% CI: 0.942- 1, p<0.001) at an optimal cut-off point of 1.76, 100% sensitivity and 98% specificity. **Conclusion:** Our findings have, for the first time, demonstrated that circulating LINC01538 are highly expressed in patients with MI, functioning as potential novel biomarker for diagnosis.

**Keywords:** LncRNA; LINC01538; novel biomarker; myocardial infarction

**ÖZET Amaç:** Kodlamayan RNA'lar protein kodlama işlevi olmayan RNA'lardır, uzun kodlamayan RNA'lar (LncRNA'lar), 200'den fazla nükleotid içeren kodlamayan RNA'lardır. Miyokard enfarktüsünün (MI) tanısı için yeni biyobelirteçleri keşfetme yolculuğu sırasında, LncRNA'yı bir potansiyel olarak inceleme ilkesi, kolay örnek toplamaya ihtiyaç duyduğundan, yüksek algılama duyarlılığına ve yüksek miyokardiyal dolarak LINC01538'in potansiyelini araştırmaktır. **Gereç ve Yöntemler:** Elli ST yükselmeli MI (STEMI) hoku özgüllüğüne sahip olduğundan her zaman çekici olacaktır. Asıl amacımız MI için yeni bir biyobelirteç astası ve 48 kontrolde serum LINC01538 ekspresyonunu değerlendirmek için kantitatif gerçek zamanlı polimeraz zincir reaksiyonu (RT-qPCR) kullanıldı. **Bulgular:** MI hastalarında kontrollere kıyasla serum LINC01538 düzeylerinde anlamlı artış olduğu görüldü [12 (6.6-21.8) vs. 0.07 (0.01-0.2), p<0.001]. MI grubunda LINC01538 ekspresyon düzeyi kreatin kinaz MB ve yüksek sensitif kardiyak troponin I (hs-cTnI) ile pozitif olarak körele idi [sırasıyla, 12 (6.6-21.8) vs. 0.07 (0.01-0.2), p<0.001]. Hs-cTnI, eğri altında kalan alan (AUC) 0,917 [%95 güven aralığı (GA): 0,855-0,979, p<0,001] ile 1,45 ng/L optimal kesme noktasında, %90 duyarlılık ve %91 özgüllük ile MI için tanısal değere sahip bulundu. Bununla birlikte, LINC01538, 1,76 optimal kesme noktasında, %100 duyarlılık ve %98 özgüllükte AUC 0.980 (%95 CI: 0.942-1, p<0.001) ile en yüksek tanı değerini göstermiştir. **Sonuç:** Bulgularımız, ilk kez, dolaşımdaki LINC01538'in MI hastalarında yüksek oranda eksprese edildiğini ve tanı için potansiyel yeni bir biyobelirteç olarak işlev gördüğünü göstermiştir. Bununla birlikte, potansiyel uygulamayı doğrulamak ve tutarlılığı belirlemek için ST elevasyonsuz akut koroner sendromda test edilmesi gerekir.

**Anahtar Kelimeler:** LncRNA; LINC01538; yeni biyobelirteç; myokard enfarktüsü

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Myocardial infarction (MI) results from acute severe mismatch between myocardial tissue demand and blood supply, leading to sequence of biochemical and inflammatory reactions with subsequent myocardial cell death and necrosis.[1,2]

One of the most challenging difficulties facing health care system when dealing with a case of MI is rapid and accurate diagnosis. Acute myocardial infarction (AMI) is a critical and fatal medical condition, any delay in accurate diagnosis and management can lead to mortality or subsequent disabling morbidity; hence, the focus of many research projects is to rapidly and accurately diagnose this condition.[3,4]

When we are facing a case of acute chest pain with suspected MI, rule in/rule out laboratory test is needed. Optimal biomarker for diagnosis of MI should be not only sensitive and specific, but also accessible and non time consuming. In the current practice, cardiac troponins (cTns) and creatine kinase MB (CK-MB) are the conventional biomarkers for diagnosis and risk stratification of MI.[5] However, these biomarkers have their own pitfalls. One of the pitfalls is that other medical conditions can lead to false positive rise in cTns levels.[6] Therefore, there is an imminent need to discover novel biomarkers with high sensitivity and better specificity to improve the clinical outcome.

RNAs with no protein coding function called non-coding RNAs. Those with 21-25 nucleotides are called microRNAs, while those with more than 200 nucleotides are called long non-coding RNAs (LncRNAs).[7,8] Although these RNAs have no protein coding functions, they found to have many roles in regulation and control of cellular functions.[9-11] For example, roles in myocardial cell functions, apoptosis and metabolism found to be regulated by LncRNAs.[12] Moreover, it was found that many LncRNAs are highly specific to myocardial tissue. The high specificity of the LncRNAs to myocardial tissue was the trigger for the idea of testing its potentials as a novel biomarker of MI.[13] In addition, LncRNA found to have better detection sensitivity using quantitative real time polymerase chain reaction (RT-qPCR) in comparison to routine protein biomarkers.[14] Many studies tested also the use of the LncRNAs biomarkers for atrial fibrillation and heart failure.[15,16] Considerable number of research projects was focusing on studying the role of the microRNAs as novel biomarker for AMI.[17,18] On the other hand, LncRNAs still not largely studied yet for this potentiality.

LINC01538 is one of the intergenic LncRNAs which was tested in patients after having off pump coronary artery bypass grafting (CABG) as a potential novel diagnostic biomarker and predictor for myocardial tissue reperfusion injury and inflammation.[19] Hence, our study aims to investigate the potential of LINC01538 as a novel diagnostic biomarker for MI.

## MATERIAL AND METHODS

Our study is a prospective study done from November 2019 till October 2020. We recruited 50 acute ST elevation myocardial infarction (STEMI) patients from university hospitals of Ain Shams to MI group. We recruited another 48 healthy volunteers as controls with normal electrocardiogram (ECG) and no history of ischemic heart disease seeking routine health check-ups with matched sex and age to the MI group, the recruitment of controls was done after inclusion of the entire MI group, to allow inclusion of controls with matched age and sex to MI group. Helsinki Declaration principles were considered and applied during our study. All participants gave informed written consent and we got an approval for our study from Research Ethics Committee of Ain Shams University, Faculty of Medicine (date: May 13, 2019, no: FMASU R 30/2019).

Diagnosis of STEMI was made when acute symptoms suggestive for myocardial ischemia were combined with new ECG changes consistent with STEMI criteria according to European Society of Cardiology guidelines 2017.[20] All patients diagnosed with STEMI underwent emergency percutaneous coronary intervention.

We excluded patients with chronic kidney disease, liver disease, cardiomyopathy, history of ischemic heart disease, and history of chemotherapy or chest radiotherapy.

In MI group, blood samples were collected in the first 6 hours of chest pain onset for measuring high sensitive cardiac troponin I (hs-cTnI), CK-MB and LncRNA LINC01538 expression levels.

Serum hs-cTnI was measured using ELISA kit (Cat.no.E-EL-H1420), manufactured by (Elabscience, Houston, TX, USA).

Measurement of LncRNA LINC01538 relative expression level was done as following: first, centrifugation of blood samples was done for 20 minutes at 4k rpm, then we stored serum samples at a temperature of -70°C into DNase-free and RNase-free eppendorf tubes, then we used extraction kit miRNEasy (Qiagen, Hilden, Germany) for purification of Total RNA from sera samples. The second step was using NanoDrop (Thermo Scientific, Waltham, MA, USA) and Invitrogen™ Qubit™ 3.0 Fluorometer (Thermo Fisher, Waltham, MA, USA) to assess the concentration of the RNA. In the third step, we used miScript II RT Kit (Qiagen, Hilden, Germany) to assess LINC01538 LncRNA (LPH25210_200; human LINC01538 LOT 20160531004) in the sera samples. The fourth step was using RT2 SYBR Green ROX qPCR Master mix (Qiagen, Germany) and quantitative PCR to assess levels of LncRNAs. The used reference gene for normalisation was ACTB-1/beta actin (Hs-ACTB-1-RT2 QuantiTect Primer Assays, Qiagen, Germany). The used qRT-PCR system in our study was Applied Biosystems 7500 FAST. The final step was calculation of relative quantification of RNA panel expression including LINC01538 using Livak method with an equation $RQ=2^{-\Delta\Delta Ct}$.

We collected blood samples for all participants after 12-14 fasting hours. In order to test for lipid profile panel including total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG). In control group, single blood sample was taken to do all the tests. While in MI group, the second blood sample was needed during hospital stay after fulfilling the 12-14 fasting hours.

### STATISTICAL ANALYSES

Software package of statistical analysis version 20 (IBM SPSS Statistics for Windows, Version 20. Armonk, NY: IBM.Corp) was used for our data statistical analysis. Regarding quantitative data, pattern of data distribution was determined first by Shapiro-Wilk test. We expressed normally distributed data as mean±standard deviation (SD); on the other hand, median and interquartile range (25$^{th}$ and 75$^{th}$ percentile) was the way to express non-normally distributed data. Regarding continuous variables, we used independent T test or Mann-Whitney U test as appropriate. Regarding categorical data, we presented descriptive measures in the form of frequencies and percentages. Spearman's correlation test was the one used for correlation analysis. All p-values were 2 sided and those ≤0.05 was considered significant. Finally, to evaluate the diagnostic value of LINC01538 and hs-cTnI we constructed receiver operating characteristic (ROC) curves and subsequently we could evaluate the sensitivity and specificity of each for diagnosis of MI.

## RESULTS

Table 1 presents the demographic data and characteristics of the studied groups. Age, sex, hypertension and diabetes were not statistically different among the groups, while smoking was statistically higher in MI group when compared to the control group (p=0.026). In addition, MI patients showed a significant elevation in Body Mass Index (BMI) when compared to controls (p=0.043). When we evaluated the medications taken before recruitment of the participants to the study, we found the number of participants taking beta receptor blockers (BB) and the number of those taking angiotensin converting enzyme inhibitors or angiotensin II receptor blockers were not significantly different between the 2 groups (p-value 0.72 and 0.23 respectively). However, we found the number of participants taking statins was significantly higher in MI group (p-value <0.001) (Table 1).

Table 2 presents both molecular and biochemical characteristics of the studied groups. Among studied parameters of the lipid profile panel, only TG was significantly higher in MI group in comparison to controls (p-value <0.001). Moreover, as expected there was a significant increase in serum levels of hs-cTnI and CK-MB in MI group vs. controls (p-value <0.001).

Regarding LINC01538 expression levels, significant relative over-expression of LINC01538 was found in serum of MI patients when compared to con-

**TABLE 1:** Baseline characteristics of the studied groups.

| | Control (n=48) | MI (n=50) | p-value |
|---|---|---|---|
| Age (years) | 55.8±10.1 | 54.6±9.9 | 0.550 |
| Sex (male/female), n (%) | 39 (81.2) | 40 (80) | 0.876 |
| BMI (kg/m$^2$) | 26.2±2.4 | 27.3±2.6 | 0.043 |
| Smoking, n (%) | 23 (47.9) | 35 (70) | 0.026 |
| Hypertension, n (%) | 14 (29.2) | 21 (42) | 0.185 |
| Diabetes, n (%) | 15 (31.2) | 19 (38) | 0.483 |
| Ejection fraction mean±SD | 58.1±7.4 | 33.6±14.2 | <0.001 |
| Infarct related artery in MI group | | LAD 29<br>RCA 13<br>LCX 8 | |
| Medications | | | |
| β-blockers, n (%) | 14 (29.2) | 13 (26) | 0.726 |
| ACE I or ARBs, n (%) | 7 (14.6) | 12 (24) | 0.238 |
| Statins, n (%) | 9 (18) | 32 (46) | <0.001 |

Data are expressed as mean±SD for Gaussian variables, and frequencies (percentages) for categorical variables. SD: Standard deviation; MI: Myocardial infarction; BMI: Body Mass Index; ACEI: Angiotensin converting enzyme inhibitors; LAD: Left anterior descending; RCA: Right coronary artery; LCX: Left circumflex.

**TABLE 2:** Biochemical and molecular variables among the studied groups.

| Variables | Control (n=48) | MI (n=50) | p-value |
|---|---|---|---|
| TC (mg/dL) | 200.3±27.7 | 210±33.5 | 0.106 |
| TG (mg/dL) | 67.1±29 | 138±38.8 | <0.001 |
| HDL-C (mg/dL) | 30 (25-40) | 30 (26-35) | 0.552 |
| LDL-C (mg/dL) | 118.2±22.4 | 126.6±30.6 | 0.125 |
| CK-MB (IU/l) | 13 (2.3-33.8) | 33 (24-69) | <0.001 |
| Hs-cTnI (ng/L) | 0.5 (0.2-0.9) | 55 (29-89) | <0.001 |
| LINC01538 relative expression | 0.07 (0.01-0.2) | 12 (6.6-21.8) | <0.001 |

Gaussian variables are expressed as mean±SD and non-Gaussian variables as median (inter-quartile range). SD: Standard deviation; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein-cholesterol; hs-cTnI: High sensitive cardiac troponin I.

trols [12 (6.6-21.8) vs. 0.07 (0.01-0.2), p-value <0.001] (Table 2).

Regarding correlation of LINC01538 expression levels with serum levels of CK-MB and hs-cTnI, LINC01538 expression level in MI group was positively correlated with CK-MB and hs-cTnI (r=0.39, p-value=0.006 and r=0.22, p-value=0.007, respectively). Correlation of LINC01538 with CK-MB and hs-cTnI was calculated from the serum level values of blood sample collected in first 6 hours of chest pain (41 of them were collected in first 3 hours of chest pain). Serial blood testing could show stronger correlation.

On the other hand, we found no significant correlation between LINC01538 relative expression levels and CK-MB or hs-cTnI levels in control group (p-value=0.61 and 0.25, respectively) (Table 3).

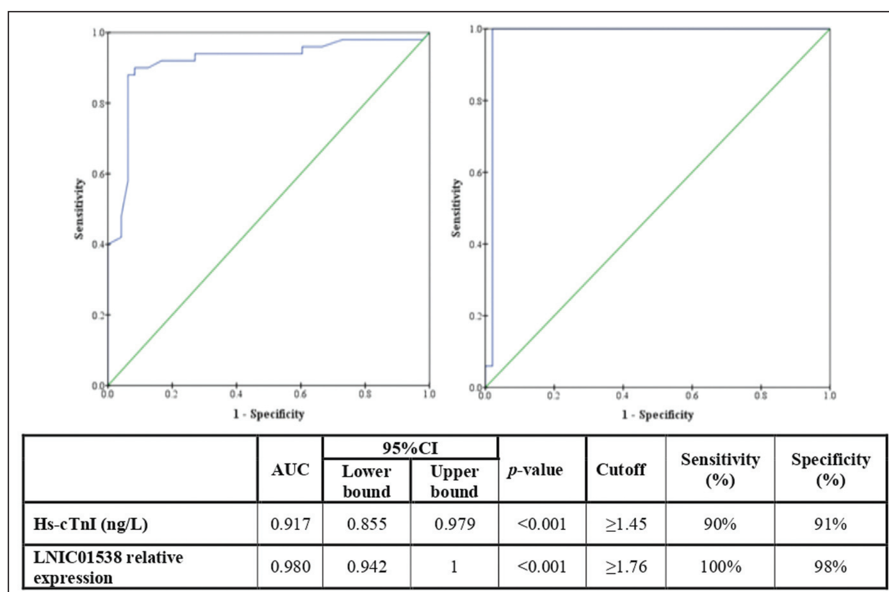
There were non-significant correlations between LINC01538 expression levels and other variables (age, BMI, TC, TG, HDL and LDL) in the studied groups (Table 3).

ROC curves were constructed for hs-cTnI and LINC01538 relative expression levels to discriminate MI patients from the control group (Figure 1). Analysis of the ROC curves showed that diagnostic value of hs-cTnI for AMI had an area under curve (AUC) of 0.917 [p-value <0.001 with 95% confidence interval (CI) 0.855-0.979] the optimal cut-off point was 1.45 ng/L at which sensitivity was 90% and specificity was 91%. However, diagnostic value of LINC01538 for AMI had an AUC of 0.980 (p-value <0.001 with 95%, CI 0.942-

**TABLE 3:** Correlation analysis of the studied parameters with LINC01538 relative expression.

| | Control (n=48) | | MI (n=50) | |
|---|---|---|---|---|
| | r | p-value | r | p-value |
| Age (years) | 0.30 | 0.06 | 0.05 | 0.752 |
| BMI (kg/m$^2$) | -0.08 | 0.56 | -0.27 | 0.06 |
| TC (mg/dL) | 0.19 | 0.17 | -0.01 | 0.935 |
| TG (mg/dL) | -0.17 | 0.25 | 0.02 | 0.905 |
| HDL-C (mg/dL) | 0.11 | 0.47 | -0.1 | 0.483 |
| LDL-C (mg/dL) | -0.10 | 0.49 | -0.02 | 0.9 |
| CK-MB (IU/L) | 0.08 | 0.61 | 0.39 | 0.006 |
| Hs-cTnI (ng/L) | 0.39 | 0.25 | 0.22 | 0.007 |

BMI: Body Mass Index; TC: Total cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein-cholesterol; CK-MB: Creatine kinase MB; hs-cTnI: High sensitive cardiac troponin I.




| | AUC | 95%CI Lower bound | 95%CI Upper bound | *p*-value | Cutoff | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|---|
| **Hs-cTnI (ng/L)** | 0.917 | 0.855 | 0.979 | <0.001 | ≥1.45 | 90% | 91% |
| **LNIC01538 relative expression** | 0.980 | 0.942 | 1 | <0.001 | ≥1.76 | 100% | 98% |

**FIGURE 1:** ROC curves of a) hs-cTn I concentration and b) LNIC01538 relative expression for discriminating MI patients.
AUC: Area under curve; CI: Confidence interval; hs-cTnI: High sensitive cardiac troponin I; MI: Myocardial infarction; ROC: Receiver operating characteristic.

1) the optimal cut-off point was 1.76 at which sensitivity was 100% and specificity was 98%.

## DISCUSSION

During the journey of discovering novel biomarkers for diagnosis of MI, the principle of studying the LncRNA as a potential would be always attractive for many reasons.[21,22] First, testing for LncRNAs needs just a venous blood sample with no special precautions in collection. Second, the detection sensitivity of the target LncRNA using real time quantitative PCR (RT-qPCR) found to be higher than other protein biomarker.[14] Third, the existence of LncRNAs highly specific myocardial tissue.[13]

The website of LncRNA disease database contains 166 diseases with 478 LncRNAs found to be associated with these diseases. Cardiovascular disease represents 10.8% of the included diseases.[23] Moreover, some of the LncRNAs in this database were found to be significantly different in MI patients when compared to healthy individuals.[24]

Many studies investigated the LncRNAs roles in MI. Gao et al. tested for the serum level expression of

LncRNA HOTAIR in 50 MI patients vs. 50 controls, they found significant decrease in serum expression of HOTAIR in MI patients when compared to control group.[25] In addition, Liu et al. found in post-MI cells down regulation of LnRNA miR-143-3p and upregulation in expression of LncRNA LINC00528.[26] Moreover, Wang et al. tested for 10 different LncRNA in 132 MI patients and 104 controls, their study found a significant increase in expression levels of 3 LncRNAs (H19, MIAT and MALAT1) in MI group when compared to healthy controls.[3] Also, Li et al. tested for quantitative expression of different LncRNAs in 46 MI patients and 40 controls, they showed a significant increase in serum expression levels of 3 LncRNAs (aHIF, KCNQ1OT1 and LIPCAR) in MI group when compared to control group.[13]

In a study done by Vausort et al., 5 LncRNAs (aHIF, ANRIL, KCNQ1OT1, MIAT, MALAT1) were tested in blood samples of MI patients, they found that expression levels of MALAT1, aHIF and KCNQ1OT1 were higher in MI patients when compared to controls ($p<0.01$). On the other hand, expression level of ANRIL were lower in MI patients vs. controls ($p=0.003$).[12]

Moreover, Kumarswamy et al. concluded that LIPCAR could be a novel biomarker of cardiac remodeling and predictor of future death in patients with heart failure after MI. (LIPCAR) was initially downregulated early after MI but upregulated during later stages with remodeling.[27]

In the present study, the AUC of ROC, as well as the value of specificity and sensitivity showed that LINC01538 is considered a useful diagnostic biomarker for AMI when compared to traditional protein biomarkers like troponin I. Although we found that LINC01538 was differentially expressed between MI and healthy control group, additional studies with larger sample size are needed to determine the consistency of LINC01538 expression and further confirm the potential applications of LINC01538 as a novel biomarker for diagnosis of MI.

## LIMITATIONS

1. Testing LINC01538 as a potential novel biomarker was done in STEMI patients, in order to have true negatives and true positives (based on definite ECG diagnosis) and answer the question of sensitivity and specificity of that test. Further studies should be done to apply this test on non-ST elevation ACS.

2. Serial measurements of LINC01538 were not done. Our main objective was to investigate the potential of LINC01538 as a novel diagnostic biomarker for MI (rule in/rule out test). Further studies should be done to evaluate the time of normalization.

3. Correlation of LINC01538 with CK-MB and hs-cTnI was calculated from the serum level values of blood sample collected in first 6 hours of chest pain. Serial testing could find stronger correlation.

## CONCLUSION

Our findings have, for the first time, demonstrated that circulating LINC01538 are highly expressed in patients with MI, functioning as potential novel biomarker for diagnosis. However, larger sample size studies and testing in non-ST elevation acute coronary syndrome are needed to confirm the potential application and determine the consistency.

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***Conflict of Interest***

*No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.*

***Authorship Contributions***

***Idea/Concept:*** *Christina Soliman, Dona Ibrahim, Sara Agwa;* ***Design:*** *Ahmed Elshazy, Shadia Fathy, Manal Emam;* ***Control/Supervision:*** *Ahmed Elshazly, Christina Soliman, Dona Ibrahim;* ***Data Collection and/or Processing:*** *Sara Agwa, Shadia Fathy, Manal Emam;* ***Analysis and/or Interpretation:*** *Dona Ibrahim, Ahmed Elshazly, Christina Soliman;* ***Literature Re-***

***view:*** *Christina Soliman, Sara Agwa, Shadia Fathy, Manal Emam, Ahmed Elshazly, Doaa Ibrahim;* ***Writing the Article:*** *Ahmed Elshazly, Dona Ibrahim, Christina Soliman;* ***Critical Review:*** *Christina Soliman, Sara Agwa, Shadia Fathy, Manal Emam, Ahmed Elshazly, Doaa Ibrahim;* ***References and Fundings:*** *Christina Soliman, Sara Agwa, Shadia Fathy, Manal Emam, Ahmed Elshazly, Doaa Ibrahim;* ***Materials:*** *Sara Agwa, Ahmed Elshazly, Dona Ibrahim.*

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