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Department of Medicine, Udayana University,
Bali, Indonesia

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High 8-OHdG compounds concentration and lymphovascular invasion are risk factors for increases invasive breast cancer behavior

Ni Wayan Tianing*

ABSTRACT

Free radicals produced by oxidative stress has long been associated as one of the triggering factor of DNA mutation that lead to oncogenesis, especially in breast cancer. Serum level of 8-hydroxy 2-deoxyguanosine (8-OHdG) is one of the most studied oxidative stress biomarker in nuclear and mithocondrial DNA damage. The objective of this study was to analyse the risk of high serum level of 8-OHdG in invasive behaviour of breast cancer based on lymphovascular invasion (LVI). This was a case-control study involving 66 breast cancer patients devided into; 33 patients with positive LVI (LVI+) as case group and 33 patients with negative LVI (LVI-) as control group. This study was conducted in Laboratory of Biochemistry, Faculty of Medicine, Udayana University and Sanglah General Hospital Denpasar, Bali. Serum level of 8-OHdG was measured by ELISA. The Results of this research: Increase of 8-OHdG concentration were associated with invasive characteristics in breast cancer patients (565.326 ± 282.655 ng/mL in case group compared with 326.336 ± 144.53 ng/mL in control group; p<0,05). Risk analysis has shown higher level of 8-OHdG were associated with increase risk of invasive behaviour of the cancer it self (OR: 13.913; 95% CI: 1.163 – 116.412). ROC curve analysis showed Area Under the Curve (AUC) was 0.764 which indicates moderate predictive value. This result emphasized the role of oxidative stress in DNA damage and oncogenesis. Conclusion: High concentration of 8-OHdG compounds and positively lymphovascular invasion was associated increases of invasive breast cancer behavior

Keywords: 8-hydroxy 2-deoxyguanosine, lymphovascular, invasion, breast cancer
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ABSTRAK

Parameter oksidatif seperti radikal bebas yang dihasilkan dari stress oksidatif telah lama dikaitkan dengan karsinogenesis terutama sebagai salah satu yang memicu mutasi atau sebagai induktor mutasi DNA dan mendorong onkogenesis. Hal ini sangat berhubungan dan berperan penting dalam proliferasi sel, peradangan, survival dan metastasis kanker khususnya kanker payudara. Tujuan penelitian adalah menganalisis hubungan parameter oksidatif darah (8-OHdG) dan *lipovascularinvassion* dengan tingkat invasive kanker payudara. Studi kasus kontrol digunakan pada penelitian ini dengan menggunakan 66 subjek kanker payudara. 33 subjek sebagai kasus dan 33 subyek sebagai kontrol. Penelitian dilakukan di Laboratorium Biokimia Fakultas Kedokteran Universitas Udayana dan Rumah Sakit Umum Sanglah Denpasar Bali. Sampel dengan limpovaskularinvassion positif (LVI) diklasifikasikan ke dalam kelompok kasus dan sebagai kontrol digunakan sampel dengan *lipovascularinvassion* negative (LVI). Kadar senyawa 8-OHdG diukur dari serum penderita menggunakan metode ELISA. Peningkatan kadar serum 8-OHdG dikaitkan dengan karakteristik invasive kanker payudara (326,336 ± 144,53 ng/mL pada kelompok kontrol vs 565,326 ± 282,655 ng/mL pada kelompok kasus; p <0,05). Analisis risiko menunjukkan bahwa kadar 8-OHdG yang tinggi dan dengan LVI positif dikaitkan dengan peningkatan risiko perilaku kanker yang lebih invasif (OR: 13,913; 95% IK: 1,163 - 116,412; p <0,05). Kurva ROC menunjukkan bahwa *Area Under the Curve AUC* sebesar 0,764 yang menunjukkan nilai prediktif moderat. konsentrasi 8-OHdG dan LVI positif berhubungan dengan meningkatnya prilaku invasive kanker payudara.

Kata Kunci: 8-hidroksi 2-deoxyguanosine, lipovascular invassion, kanker payudara, invasive
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Biochemistry Department, Faculty of Medicine, Udayana University

*Corresponding to: Ni Wayan Tianing,Biochemistry Department, Faculty of Medicine, Udayana University
wtianing@yahoo.com

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INTRODUCTION

Oxidative stress has long been linked as one mutation inductor that leads to carcinogenesis by mutating the oncogene gene or suppressor tumor gene. The causes of oxidative stress can come from outside and inside the body. Oxidative stress from the body comes from the process of energy synthesis in mitochondria, especially in the electron transfer process.[1,2] Electron transfer processes

that can produce free radicals such as oxidative uncouple phosphorylation or molecular processes in peroxisomes, but they are rapidly neutralized by the cell's antioxidant enzymes. But after carcinogenesis occurs, cancer cells tend to produce high amounts of free radicals resulting from an increase in the uncouple electron transport rate in the inner mitochondrial membrane. This increase in uncouple electron transport rates is predicted to produce more free radicals and will cause hyper mutations and genome instability in cancer cells.[2,3] The unstable genome in these cells underlies therapeutic changes in cancer characteristics. Another effect of high oxidative levels is not yet known in cancer. Recent cancer research has shown that increasing numbers of free radicals also have other functions for cancer cells, namely inducing the transformation of normal fibroblasts into cancer-related fibroblasts and contributing to regulating mitochondrial biogenesis through the Cancer Anti-fibroblast (CAF) mechanism. This CAF is forced to convert the metabolic profile into predominantly glycolytic and produce a stable supply of lactate which is used by cancer cells to produce energy or as the backbone of the biosynthetic process. In addition, free radicals are involved in many aspects of cancer development including remodeling of extracellular matrix and tumor-related degradation, inflammation, and mitogenic signal activation between MAPK, ERK-1, and JNK intermediaries.[1,4,5] While most mechanisms linking free radicals or oxidative stress with cancer development have been established, most studies in the field of cancer are in vitro.[3,6,7] Therefore, this study was conducted with the aim to investigate the relationship of oxidative damage to breast cancer with its invasive characteristics to better ascertain previous in vitro findings.

METHODS

An analytic cross-sectional study was conducted in Faculty of Medicine Udayana University and Sanglah General Hospital from January to December 2016. The sample were obtained from Surgical Oncology Clinic in Sanglah General Hospital and then examined in Biochemistry Laboratory in Faculty of Medicine Udayana University. The inclusion criteria in this study were newly diagnosed invasive breast cancer with clinical grade II and III regardless of the type. The exclusion criteria were phyllodes tumor, pathologically benign results, patient who has history of chemotherapy, secondary breast cancer, and disagree to participate. All subject then grouped into control and case group with those with invasive characteristics such as lymphovascular invasion (LVI). This was a case-control study involving 66 breast cancer patients devided into; 33 patients with positive LVI (LVI$^+$) as case group and 33 patients with negative LVI (LVI$^-$) as control group. This study was conducted in Laboratory of Biochemistry, Faculty of Medicine, Udayana University and Sanglah General Hospital Denpasar, Bali. Samples was obtained from every subject and clinicpathological data were collected from medical record. Concentration of 8-OHdG was examined by Enzyme Link Immunosorbent Assay (ELISA) technique using 8-hydroxy 2 deoxyguanosine ELISA Kit following the manufacturer instruction. All of the data obtained were analyzed descriptively to obtain the proportion of each variable in each group and the mean of 8-OHdG. Then, analytical study were conducted using independent sample T-test to evaluate the difference concentration of 8-OHdG between case and control group. Risk assessment was conducted using chi-square test by classifying the 8-OHdG concentration into high and low. this research has obtained ethical clearance with no:161/UN.14.2/Litbang/2014.

RESULT

66 subjects were participated in this study with mean age of 41.26±9.097 years. The mean age in control group was found to be younger than case group (34.73±5.80 year vs. 47.79±6.818 year). The mean concentration of 8-OHdG was found at 326.336±144.53 ng/mL in control group which was lower than case group at 565.326±282.655 ng/mL. Meanwhile, the proportion of clinical stadium (II and III) was found to be roughly comparable. At show to table 1.

Prior to analysis, the normality of the 8-OHdG concentration was examined using Kolmogorov-smirnov test. It was found that the 8-OHdG data were not normally distributed. According to normality test result, the 8-OHdG concentration between case and control group were examined using non-parametric Mann-Whitney test. The result showed that the 8-OHdG concentration between case and control group was significantly different with case group had significantly higher concentration. (*The data was analyzed using Mann-Whitney Test according to the result of the normality test).

Then, we divide the 8-OHdG concentration using 223ng/mL as cut-off point according to ROC curve analysis. Subsequently, risk analysis was conducted to determine the OR of 8-OHdG concentration toward invasive behavior of breast cancer. According to risk analysis, it was found that the increased 8-OHdG concentration was significantly elevated the risk of invasiveness of breast

Table 1 The Baseline Characteristics of The Subjects

Variables	Mean
Age	Overall : 41.26±9.097 Control : 34.73±5.80 Case : 47.79±6.818
8-OHdG	Overall : 474.129±253.27 ng/mL Control : 326.336±144.53 ng/mL Case : 565.326±282.655 ng/mL
Stadium	II : 36 (54.5%) III : 30 (45.5%)

Table 2 Normality and Bivariate Analysis Of 8-OHdG Concentration Between Case and Control Group

Normality Test	Statistic	P-value
Kolmogorov-Smirnov	0.182 (df: 66)	0.000
Group	**Serum 8-OHdG Concentration**	**P-value***
Case	326.336±144.53 ng/mL	0.000
Control	565.326±282.655 ng/mL	

Table 3 Risk Analysis of 8-OHdG Level Toward Cancer Behavior

		Group		
		Control	Case	Statistical Analysis
8-OHdG Concentration	Low	10	1	P < 0.003
	High	23	32	OR: 13.913; 95% CI: 1.163¬116.412
Total		33	33	

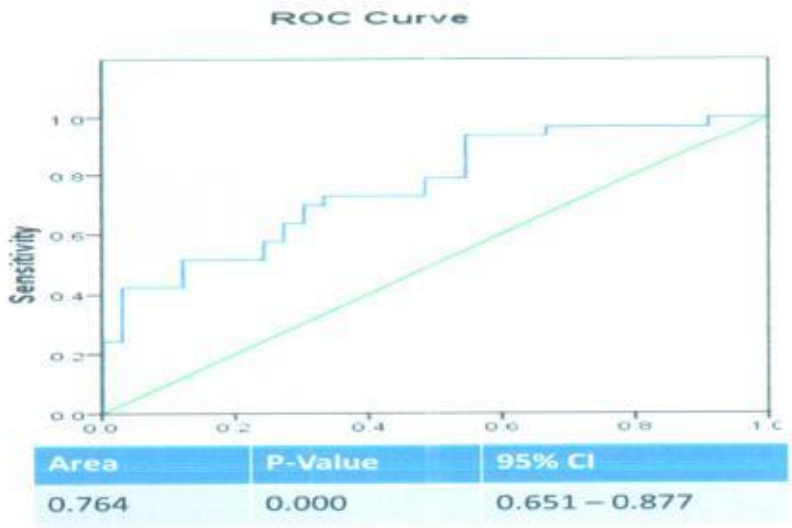

Figure 1 ROC Curve Of Plasma 8-OHdG Level to Predict The Invasiveness of Breast Cancer Reveal A Moderate Strength Prediction Value

cancer (OR: 13.91; 95% CI: 1.663-116.412). ROC curve analysis also reveals that 8-OHdG concentration had a moderate prediction value for cancer invasiveness with Area Under the Curve at 0.764 (P-value: 0.000; 95% CI: 0.651-0.877)

DISCUSSION

The advancements in cancer diagnostic and management, breast cancer still placed as the second most lethal cancer in women after cervical cancer. Traditional classification system that based only on clinical observation and pathologic examination proved insufficient to accurately determine the prognosis and behavior of breast cancer. Determination of whether the cancer is actually more or less invasive is particularly challenging.[8,9] For example, many early stage breast cancer were proved to be more invasive even when compare to the advanced stage counterpart. The triple negative breast cancer is even more challenging since this type has the worst prognosis than others as well as having the highest molecular diversity. However, recent findings in tumor biology could be the key to develop and perfect current method of prognostic determination. Free radicals have been long associated with carcinogenesis particularly as the one that triggers the mutation which drives the oncogenesis.[10,11] However, recent findings showed that free radicals had many important roles other than triggering oncogenic mutations. It begins with study that reveal cancer cells were produced high number of free radicals mainly as the result of their high metabolic rate. High metabolic rate means higher rate of electron transport in inner mitochondrial membrane and, thus, higher chance of uncouple electron transports which result in free radical formation. Then, with the advance in the study of cancer microenvironment, it was found that cancer cells were actually produced free radicals on purpose to modify the surrounding connective tissue. Connective tissue has anti-cancer properties in nature but continuous exposure to free radicals trigger the differentiation of fibroblast into cancer associated fibroblast (CAF) which is pro-carcinogenic.[1,4,5,12] CAF produce many pro-carcinogenic cytokines and support cancer cell both metabolically and immunologically. From metabolism perspective, CAF plays major part in reverse Warburg effect by increasing its glycolysis and lactate fermentation rate. It ensures the steady supply of lactate to the cancer cells for anabolic purpose. Immunologically, it participates in immune-evasion of cancer cell by suppressing and modifying nearby macrophage and lymphocyte driving their differentiation into M2 macrophage or cancer associated macrophage (CAM) and T-regulator cells or trigger the effector T-cell energy. On the other hand, free radicals also act directly on cancer cells, activating many pro-survival, mitotic, and metastatic pathways.[13,14]

ROS has been known to activate mithogenic signaling pathway mediated growth factor receptor tyrosine kinase (R) and Rac. These signaling eventually activate c-jun and c-fos which are the subunit of AP-1 nuclear transcription factor and induce the expression of mitotic related genes. ROS also known to activates autophagy in cancer cell via mTOR activation.[1,4,5] Autophagy is an essential process that recycles the macromolecules in cancer cell and enhance cell repair, the process that plays essential role in enhancing cell survivability. In addition, increased amount of ROS could induce inflammatory process in tumor micro-environment. In contrast from previous believe that inflammation would has a detrimental effect on cancer cells, current study reveals that inflammation in tumor microenvironment has many beneficial effects for cancer cells including immune evasion, proliferation and metastasis. Immune evasion is mediated by M2 macrophage and suppressor T cell while proliferation is mediated by increased production of growth factor including VEGF and IGF by M2 macrophage.[1,4,5] Increased expression of MMP-9 either by inflammatory stimuli or secondary from increased SOD2 activation mediate the metastatic process of cancer cell by degrading and remodel the extracellular matrix. In addition, ROS stimulate the internalization of E-cadherin which is essential to maintain the locality of tumor cell and, thus, loosen the inter-cellular interaction of cancer cell and initiate metastasis.[1,15,16] Consistent with findings and theory described above, our study showed that there was association between increased oxidative DNA damage with the invasiveness of breast cancer which we describe as positive LVI and high histological grade (grade III). This finding could supplement the traditional staging and grading system to give a better prediction of the prognosis of breast cancer. It is also opened a new possibility that anti-oxidant intervention could possibly have beneficial effect on cancer patient by interfering with ROS mediated signaling processes. However, this study only revealed the association between tumor invasiveness and oxidative damage parameter with no evidence of how the ROS act to enhance the invasiveness of breast cancer. Thus, further study to fill in the gap between those variables would be needed especially study that use biopsy sample to provide better understanding what process that actually happen in the real case.

CONCLUSIONS

Based on the results of research and data analysis, it can be concluded that high concentration of 8-OHdG compounds and positively lymphovascular invasion was associated increases of invasive breast cancer behavior.

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High 8-OHdG compounds concentration and lymphovascular invasion are risk factors for increases invasive breast cancer behavior

by Ni Wayan Tianing

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High 8-OHdG compounds concentration and lymphovascular invasion are risk factors for increases invasive breast cancer behavior

Ni Wayan Tianing
[1]Biochemistry Department, Faculty of Medicine, Udayana University
email: wtianing@yahoo.com

Abstract

Free radicals produced by oxidative stress has long been associated as one of the triggering[3] factor of DNA mutation that lead to oncogenesis, especially in breast cancer. Serum level of 8-hydroxy 2-deoxyguanosine (8-OHdG)[7] is one of the most studied oxidative stress biomarker in nuclear and mithocondrial DNA damage. The objective of this study was to analyse the risk of high serum level of 8-OHdG in invasive behaviour of breast cancer based on lymphovascular invasion (LVI). This was a case-control study involving 66 breast cancer patients devided into; 33 patients with positive LVI (LVI^+) as case group and 33 patients with negative LVI (LVI^-) as control group. This study was conducted in Laboratory of Biochemistry, Faculty of Medicine, Udayana University and Sanglah General Hospital Denpasar, Bali[1]. Serum level of 8-OHdG was measured by ELISA. The Results of this research: Increase of 8-OHdG concentration were associated with invasive characteristics in breast cancer patients (565.326 ± 282.655 ng/mL in case group compared with 326.336 ± 144.53 ng/mL in control group; $p<0,05$). Risk analysis has shown higher level of 8-OHdG were associated with increase risk[6] of invasive behaviour of the cancer it self (OR: 13.913; 95% CI: 1.163 – 116.412). ROC curve analysis showed Area Under the Curve (AUC) was 0.764 which indicates moderate predictive value. This result emphasized the role of oxidative stress in DNA damage and oncogenesis. Conclusion: High concentration of 8-OHdG compounds and positively lymphovascular invasion was associated increases of invasive breast cancer behavior

Keywords: 8-hydroxy 2-deoxyguanosine, lymphovascular, invasion, breast cancer

Abstrak

Parameter oksidatif seperti radikal bebas yang dihasilkan dari stress oksidatif telah lama dikaitkan dengan karsinogenesis terutama sebagai salah satu yang memicu mutasi atau sebagai induktor mutasi DNA dan mendorong onkogenesis. Hal ini sangat berhubungan dan berperan penting dalam proliferasi sel, peradangan, survival dan metastasis kanker khususnya kanker payudara. Tujuan penelitian

adalah menganalisis hubungan parameter oksidatif darah (8-OHdG) dan *lipovascularinvassion* dengan tingkat invasive kanker payudara. Studi kasus kontrol digunakan pada penelitian ini dengan menggunakan 66 subjek kanker payudara. 33 subjek sebagai kasus dan 33 subyek sebagai kontrol. Penelitian dilakukan di Laboratorium Biokimia Fakultas Kedokteran Universitas Udayana dan Rumah Sakit Umum Sanglah Denpasar Bali. Sampel dengan limpovaskularinvassion positif (LVI) diklasifikasikan ke dalam kelompok kasus dan sebagai kontrol digunakan sampel dengan *lipovascularinvassion* negative (LVI). Kadar senyawa 8-OHdG diukur dari serum penderita menggunakan metode ELISA. Peningkatan kadar serum 8-OHdG dikaitkan dengan karakteristik invasive kanker payudara (326,336 ± 144,53 ng/mL pada kelompok kontrol vs 565,326 ± 282,655 ng/mL pada kelompok kasus; p <0,05). Analisis risiko menunjukkan bahwa kadar 8-OHdG yang tinggi dan dengan LVI positif dikaitkan dengan peningkatan risiko perilaku kanker yang lebih invasif (OR: 13,913; 95% IK: 1,163 - 116,412; p <0,05). Kurva ROC menunjukkan bahwa *Area Under the Curve* AUC sebesar 0,764 yang menunjukkan nilai prediktif moderat. konsentrasi 8-OHdG dan LVI positif berhubungan dengan meningkatnya prilaku invasive kanker payudara.
Kata Kunci: 8-hidroksi 2-deoxyguanosine, lipovascular invassion, kanker payudara, invasive

Introduction

Oxidative stress has long been linked as one mutation inductor that leads to carcinogenesis by mutating the oncogene gene or suppressor tumor gene. The causes of oxidative stress can come from outside and inside the body. Oxidative stress from the body comes from the process of energy synthesis in mitochondria, especially in the electron transfer process.[1,2] Electron transfer processes that can produce free radicals such as oxidative uncouple phosphorylation or molecular processes in peroxisomes, but they are rapidly neutralized by the cell's antioxidant enzymes. But after carcinogenesis occurs, cancer cells tend to produce high amounts of free radicals resulting from an increase in the uncouple electron transport rate in the inner mitochondrial membrane. This increase in uncouple electron transport rates is predicted to produce more free radicals and will cause hyper mutations and genome instability in cancer cells.[2,3] The unstable genome in these cells underlies therapeutic changes in cancer characteristics. Another effect of high oxidative levels is not yet known in cancer. Recent cancer research has shown that increasing numbers of free radicals also have other functions for cancer cells, namely inducing the transformation of normal fibroblasts into cancer-related fibroblasts and contributing to regulating mitochondrial biogenesis through the Cancer Anti-fibroblast (CAF) mechanism. This CAF is forced to convert the metabolic profile into predominantly glycolytic and produce a stable supply of lactate which is used by cancer cells to produce energy or as the backbone of the biosynthetic process. In addition, free radicals are involved in many aspects of cancer development including remodeling of extracellular matrix and tumor-related degradation, inflammation, and mitogenic signal activation between MAPK, ERK-1, and JNK intermediaries.[1,4,5] While most mechanisms linking free radicals or oxidative stress with cancer development have been established, most studies in the

field of cancer are in vitro.[3,6,7] Therefore, this study was conducted with the aim to investigate the relationship of oxidative damage to breast cancer with its invasive characteristics to better ascertain previous in vitro findings.

Methods

An analytic cross-sectional study was conducted in Faculty of Medicine Udayana University and Sanglah General Hospital from January to December 2016. The sample were obtained from Surgical Oncology Clinic in Sanglah General Hospital and then examined in Biochemistry Laboratory in Faculty of Medicine Udayana University. The inclusion criteria in this study were newly diagnosed invasive breast cancer with clinical grade II and III regardless of the type. The exclusion criteria were phyllodes tumor, pathologically benign results, patient who has history of chemotherapy, secondary breast cancer, and disagree to participate. All subject then grouped into control and case group with those with invasive characteristics such as lymphovascular invasion (LVI). This was a case-control study involving 66 breast cancer patients devided into; 33 patients with positive LVI (LVI^+) as case group and 33 patients with negative LVI (LVI^-) as control group. This study was conducted in Laboratory of Biochemistry, Faculty of Medicine, Udayana University and Sanglah General Hospital Denpasar, Bali. Samples was obtained from every subject and clinicpathological data were collected from medical record. Concentration of 8-OHdG was examined by Enzyme Link Immunosorbent Assay (ELISA) technique using 8-hydroxy 2 deoxyguanosine ELISA Kit following the manufacturer instruction. All of the data obtained were analyzed descriptively to obtain the proportion of each variable in each group and the mean of 8-OHdG. Then, analytical study were conducted using independent sample T-test to evaluate the difference concentration of 8-OHdG between case and control group. Risk assessment was conducted using chi-square test by classifying the 8-OHdG concentration into high and low. this research has obtained ethical clearance with no:161/UN.14.2/Litbang/2014.

Result

66 subjects were participated in this study with mean age of 41.26±9.097 years. The mean age in control group was found to be younger than case group (34.73±5.80 year vs. 47.79±6.818 year). The mean concentration of 8-OHdG was found at 326.336±144.53 ng/mL in control group which was lower than case group at 565.326±282.655 ng/mL. Meanwhile, the proportion of clinical stadium (II and III) was found to be roughly comparable. At show to table 1.

Table 1. The Baseline Characteristics of The Subjects

Variables	Mean
Age	Overall : 41.26±9.097 Control : 34.73±5.80 Case : 47.79±6.818
8-OHdG	Overall : 474.129±253.27 ng/mL Control : 326.336±144.53 ng/mL Case : 565.326±282.655 ng/mL
Stadium	II : 36 (54.5%) III : 30 (45.5%)

Prior to analysis, the normality of the 8-OHdG concentration was examined using Kolmogorov-smirnov test. It was found that the 8-OHdG data were not normally distributed. According to normality test result, the 8-OHdG concentration between case and control group were examined using non-parametric Mann-Whitney test. The result showed that the 8-OHdG concentration between case and control group was significantly different with case group had significantly higher concentration. (*The data was analyzed using Mann-Whitney Test according to the result of the normality test).

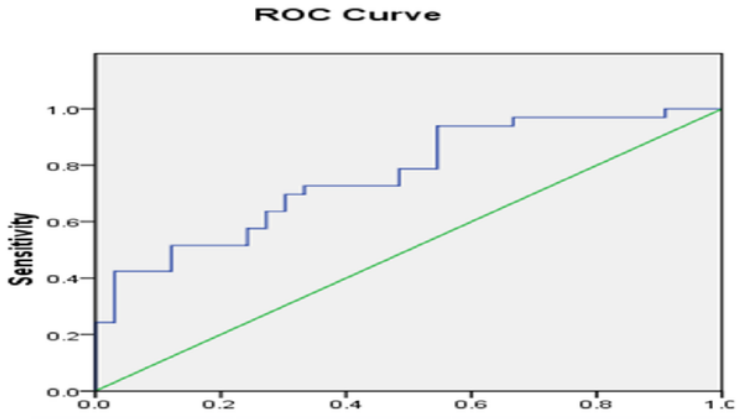
Table 2. Normality and Bivariate Analysis Of 8-OHdG Concentration Between Case and Control Group

Normality Test	Statistic	P-value
Kolmogorov-Smirnov	0.182 (df: 66)	0.000
Group	**Serum 8-OHdG Concentration**	**P-value***
Case	326.336±144.53 ng/mL	0.000
Control	565.326±282.655 ng/mL	

Then, we divide the 8-OHdG concentration using 223ng/mL as cut-off point according to ROC curve analysis. Subsequently, risk analysis was conducted to determine the OR of 8-OHdG concentration toward invasive behavior of breast cancer. According to risk analysis, it was found that the increased 8-OHdG concentration was significantly elevated the risk of invasiveness of breast cancer (OR: 13.91; 95% CI: 1.663-116.412). ROC curve analysis also reveals that 8-OHdG concentration had a moderate prediction value for cancer invasiveness with Area Under the Curve at 0.764 (P-value: 0.000; 95% CI: 0.651-0.877)

Table 3. Risk Analysis of 8-OHdG Level Toward Cancer Behavior

		Group		Statistical Analysis
		Control	Case	
8-OHdG Concentration	Low	10	1	P < 0.003
	High	23	32	OR: 13.913; 95%CI: 1.163 – 116.412
Total		33	33	



Area	P-Value	95% CI
0.764	0.000	0.651 – 0.877

Figure 1. ROC Curve Of Plasma 8-OHdG Level to Predict The Invasiveness of Breast Cancer Reveal A Moderate Strength Prediction Value.

Discussion

The advancements in cancer diagnostic and management, breast cancer still placed as the second most lethal cancer in women after cervical cancer. Traditional classification system that based only on clinical observation and pathologic examination proved insufficient to accurately determine the prognosis and behavior of breast cancer. Determination of whether the cancer is actually more or less invasive is particularly challenging.[8,9] For example, many early stage breast cancer were proved to be more invasive even when compare to the advanced stage counterpart. The triple negative breast cancer is even more challenging since this type has the worst prognosis than others as well as having the highest molecular

diversity. However, recent findings in tumor biology could be the key to develop and perfect current method of prognostic determination. Free radicals have been long associated with carcinogenesis particularly as the one that triggers the mutation which drives the oncogenesis.[10,11] However, recent findings showed that free radicals had many important roles other than triggering oncogenic mutations. It begins with study that reveal cancer cells were produced high number of free radicals mainly as the result of their high metabolic rate. High metabolic rate means higher rate of electron transport in inner mitochondrial membrane and, thus, higher chance of uncouple electron transports which result in free radical formation. Then, with the advance in the study of cancer microenvironment, it was found that cancer cells were actually produced free radicals on purpose to modify the surrounding connective tissue. Connective tissue has anti-cancer properties in nature but continuous exposure to free radicals trigger the differentiation of fibroblast into cancer associated fibroblast (CAF) which is pro-carcinogenic (1,4,5,12). CAF produce many pro-carcinogenic cytokines and support cancer cell both metabolically and immunologically. From metabolism perspective, CAF plays major part in reverse Warburg effect by increasing its glycolysis and lactate fermentation rate. It ensures the steady supply of lactate to the cancer cells for anabolic purpose. Immunologically, it participates in immune-evasion of cancer cell by suppressing and modifying nearby macrophage and lymphocyte driving their differentiation into M2 macrophage or cancer associated macrophage (CAM) and T-regulator cells or trigger the effector T-cell energy. On the other hand, free radicals also act directly on cancer cells, activating many pro-survival, mitotic, and metastatic pathways (13,14). ROS has been known to activate mithogenic signaling pathway mediated growth factor receptor tyrosine kinase (R) and Rac. These signaling eventually activate c-jun and c-fos which are the subunit of AP-1 nuclear transcription factor and induce the expression of mitotic related genes. ROS also known to activates autophagy in cancer cell via mTOR activation (1,4,5). Autophagy is an essential process that recycles the macromolecules in cancer cell and enhance cell repair, the process that plays essential role in enhancing cell survivability. In addition, increased amount of ROS could induce inflammatory process in tumor micro-environment. In contrast from previous believe that inflammation would has a detrimental effect on cancer cells, current study reveals that inflammation in tumor microenvironment has many beneficial effects for cancer cells including immune evasion, proliferation and metastasis. Immune evasion is mediated by M2 macrophage and suppressor T cell while proliferation is mediated by increased production of growth factor including VEGF and IGF by M2 macrophage (1,4,5). Increased expression of MMP-9 either by inflammatory stimuli or secondary from increased SOD2 activation mediate the metastatic process of cancer cell by degrading and remodel the extracellular matrix. In addition, ROS stimulate the internalization of E-cadherin which is essential to maintain the locality of tumor cell and, thus, loosen the inter-cellular interaction of

cancer cell and initiate metastasis (1,15,16). Consistent with findings and theory described above, our study showed that there was association between increased oxidative DNA damage with the invasiveness of breast cancer which we describe as positive LVI and high histological grade (grade III). This finding could supplement the traditional staging and grading system to give a better prediction of the prognosis of breast cancer. It is also opened a new possibility that anti-oxidant intervention could possibly have beneficial effect on cancer patient by interfering with ROS mediated signaling processes. However, this study only revealed the association between tumor invasiveness and oxidative damage parameter with no evidence of how the ROS act to enhance the invasiveness of breast cancer. Thus, further study to fill in the gap between those variables would be needed especially study that use biopsy sample to provide better understanding what process that actually happen in the real case.

Conclusions

Based on the results of research and data analysis, it can be concluded that high concentration of 8-OHdG compounds and positively lymphovascular invasion was associated increases of invasive breast cancer behavior.

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High 8-OHdG compounds concentration and lymphovascular invasion are risk factors for increases invasive breast cancer behavior

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