OXIDATIVE PARAMETERS OF 8-HYDROXY 2-DEOXYGUANISINE (8-OHdG) COMPOUNDS AND LIPOVASCULAR INVASION INCREASE INVASIVE BREAST CANCER BEHAVIOR

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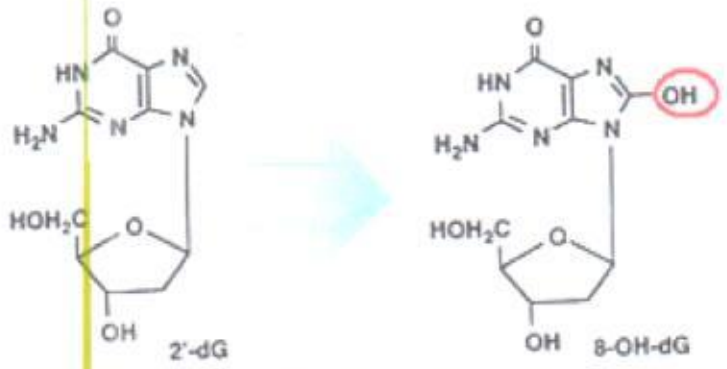
INTRODUCTION

- Breast cancer is the second most prevalent disease in women after cervical cancer.
- It also associated with significant amount of mortality above than cervical cancer
- Breast cancer also a challenge for oncologists because most patients come with advanced stage (Clinical Stage III-IV) and there is still no known methodology to predict the invasiveness of breast cancer.

- The free radicals produced by oxidative stress have long been linked to as a trigger factor for DNA mutations that cause oncogenesis, especially in breast cancer.
- Oxidative stress: a higher oxidant concentration than antioxidants → DNA oxidation → 8-hydroxy 2-deoxyguanosine (8-OHdG).
- 8-hydroxy 2-deoxyguanosine (8-OHdG) compund is one of the biomarkers of DNA damage due to DNA oxidation by Reactive Oxygen Species (ROS) or free radicals.

STRUCTURE OF 8-HIDROXY 2-DEOXYGUANOSINE (8-OHdG)



Formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) by oxygen radicals
H Kasai Environmental Mutagen Research, Vol. 10, p75-78 (1988)

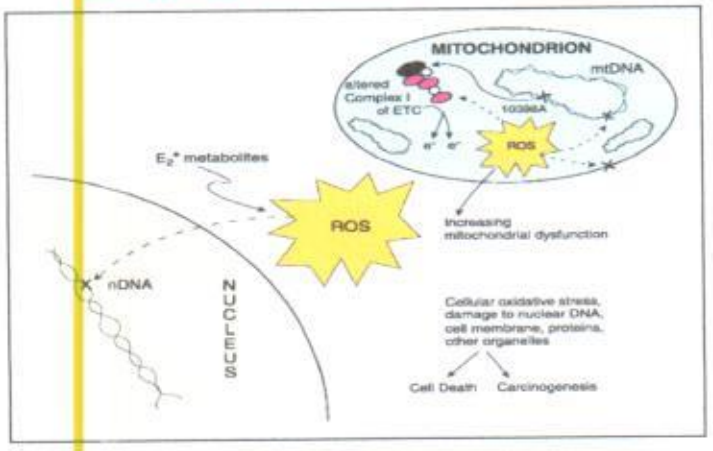
- Oxidative stress: is undeniably plays important role in initiating carcinogenesis and cancer progression.
- Inside the cell, mitochondria are the primary site where most free radical form especially when there is an alteration in the electron transport chain process.
- Normally, the free radicals produced would be neutralized by endogenous antioxidant systems.

- The presence of the polymorphism result in the alteration of mitochondrial complex-1 function.
- Polymorphisms of ND_3 gene had been associated with increased risk of breast cancers.
- Polymorphisms in mitochondrial especially ND_3 genes had been proved to be associated with the occurrence of breast cancer.
- The genes that encode the protein involved in the electron transport chain are particularly important because the defect in this process could result in increased production of ROS.
- ND3 gene: The gene that encodes NADH dehydrogenase of the complex-1 of the electron transport chain.
- ND3 gene had been associated with several kinds of cancer namely bladder, prostate, and thyroid cancer.

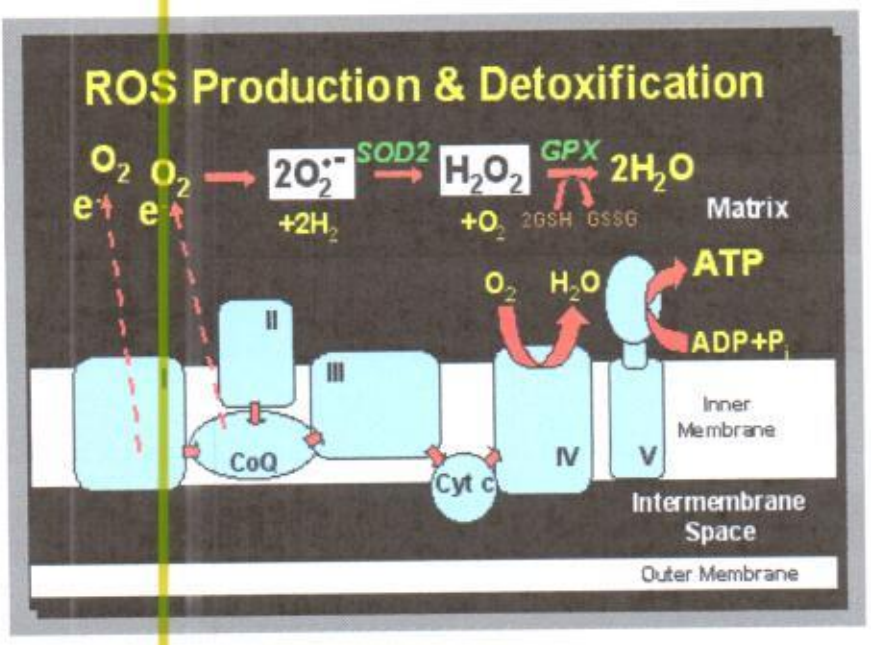
- **However, several studies that evaluate its association in the population showed non-conclusive findings, still controversial.**
- **Czarneka et.al reported that the ND_3 polymorphism A10398G was significantly associated with sporadic breast cancer in Poland.**
- **Then, Canter et.al found that the same polymorphism might also contribute to the risk of breast cancer in African-American women (OR: 1.6; 95%CI: 1.10-2.31).**
- **Jiang et.al also supported those findings, stated that the A10398G ND_3 polymorphism also contributed significantly to the breast cancer risk among Chinese Han women (OR: 1.49; 95%CI: 1.05-2.11).**

- Considering the important role of ROS in the development of cancer and its relationship to the ND3 gene polymorphism that has been widely associated with breast cancer makes this relationship very important for further analysis.

ROS PRODUCTION AND DNA DAMAGE



Jeffrey Canter 2005



RESEARCH PURPOSES

- To analyze the relationship between 8-hydroxy 2-deoxyguanosine (8-OHdG) and lymphovascular invasion status with invasive breast cancer behavior.

METHODS

- This study 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.

- ***DNA Isolation and Sequencing***
- The DNA was isolated using Promega Blood DNA Isolation Kit, the isolated DNA was subsequently amplified by PCR and sequencing.
- ***Examination of Serum 8-OHdG Concentration*** was examined by Enzyme link Immunosorbent Assay (ELISA) Kit.

STATISTICAL ANALYSIS

- 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 one-way annova 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 and assess whether the presence of ND_3 polymorphism increased the risk of invasive morphology in breast cancer.

RESULTS AND DISCUSSION

- Increase of 8-OHdG serum level 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 <0,03).
- Risk analysis has shown higher level of serum 8-OHdG were associated with increase risk of invasive behaviour of the cancer itself (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.

BASIC CHARACTERISTICS OF THE SUBject

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%)

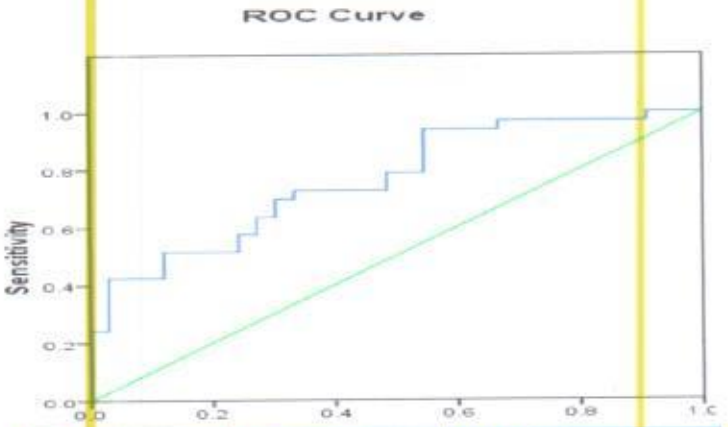
NORMALITY AND BIVARIATE ANALYSIS OF SERUM 8-OHdG CONCENTRATION BETWEEN CASE AND CONTROL GROUP

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	

ANALYSIS OF 8-OHdG CONCENTRATION BETWEEN CASES AND CONTROL GROUPS

		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	

8-OHdG CURVE RATIO TO PREDICT BREAST CANCER INVASION



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

CONCLUSION

- HIGH CONCENTRATIONS OF 8-HYDRODXY 2-DEOXYGUANOSINE (8-OHdG) AND LYMPHOVASCULAR INVASION ARE POSITIVELY ASSOCIATED WITH INCREASED INVASIVE BREAST CANCER BEHAVIOR.

THANK YOU