DNA INTEGRITY INDEX AS A MOLECULAR MARKER FOR PREDICTION OF TUMOR BURDEN IN BREAST CANCER

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ABSTRACT:

Background: *Globally, the incidence and death rate from of breast cancer (BC) are rising quickly; by 2040, there may be one million deaths and over 3 million new cases of BC. Various studies assessed the usefulness of DNA integrity index (DII) as marker for diagnosis and prognosis in variety of solid tumors.*

Aim of the work: *We aimed to evaluate DII's clinical usefulness as a molecular marker for BC detection, prognosis prediction and assessment of tumor burden in Egyptian BC female patients.*

Patients and methods*: This study included 150 female patients with BC, and 75 healthy controls. Measurement of cfDNA ALU 115 and 247 fragments was by real-time polymerase chain reaction and DII was calculated.*

Results: *In comparison to controls, BC patients had a highly statistically significant rise in DII (p=<0.001). A highly significant increase in DII was observed in cases with early stages compared to controls (p=<0.001). Also, it was showed that patients with metastasis had greater levels of DII than patients without metastasis (p=0.002). DII was positively correlated with tumor size (r=0.64, p= <0.001) and with stage (r=0.73, p=<0.001). ROC curve was constructed using DII to discriminate between BC cases and controls. The optimal cut off was greater than 0.51 with sensitivity 88%, specificity 92%, PPV 96%, NPV 79% and the AUC was 0.95 with 95% CI (0.90-0.99).*

Conclusions*: This study suggests the possible use of DII as promising adjuvant marker in early BC detection, and it may be used as a prognostic biomarker for detection of BC tumor progression and tumor burden.*

Keywords: *Egyptian, tumor size, Alu repeats.*

INTRODUCTION:

Breast cancer (BC) is the most frequently diagnosed cancer in females, representing 25% of all cancer cases by 2020. Its burden has been rising in many countries and in 2040, more than three million new cases and one million deaths were expected [(1)]. In Egypt, BC accounts for 35.1% of cases among females and reports stated that it carries bad prognosis with 29% mortality rate[(2)]. Many studies have found increased concentrations of cfDNA and its integrity in various types of malignancy including BC [(3,4)]. Because the advantage of cfDNA as a non-invasive biomarker, numerous samplings can be obtained to follow up dynamic changes in tumor burden [(5)]. Several trials have reported that elevated concentrations of cfDNA in patients with cancer, are correlated with high tumor progression and more aggressiveness of tumor in the form of large tumor size, high grade, advanced stages and hence reduced

overall survival rate with poor outcomes. Additional studies are needed to identify the dynamic of cfDNA along the course of the disease to predict prognosis and tumor progression in cancer cases [6]. However, it was found that cfDNA levels can be affected by presence of other illnesses as inflammation or infection and other comorbidities. So, measurement of the DNA integrity as an alternative specific approach can be used. In this regard, the Arthrobacter luteus (ALU) repeat family that are usually be found in cfDNA can be used as marker for DNA Integrity Index (DII). The ALU repeats are composed of nearly 300 bp and accounted for more than 10% of the genome, representing the most repeated sequence along the genome [7&8]. Bloodstream cfDNA is assumed to be released from necrotic or apoptotic cells. The primary source of cfDNA in a healthy individual is apoptosis, which yields short sized DNA fragments of about 180 bp. However, in cancer, tumor necrosis produces unequal longer DNA fragments that are generally >200 bp. Hence, fragmentomic analysis and obtaining idea of DNA length could predict cfDNA source. Accordingly, higher concentrations of longer necrotic circulating DNA fragments have been proposed to be a convenient parameter of malignancy [4]. Various studies used Alu-quantitative PCR that is based on using Alu115 primer to amplify short apoptotic DNA fragments and ALU247 primer to amplify long necrotic DNA fragments. They calculated DII by dividing ALU long fragments (247 bp) concentrations by ALU short fragments concentrations. ALU (115 bp)[6,9&10].

AIM OF THE WORK:

This study trial aimed to evaluate the DII as an adjuvant marker in early BC detection and to predict its potentiality as prognostic marker in assessment of tumor progression and tumor burden in Egyptian BC female patients.

PATIENTS AND METHODS:

Study participants:

Before beginning the study, each participant wrote informed consent. The Research Ethics Committee at the Faculty of Medicine at Ain Shams University in Cairo, Egypt, approved our work. with IRB approval number FMASU MS627/2022.

This study included 150 adult female patients with histopathologically confirmed BC, and 75 age-matched female healthy controls. Clinical examination, sonomamogram and relevant imaging were used for staging for all cases as guided by American Joint Committee of Cancer (AJCC) TNM staging and lymph nodal status. Also, immunohistochemistry for all patients were done for molecular subtyping. All BC stages were involved in this study. This study excluded BC patients with a history of tissue injury or autoimmune disease at the time of diagnosis, as well as those who had radiation therapy, chemotherapeutic drugs, or had other forms of malignant tumor.

Sample collection:

Two milliliters (2 mL) of venous blood were collected into ethylenediaminetetraacetic acid dipotassium salt (K2- EDTA) vacutainer tubes from all study participants for PCR. Within two to maximum four hours of collection, two steps of centrifugation were performed. The first stage centrifugation was at low speed, 1600×g for 10 minutes. The created plasma supernatant was carefully collected into a second tube and was then centrifugated at high speed, i.e., 16000×g for 10 minutes, The final obtained plasma was kept at -80 ° until processing.

CfDNA was extracted from 400 uL of plasma using the QIAamp DNA blood Mini kit (QIAGEN, Germany) as instructed in the kit handbook. Using a Nanodrop One

Spectrophotometer (Thermo Fisher Scientific Inc., USA), the absorbance at 260 nm was measured to obtain the concentration of extracted cfDNA. Absorbance value obtained at 260 was divided by absorbance value at 280 nm to create a ratio for assessment of cfDNA purity. Ratios of any value equal to 1.8 were considered acceptable.

PCR analysis of Alu fragment concentrations:

Maxima SYBR Green qPCR Master Mix (2X) (Thermo scientific, USA) , Alu 115 and Alu 247 primer sets (Invitrogen, USA) were used for qPCR amplification of Alu 115 and Alu 247 fragments.

The sequence of used primers was as follow: ALU 115 Forward primer: 5` CCTGAGGTCAGGAGTTCGAG`3, ALU 115 Reverse primer: 5`CCCGAGTAGCTGGGATTACA`3, ALU247 Forward primer: 5` GTGGCTCACGCCTGTAATC`3, ALU 247 Reverse primer: 5` CAGGCTGGAGTGCAGTGG`3.

Taqman Control Genomic Human DNA (10 ng/µL) (Applied Biosystems, Thermofisher, USA) was used for construction of calibration curve to quantify Alu 115 and Alu 247 concentrations.

Each sample was prepared in two reaction tubes. The first reaction was conducted using the Alu115 primer set, while the second reaction was conducted using the Alu 247 primer set. The final reaction volume for the qPCR was 20 µL, and it was set up as follows: 10 µL of the mentioned Master Mix, 0.6 µL each of the forward and reverse primers, 3.8 µL of nuclease-free water, and 5 µL of the cfDNA extract.

The PCR amplification was carried out using real-time PCR using the DT-Lite equipment (DNA technology, Russia) in accordance with the subsequent protocol: initial activation at 95°C for 10 minutes followed by 35 cycles of: denaturation at 95°C for 30 seconds, annealing at 64°C for 30 seconds, and extension at 72°C for 30 seconds. Following PCR amplifications, we did melt curve analysis for each PCR amplicon to verify the reaction products' specificity.

The concentration of both Alu 115 and Alu 247 amplicons was obtained using the constructed standard curve by 10-fold serially diluted Taqman Control Genomic Human DNA concentrations (10, 1, 0.1, 0.01, 0.001 ng/µL) and DII was calculated as the ratio of Alu 247 concentration to Alu 115 concentration.

Statistical methodology:

Statistical analysis was evaluated by the statistical software named GraphPad Prism version (8.0.1). Mean and standard deviation (SD) are used to represent Gaussian parametric data, while median and interquartile range (IQR) are used to represent skewed non-parametric data. Data that are categorical are shown as percentages and numbers. The Mann Whitney-U test was utilised for group comparison. The correlation coefficient between numerical variables is measured using Spearman's rank (rs). The diagnostic performance of the DII marker was evaluated using the receiver operating characteristic (ROC) curve. A comparison is considered significant if the p value is less than 0.05, and highly significant if it is less than 0.001.

Ethical consideration:

The approval of the study was taken from the Institutional Ethics Committee of the Faculty of Medicine, Ain Shams University (FWA 000017585), (Ethical Committee's reference number: FMASU MS627/2022.). Written informed consent was taken from all patients who were invited to participate in the research.

RESULTS:

The mean age for BC cases was 33.3±10 years. As for healthy female controls, the

mean age was age 31.6±7.8 years. Among BC patients' group, there was 14% with positive family history of BC and 86% with negative family history. While all healthy controls were having negative family history of BC. Clinical and tumor characteristics of the patient group were described in Table (1).

Table 1: Descriptive Data of tumor characteristics

Parameter	BC Patients (n=150)
Tumor stage	
I (%)	15/150 (10%)
II (%)	60 /150(40%)
III (%)	45 /150(30%)
IV (%)	30 /150(20%)
Tumor site	
Rt (%)	78/150 (52%)
Lt (%)	72 /150(48%)
Tumor size (cm)	
(Mean ± SD)	4 (±2)
BIRAD sonography	
BIRAD 4 (%)	45/150 (30%)
BIRAD 5(%)	93/150 (62%)
BIRAD 6 (%)	12/150 (8%)
Lymph node metastasis	
Absent (%)	54/150 (36%)
Present (%)	96/150 (64%)
Histopathological examination	
Ductal Invasive Carcinoma (%)	147/150 (98%)
Invasive pleomorphic lobular carcinoma (%)	3 /150 (2%)
Tumor grade	
2 (%)	114/150 (76%)
3 (%)	36 /150(24%)
Estrogen Receptors (ER)	
Negative (%)	30/150 (20%)
Positive (%)	120/150 (80%)
Progesterone Receptors (PR)	
Negative (%)	42/150 (28%)
Positive (%)	108/150 (72%)
Human epidermal growth factor receptor 2 (HER-2)	
Negative (%)	102/150 (68%)
Positive (%)	48/150 (32%)
Ki67	
Low (%)	75/150 (50%)
High (%)	75/150 (50%)
Distant metastasis	
Absent (%)	114/150 (78%)
Present (%)	36/150 (22%)

BC: breast cancer.

DII and Alu 247 were higher in BC cases than healthy controls.

When comparing BC patients to controls, there was a statistically significant rise in both DII and Alu247 concentrations (p=0.001). As for Alu 115, however, there was no discernible difference between the two groups (p=0.339). Table (2) and diagram (1)

Table 2: Comparison between controls and BC patients regarding ALU115, 247 and DII:

Parameter	Healthy controls (N= 75)	BC patients (N=150)	Mann-Whitney Test		
	Median (IQR)	Median (IQR)	U Value	p-Value	Sig.
Alu 115 (pg/µl)	24.11 (12.8 - 36.79)	31.96 (7.82 - 98.6)	540	0.339	NS
Alu 247 (pg/µl)	5.51 (2.55 - 11.15)	32.15 (5.51 - 105.27)	319	<0.001	HS
DII	0.35 (0.15 - 0.42)	0.91 (0.71 - 1.10)	65.4	<0.001	HS

DII: DNA integrity index, Alu: Arthrobacter luteus, BC: breast cancer, IQR: Interquartile range, p <0.05 significant, p<0.001 highly significant

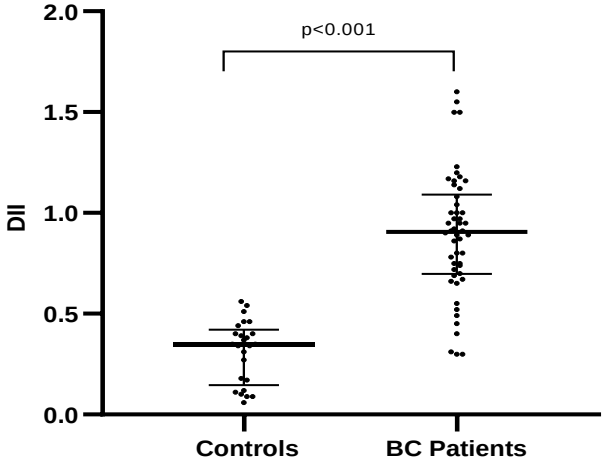


Diagram 1: Scatter dot plot for comparative statistics between controls and cases regarding DII

DII and Alu 247 were increased in BC cases with early stages.

The efficacy of three indicators as a tool for early diagnosis was evaluated by comparing the amounts of Alu115, Alu247, and DII in patients with early stages to a control group. In patients with early stages, we identified a statistically significant rise in Alu247 and DII levels when compared to controls (p=<0.001). In the meantime, there was no discernible difference with regard to Alu115 (p=0.497). Table (3) and diagram (2).

Table 3: Statistical comparative study between controls and early stages BC patients regarding ALU115, 247 and DII

Parameter	Healthy controls (N= 75)	Early stages patients (N=75)	Mann-whiteny test		
	Median (IQR)	Median (IQR)	U value	P value	Sig.
Alu 115 (pg/µl)	24.11 (12.8 - 36.79)	31.96 (9 - 121.79)	277.5	0.497	NS
Alu 247 (pg/µl)	5.51 (2.55 - 11.15)	32.15 (5.35 – 108.9)	173.5	<0.001	HS
DII	0.35 (0.15 - 0.42)	0.74 (0.50 – 0.91)	65.5	<0.001	HS

DII: DNA integrity index, Alu: Arthrobacter luteus, IQR: Interquartile range, p <0.05 significant, p<0.001 highly significant.

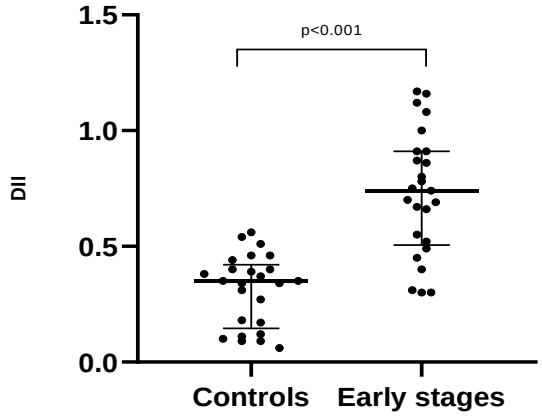


Diagram 2: Scatter dot plot for comparison between controls and early stages regarding DII.

Assessment of Alu115, 247 and DII as markers for prognosis

We did comparison between the levels of three studied markers in early and late stages. Alu115 and Alu247 did not show a significant difference (p=0.977 and p=0.722, respectively), but DII was the only one that was significantly greater in the late stages compared to the early stages (p=<0.001). Table (4) and diagram (3).

Table 4: Statistical comparative study early stages BC cases and late stages BC cases regarding ALU115, 247 and DII

Parameter	Early stage (N= 75)	Late stage (N= 75)	Mann-whiteny test		
	Median (IQR)	Median (IQR)	U value	P value	Sig.
Alu 115 (pg/µl)	31.96 (7.82 - 85.65)	31.96 (9 - 121.79)	311	0.977	NS
Alu 247 (pg/µl)	25.67 (5.87 - 86.84)	33.18 (4.84 - 105.27)	294.5	0.727	NS
DII	0.74 (0.51 – 0.91)	0.97 (0.89 - 1.2)	118.5	<0.001	HS

DII: DNA integrity index, Alu: Arthrobacter luteus, IQR: Interquartile range, p <0.05 significant, p<0.001 highly significan**.**

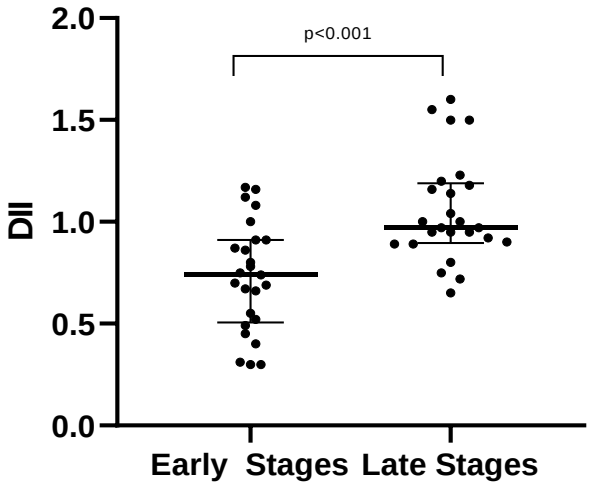


Diagram 3: Scatter dot plot for comparison between early and late stages regarding DII.

Additionally, we examined the parameters in patients who were positive for LN vs negative for LN. DII levels were significantly greater in LN positive cases than in LN negative cases (p=0.03). Alu115 and Alu247, however, did not reveal any significant variations between the two groups (p=0.73 and p=0.61, respectively). Table (5) and diagram (4).

Table 5: Comparison between LN negative BC patients and LN positive patients regarding ALU115, 247 and DII

Parameter	LN negative (N= 54)	LN positive (N=96)	Mann-whiteny test		
	Median (IQR)	Median (IQR)	U value	P value	Sig.
Alu 115 (pg/µl)	24.65 (8.57 – 86.62)	38.7 (6.7 – 116)	270.5	0.73	NS
Alu 247 (pg/µl)	24.98 (5.4 – 99.1)	34.28 (5.0 – 116)	262.5	0.61	NS
DII	0.72 (0.48 – 1.09)	0.95 (0.80 – 1.11)	179	0.03	S

DII: DNA integrity index, Alu: Arthrobacter luteus, LN: lymph node, IQR: Interquartile range, p <0.05 significant, p<0.001 highly significant.

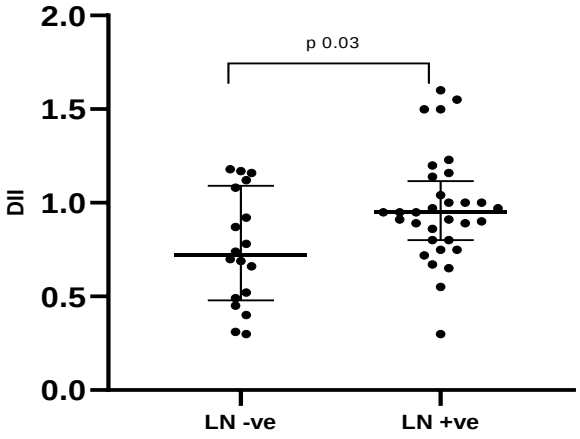


Diagram 4: Scatter dot plot for comparison between LN +ve and LN -ve patients regarding DII.

Furthermore, only DII was discovered to be significantly greater in metastatic cases than in non-metastatic ones (p=0.002), and there was no significant difference between the two groups with regard to Alu115 and Alu247 (p=0.351 and p=0.325, respectively). Table (6) and diagram (5).

Table 6: Comparison between non metastatic BC patients and metastatic BC patients regarding ALU115, 247 and DII

Parameter	Non-Metastatic (N= 36)	Metastatic (N= 114)	Mann-whiteny test		
	Median (IQR)	Median (IQR)	U value	P value	Sig.
Alu 115 (pg/µl)	19.89 (5.12 - 82.02)	38.7 (9.65 - 121.79)	187	0.351	NS
Alu 247 (pg/µl)	19.01 (3.9 – 89.)	33.25 (6.47 - 119.67)	184	0.325	NS
DII	0.8 (0.66 - 1.00)	1.02 (0.91 - 1.43)	93.5	0.002	S

DII: DNA integrity index, Alu: Arthrobacter luteus, IQR: Interquartile range, p <0.05 significant, p<0.001 highly significant.

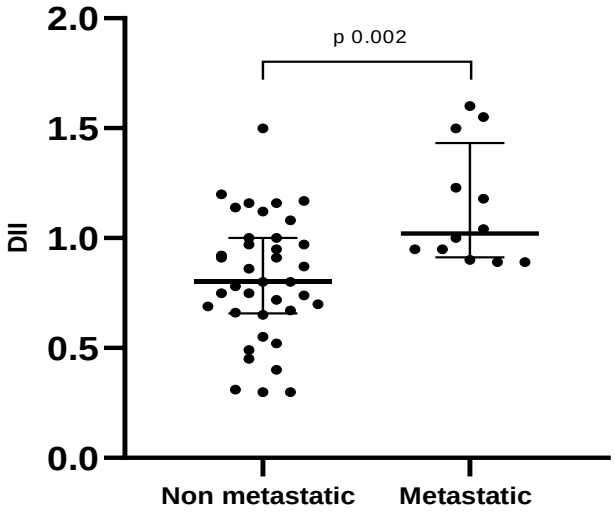


Diagram 5: Scatter dot plot for comparison between non metastatic and metastatic regarding DII

DII correlation with tumor size and tumor stage.

A highly significant positive correlation was observed between DII and tumor size (r=0.64, p= <0.001) and with tumor stage (r=0.73, p=<0.001). Diagrams (6 & 7), respectively.

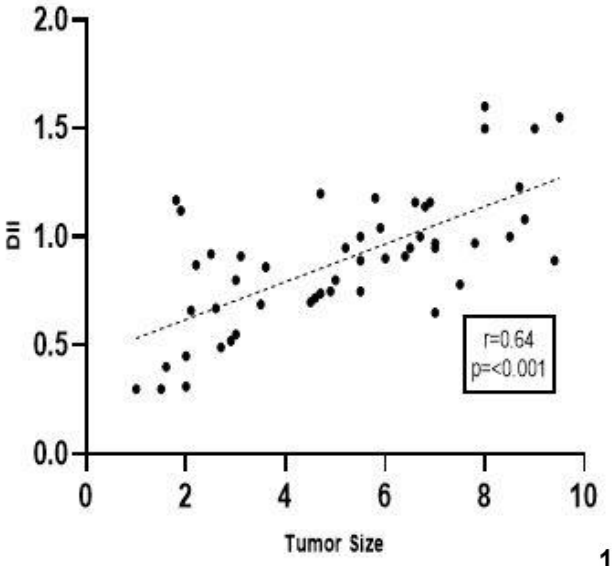


Diagram 6: Correlation between DII and tumor size

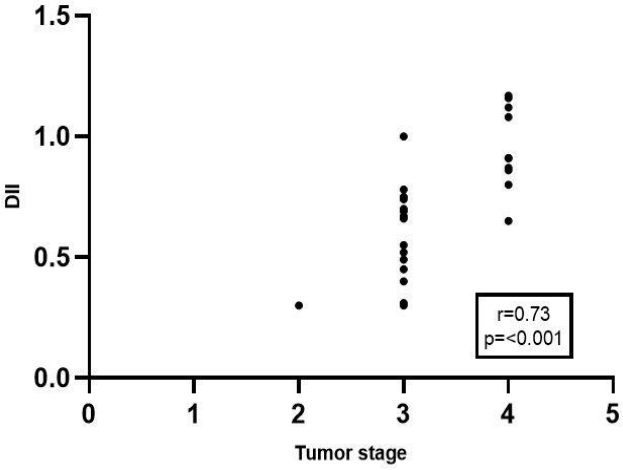
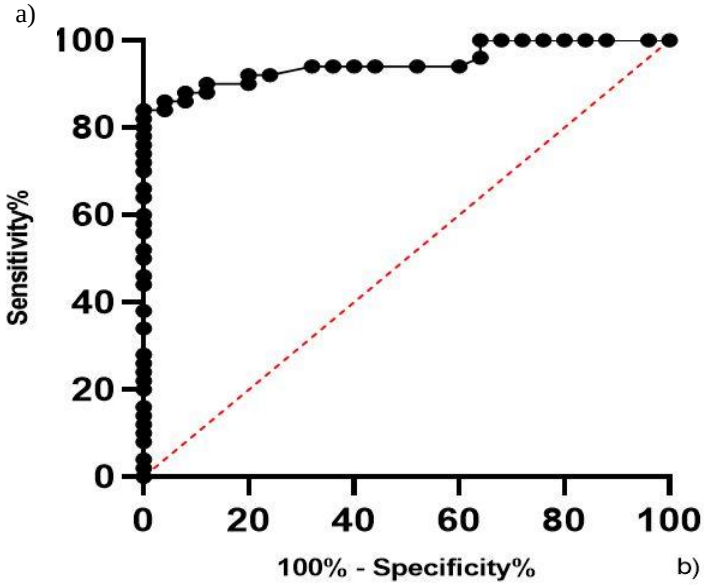


Diagram 7: Correlation between DII and tumour stage

a)



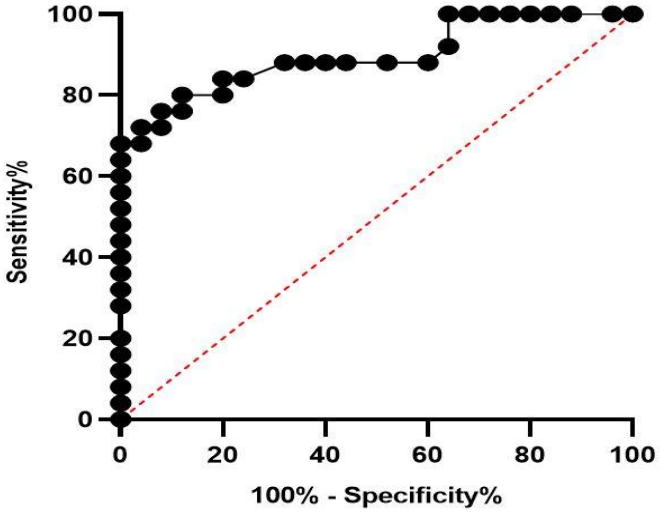
Diagnostic performance of DII as regard discrimination between BC patients and controls.

Using DII, a ROC curve was created to distinguish between BC patients and controls. The optimal cut off was > 0.51, with sensitivity of 88%, specificity of 92%, positive predictive value (PPV) of 96%, negative predictive value (NPV) of 79%, and area under the curve (AUC) of 0.95 with a 95% confidence interval (CI) (0.90-0.99). Diagram (8a).

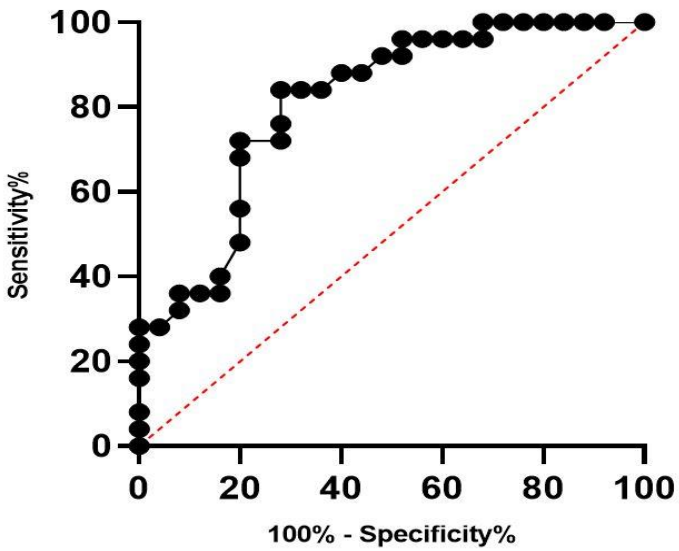
Diagnostic Performance of DII as an early marker for BC detection.

More than 0.44 was the optimal cut-off point for the DII to differentiate between the early phases and controls, with 84% sensitivity, 80% specificity, 81% PPV, 86% NPV, and an AUC of 0.90 with a 95% confidence interval. (0.80-0.98). Diagram (8b)

b)



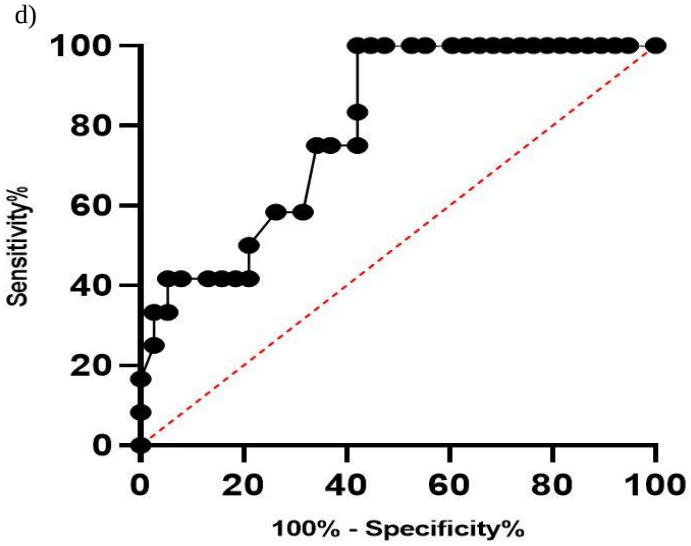
c)



Diagnostic performance of DII to discriminate between early staged and late staged patients.

To distinguish between early and late stages, another ROC was produced using DII; the optimal cut off was >0.88, with diagnostic sensitivity of 84%, specificity of 72%, PPV of 100%, NPV of 56%, and AUC of 0.81 with 95% CI (0.70-0.93). Diagram (8c)

d)



The best DII performance to differentiate between groups that are metastatic and those who are not.

To distinguish between the metastatic and non-metastatic groups, DII was also employed in another ROC curve. The optimal cut off was >0.93, with diagnostic sensitivity of 75%, specificity of 66%, PPV of 41%, NPV of 88%, and AUC of 0.79 with a 95% confidence interval (0.66-0.92). Diagram (8d)

Diagram 8: ROC curve for determination of best cut off to discriminate between BC patients and healthy controls (a), BC patients with early stages and controls (b), and early stages BC patients and late stages BC patients (c), Metastatic BC and non-metastatic patients (d).

DISCUSSION:

Because cfDNA is non-invasive, simple to sample, and may be tumour specific, much cancer research has concentrated on analysing cfDNA and its integrity as a promising biomarker in early tumour diagnosis, prognosis, and assessment of tumour burden[11&12].

Research has demonstrated that necrosis and/or apoptosis may be the source of cfDNA found in blood. Necrosis, the primary mechanism of cell death in malignancy, produces longer DNA fragments >250 bp, whereas apoptosis, the major cause of death in normal cells, releases short DNA fragments 180-200 bp or less. Consequently, the ratio of long to short DNA fragments can be used to identify DII and may be used in the future as a potential malignancy predictor[13&14].

Most of genes used for measurement of cfDNA and its integrity in various BC studies are related to repetitive DNA elements e.g., Alu and LINE, but other housekeeping genes as B- actin and GADPH are also used. The levels of DII in these studies are controversial and both increase and decrease in DII were reported[15].

In this study, we targeted Alu repeats for measurement of levels of DII in Egyptian BC patients, and we aimed to evaluate its clinical utility as adjuvant marker for BC diagnosis, prognosis, monitoring of tumour progression and tumour burden.

The DII was assessed in 150 BC patients and 75 healthy controls using two primer sets - Alu 115 and Alu 247 - to amplify short and long DNA segments of Alu repeats by real-time PCR. Because the annealing region of Alu 115 is inside the annealing region of Alu 247 fragment, the Alu short fragment i.e., 115 bp represents the total DNA concentration and the Alu long fragments i.e., 247 represents long necrotic DNA[9].

When BC patients were compared to the control group, we observed that their DII levels were noticeably higher. Furthermore, it was discovered that DII was both significantly greater in patients with late stages as compared to early stages and in BC patients with early stages as compared to the control group.

As regard the role of DII as prognostic marker for assessment of tumor progression and tumor burden. Patients who had lymph node metastasis had significantly greater DII levels than those who did not. Additionally, it was significantly greater in BC patients with metastasis than in those without. Moreover, DII levels positively correlated with the tumor's size and stage as well.

In line with our research, Umetani and colleagues measured DII in BC patients using Alu qPCR. They discovered that BC patients had much higher DII levels than controls, and that these levels increased as the patients progressed from stage II to stage IV. Moreover, tumor size and the presence of LNs were positively correlated with DII in the same study[16].

Another study by *Iqbal et al.,*[17] identified significantly elevated DII in BC patients compared with controls as well as positive correlation of DII with tumor stage. In the same study, they observed significant decrease in the levels of DII after tumor surgery. The previous findings were further confirmed by *Hussein et al,*[18]and by *Elhelaly et al.,*[19]. Furthermore, Lamminhao and his coworkers in 2021 highlighted that high DII level has a prognostic significance and elevated level of DII was associated with poor BC outcomes[20].

In 2023, a study conducted in India by *Nair et al*.[8] also showed that BC patients with metastasis had significantly higher DII levels than post-operative patients. *Nair et al*.,[8]and Hussein et al.,[18]found positive correlation of DII with HER 2 positivity, and this may be owing to rapid tumour proliferation associated with HER2 positive subtypes. In that same year, research conducted in Egypt with 80 patients with BC and 40 healthy individuals by *Zaki et al.,*[21]showed that BC patients had higher levels of DII than controls. Furthermore, in agreement with our research, they showed a positive correlation between DII and both tumor size and TNM staging.

Contrary to what we found, Madhaven et al.[13] used both ALU and LINE1 markers to compare the plasma levels of DII in 82 BC cases and 201 metastatic BC cases with 100 healthy controls. and they reported that DII was lower in BC cases than healthy group and lower. DII was associated with poor survival and outcomes.

Cheng et al., [22]also reported that reduced level of DII can be used as prognostic biomarker in metastatic BC. Prior to chemotherapy, they found lower baseline levels of DII in metastatic BC patients, and following one cycle of chemotherapy, they found significantly higher levels of DII in BC patients. Also, the increased DII levels were correlated with a better overall survival. Cheng and his colleagues described the low levels of DII observed in cancer patient may be caused by differences in the degree of DNA fragmentation that occurs during apoptosis between malignant and non-malignant cells., being higher fragmented in apoptosized malignant cell i.e., shorter than those produced by apoptosized healthy cells [22].

The discrepancy and the opposite results among studies may be related to biological, technical, and statistical issues First, the length of cfDNA released from tumor is a matter of debate; some research postulate that tumors release long DNA fragments, while others make the opposite assumption [23]. Therefore, the precise DII levels in malignancy are still under discussion. Second, the different gene markers used to assess the cfDNA short and long fragments, some studies targeted the repetitive DNA elements e.g., Alu and LINE, however, others used housekeeping genes like B-actin, GADPH and TERT as their target. Third, different sample types, some trials used plasma and other used serum. Additionally, non-standardized workflow starting from preanalytical steps, processing of the sample, storage conditions to the selection of extraction kits that may differ in

efficiency[24]. Fourth, as regard quantitative analysis by real time PCR, some studies used absolute quantitation methods and other used relative comparative delta CT method [15]. Moreover, different PCR efficiencies between short and long fragments amplification, different cycling conditions and different primer designs for the same targets [13]. Fifth, different sample sizes, different population, different tumor staging, grading and molecular subtypes of BC patients in between studies.

Conclusion:

Our study highlighted the potential use of DII as promising biomarker in BC. DII could be a used as an adjuvant marker for early BC detection. It also can be a suitable prognostic biomarker for detection of BC tumour progression and tumour burden as it was correlated with tumour stage and tumour size. Further studies are recommended on the exact nature of cfDNA fragment sizes during malignancy to decide which DNA fragment is malignant, the long or the short ones. Also, standardization of the cfDNA preanalytical and analytical processes are needed for accurate calculation of DII levels during malignancy.

Declarations:

Availability of data and materials

The data within this paper and other finding of this study are available from the corresponding author upon reasonable request.

Competing interests:

The authors declared no potential conflicts of interest with respect to the research, authorship, or publication of this article.

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Authors' contributions

Sayeda Abd El-Rahiem Saleh and, Wessam Elsayed Saad: designed and approved the whole research protocol and amended the final paper version to be published.

Heba Hassan Aly contributed to the protocol design, revised laboratory work, and revised the manuscript draft version to be published.

Mariam Mohamed Hussein: supervised sample collection according to inclusion criteria, revised clinical data, diagnosis, and patient classification.

Rasha Ahmed Ghorab: monitored data collection process and supervised on the laboratory work, interpreted the data, and drafted manuscript.

Rana Amr Abd-Elmotalib Mansy: collected the samples and patient's clinical data, carried out the laboratory work and statistical analysis of the results, and participated in the manuscript draft.

All authors have read and approved the manuscript.

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مؤشر سلامة الحمض النووي كعلامة للتنبؤ بعبء الورم في سرطان الثدي

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الخلفية: على الصعيد العالمي، ترتفع سريعا معدلات الإصابة والوفيات الناجمة عن سرطان الثدي ؛ و بحلول عام 2040، قد يكون هناك مليون حالة وفاة وأكثر من 3 ملايين حالة جديدة من مرض سرطان الثدي. قامت دراسات مختلفة بتقييم فائدة مؤشر سلامة الحمض النووي كعلامة للتشخيص والتنبؤ بتطور المرض في مجموعة متنوعة من الأورام الصلبة

الهدف: نحن نهدف إلى تقييم الفائدة السريرية لسلامة الحمض النووي كعلامة جزيئية للكشف عن سرطان الثدي والتنبؤ بالتشخيص وتقييم عبء الورم لدى المريضات المصريات.

الطريقة: شملت هذه الدراسة 150 مريضة مصابة ببسرطان الثدي، و75 من الأصحاء. تم قياس أجزاء الحمض النووي الحر بواسطة التفاعل البلمري التسلسلي و حساب سلامة الحمض النووي

النتيجة: بالمقارنة مع الضوابط، كان لدى مرضى سرطان الثدي ارتفاع لمعدل سلامة الحمض النووي بدلالة إحصائية عالية. وقد لوحظت زيادة كبيرة للغاية في سلامة الحمض النووي في الحالات ذات المراحل المبكرة مقارنة بالضوابط .كما تبين أن المرضى الذين يعانون من ثانويات الورم لديهم مستويات أعلى من سلامة الحمض النووي من المرضى الذين لا يعانون من ثانويات الورم كما يوجد ارتباط ايجابي بين سلامة الحمض النووي مع حجم الورم و تطور مراحل الورم

الخلاصة: تقترح هذه الدراسة إمكانية استخدام سلامة الحمض النووي كعلامة مساعدة واعدة في الكشف المبكر عن سرطان الثدي، ويمكن استخدامه كمؤشر حيوي إنذاري للكشف عن تطور الورم والتنبؤ بعبء الورم.