Catalase Enzyme and Unexplained Infertility

Noorhan Shaker * M.B.ch.B. D.G.O. F.I.C.O.G.

Summary:

Background: *To assess the activity of catalase enzyme in erythrocytes as a predictor of oxidative stress in female with unexplained infertility.*

Setting: *Maternity and Childhood Hospital in Ai-Najaf for the period between August 2004 to March 2005.*

Patients & Methods: *Blood samples was taken from two groups, 30 cases selected to have unexplained infertility for about 2-10 years (test group), other samples was taken from 30 cases selected to have normal fertility. The age of the cases taken are between 20-40 years old. Venous blood was drawn into EDTA-treated tubes and packed red blood cells were prepared and washed with physiological serum 3 times to eliminate leukocytes and platelets. Hemolyzates were prepared . The activity of cataiase enzyme determined by the method of Aebi which measured by 2100 spectrophotometer at wave length 240 nm.*

Results: *This study found that cases with unexplained infertility have a significant decrement in cataiase enzyme activity which is equal to 23(77%), X"= 5.71, p <0.05. Also there was a significant correlation with the Hb concentrations of the body r—0.9526 , p<o.o5; Cases with higher Hb concentrations have higher enzyme activity. In addition it was found that cases with high parity nave higher enzyme activity r=0.9589. p<0.05 . The maximum enzyme activity was found in the age group between 26-29 years old and the minimum level of activity is at age more than 36 years old. Maximum cataiase activity is found to occur in the first 15 seconds after liberation of the free radicals.*

Conclusion: *There is a significant relationship between oxidative stress in form of decrease catalase enzyme activity and unexplained infertility.*

Key words: *Catalase, unexplained infertility, oxidative stress*

Introduction:

Fertility is a physiological process result from interaction of both male & female, any disturbance to this interactive system can results in an inability to have a child.

Infertility can be defined as a lack of pregnancy after one year of regular unprotected intercourse. Approximately 15-20% of couples of reproductive age are infertile which can be attributed equally to male &female factors (l).

Etiology of infertility still not fully understood, it can be due to the following causes :Central 40% . tubal/ovarian 30-50%, pelvic/peritoneal 5-10%,endometrial/utenne,2_3%,cervicalmiucus23%, unexplained! 0%(2)

Unexplained infertility is diagnosed when the standard investigations of both male & female have ruled out other causes of infertility(3), it doesn't mean that there's no reason for the infertility; but that the reason still unable to be identified at that time(4).

* *Department of Gynaecology and Obstetric/Al-Zahraa teaching hospital for Maternity and childhood-Collage of Medicine-Kufa University.*

Recently discovered that reactive oxygen species (ROS) have an important role in the normal function of reproductive system &in the pathogenesis of female infertility(l).

(ROS) are free radicals(5,6,7) and these are unstable atoms with one or more unpaired electrons formed as a result of many physiological &pathological cellular metabolic processes, they can interfere with the biological components of the cells like cell membrane, PUFA, proteins, carbohydrate and even degradation of DNA. The major free radicals are H2O2" (hydrogen peroxide), OH' (hydroxyl radical) ,02' (Superoxide anion), hydrogen peroxide radical H2O2" is highly toxic. It plays an important role in sperm survival within the female reproductive tract; therefore its' level can be considered as a marker of normal tubal function(S). However, it is converted to oxygen & (H2O) by antioxidants. Free radicals can be formed by internal and external factors like: Tobacco smoke, excessive alcohol, radiation including ultraviolet radiation from the sun, pesticides, herbicides& pollution, drugs, surgery, stress, any trauma, hypoxia in addition to shock state(9).

Antioxidants are chemical substances that help to prevent cell damage by neutralization of free radicals or prevention of there production , they are either enzymatic which synthesized naturally in the body such as catalase enzyme, superoxide

dismutase, glutathione peroxidase or non enzymatic nutritional antioxidants such as vitamin A, vitamin C, vitamin E, Alpha Lipoid acid, niacin, vitamin B6, etc(5,10,11,12,13). A delicate balance is presents between these antioxidants and free radicals in the ceil environmental4).

Oxidative stress occurs when there is an imbalance between the production of these free radicals &. the body's antioxidants. It is the cause of more than 50 diseases including cancer, hypertension, stroke, coronary heart disease, diabetes, cataract, depression, Alzheimer dementia & aging(13,14)

Summary of possible mechanisms of how oxidative stress can induce infertility in women:

1. Direct damage to oocytes due to oxidative stress in ovarian follicles.
2. Direct damage to oocyte &spermatozoa due to oxidative stress in peritoneal cavity.
3. Direct damage to embryo due to oxidative stress in the fallopian tubes.
4. ROS-antioxidant imbalance leading to defective endometrium for supporting embryo.
5. ROS-antioxidant imbalance leading to luteal regression &lack of luteal hormonal sucDort for continuation of pregnancy.
6. Even when fertilization occurs; there will be failure of implantation or the embryo will undergo fragmentation leading to abortion or congenital anomalies in the offsprings(l).

Catalase enzyme : It is one of the most potent endogenous antioxidants known, it is a heme containing enzyme(15), it is an extra ordinary enzyme perform the trick of converting toxins & the potentially harmful H_2O_2 & recombining them into harmless or even useful products(16).In humans, the highest levels of catalase are found in liver, kidneys & erythrocytes(17). Normal level of catalase enzyme in erythrocyte = 394.9+/-22.SIU/gHb(18)

l. Hydrogen peroxide disproportionate: $2H_2O_2' \longrightarrow 2H_2O + 0_2$

2 *Peroxidative reaction* $L\_ H_2O_2 ' + RH_2 — 2H_2O- R$ (15,17,19,20)

Patients & Methods:

The study was carried out in Maternity and Childhood Teaching Hospital in Al-Najaf for the period from august to march during 2004-2005. It consists of 30 women with primary infertility 2-14 years as " test group", they are with normal ovulation, patent fallopian tubes bilaterally, no cervical hostility and normal semen analysis. Another 30 women has been taken as a "control group" selected with a parity between 2 -. 7 who delivered vaginaily with no pervious history of primary' or secondary infertility' nor missed abortion, intra-utenne death or still birth

Catalase assay: A: The catalase assay takes the advantages of the fact that hydrogen peroxide has a LJ.V. absorbance maximum at 240 nm The disappearance of H_2O_2 caused by the action of catalase can be monitored with respect to time by following the absorbance at 240 nm of a sample containing a known concentration of H2O2 & a known amount of the test materialfhaemoiysed blood) so a decrease in absorbancefAA) of 0.02 corresponds to consumption of 1 11 mole of H_2O_2 [1]9V if fAA) equal to 0.50-0.55 it means normal enzyme activity T2 1 V The instrument used is 240 nm U.V. 2100 spectrophotometer , a beam of light will split into two separate paths passing through a blank and sample tubes ; automatically & continuously subtracts the blank reading from the sample, the blank sample must include the same amount of enzyme and buffer as the sample, the sample will contain H2O2 whereas the blank will not The instrument will scan the samples at 240 nm at "0" time & at "15","30","45"."60","75" and "80"seconds [a2)]

B: Preparation :

Reagents used :

1. Phosphate buffer solution in a concentration of 50 micro mole &PH =7.0 , it consist of:

Solution "A" which consist of KH_2PO_4 by weighting 6.8 Ig _of KH_2PO_4 dissolved in 1 liter of distal water.

Solution "B" which consist of Na_2HPO_4-$2H_2O$ by taking 6.90 g & dissolve in 1 liter of distal water. **A mixture is done 390 ml from solution "A" and 610 ml from solution "B" then standard the PH at 7.0.

2. Hydrogen peroxide solution in a concentration of 30 uomole, it's prepared by diluting 0.34ml of H_2O_2 30% with phosphate buffer solution to make 100ml

Preparation of the sample;

1. Venous blood is aspirated around 4 ml & put in an EDTA-treated tubes.
2. Centrifugation is done about 200 rpm for 20 minutes.
3. We discard the plasma & the buffy coat above the sediment PJ3C.
4. Sedimented RBC are washed with saline solution 0.9% three times then centrifugation is done after each wash with discarding of the saline layer.
5. Haemoiysed blood is prepared by adding distal water to the precipitated RBC in a ratio of 4:1 and mixing them carefully.
6. Hemoglobin measurement is done to each sample of the haemoiysed blood by Hb meter.
7. We mix 20 ii liter from the haemoiysed RBC with 10 ml of phosphate buffer solution
8. Activity of catalase enzyme determined by the method of Aebi measured by 2100 spectrophotometer at wave length of 240 nm (21,23,24)

C:Procedure: Catalase enzyme can be detected by measuring the decrease in absorbance of the tested materials which contain the enzyme (haemolysed blood and the substrate which is H2O2), the decrease in absorbance is due to braking of H2O2 molecules by catalase enzyme into water and oxygen; Therefore, the more the decrease in absorbance the more the catalase enzyme activity. Blank tube contain . ml of phosphate buffer solution +2 ml of haemolysed blood diluted in phosphate buffer, it is inserted in the spectrophotometer to subtract the difference in absorption in different [illegible] and absorption of suspensions. The test tube contain 2 ml of Haemolysed blood diluted in phosphate buffer - ! ml of H_2O: . 30 uomole solution; The reaction start by adding H_2O_2 solution to the cuvette tube & measuring the absorption at 240 nm at time zero *Sc* at (15-30-45-60-75)seconds, the decrease in absorption(A absorbance) between the readings recorded at different times reflect the **enzyme activity** [illegible].

	Blank	Test
Phosphate buffer solution	*1.0 ml*	—
Haemolysed blood diluted in phosphate buffer	*2.0ml*	*2.0 ml*
H_2O_2, 30 umole solution		*1.0 ml*

Results:

Table 1-: Demonstrate comparison of normal & abnormal catalase activity in both test & control groups.

In test group 23 (77%) patients out of 30 were found to have abnormal enzyme activity and 7(23%) of cases were found to have normal enzyme activity; while in control group 14 (47%) women have abnormal enzyme activity and 16 (53%) have normal catalase enzyme activity. X^2=5.71, p<0.05, this means that there is a significant difference in catalase enzyme activity between the two groups.

Table 2-figure 2 :Demonstrates the amounts of decomposed H2O2 umol as indicator of catalase enzyme activity in relation to time factor in two cases selected to have the same Hb concentration (13mg/dl), one with normal enzyme activity from control group/A absorbance 2.0,and the other with abnormal enzyme activity from test group/A absorbance 0.4. This table shows that the maximum activity of the enzyme is carried out at the first [illegible] seconds in both cases then it decrease till the reaction stops at 60 seconds.

Table3-figure 3: Denote the relationship [illegible] enzyme activity predicted [illegible] average [illegible] of decomposed H2O2 [illegible] Hb concentrations of the haemolysed blood of the whole cases (control and test groups). It shows that cases with average Hb concentration between 1.5-1.9mg/dl appear have the lowest average A absorbance which is equal to 0.02 (10umol)of decomposed H2O2.

Cases with average Kb concentration between 5-5.4mg/dl have maximum average A absorbance equal to 2.0 (100umol)of decomposed H_2O_2. This reflects that cases with higher Hb concentrations have higher catalase enzyme activity i.e. there is significant correlation. r=0.9526, p<0.05.

Table 4-figure 4: Demonstrate the relationship between parity and the average amount of decomposed H_2O? urnole of the control group.

Cases who were P2 found to have the lowest average A absorbance which is equal to 0.4 (20 umol) of decomposed HiO? and cases who were P7 found to have higher level of average A absorbance which is equal to 1.7 (85 umol) of decomposed H2O0. This reflects that cases with higher parity have been found to have higher enzyme activity i.e. there is significant correlation. r=0.9589.p<0.05.

Table 5-figure 5 : Shows a correlation between the age of the whole cases taken and the activity of catalase enzyme represented by the average amount of decomposed JHUOo.umol.

This tabie reveal that maximum catalase enzyme activity were found in the age groups between 26-29 years old and that minimum activity were found in the age groups above 36 years old.

Table 6-: Denote correlation between years of infertility and cataiase enzyme activity determined by the average amount of decomposed H2O2 umol in the test group. This table reflects that enzyme activity has no relation with periods of infertility p>0.05

Discussion:

The role of oxidative stress in human reproduction have been expressed by many studies. Regarding female infertility, the role of excess ROS have been implicated as one of the causes of female unexplained infertility [1]. There are many studies that measure the amount of ROS in human embryos, fluid of hydrosalpinx, follicular fluid, peritoneal fluid & media prepared for IVF culture especially in cases of endometriosis and unexplained infertility [25] This study involves the measurement of this enzyme which considered as one of the powerful endogenous antioxidant enzymes present in the body[26].

There is -a significant decrease in catalase enzyme activity in cases with unexplained

infertility X^2= 5.71, p <0.05 as shown in table 1. Seven(23%) of patients with unexplained infertility have normal enzyme activity, they may have either other cause for their unexplained infertility or have other antioxidant enzymes abnormality like superoxide dismutase or glutathione peroxidase. There were no available data found to compare the results of this study with others. Cases with normal fertility and abnormal enzyme activity although they have good fertility, they may be exposed to other late onset diseases or disorders caused by the damaging effects of HoCb like: Coronary heart disease, stroke, cataract, macular degeneration[27] depression[28] alzheimer disease which is due to neuronal damage by oxidant agents [29] suppression of immunity with susceptibility to HIV infection [30] inflammatory bowel disease[31] also they are exposed to type 2 diabetes due to damage of pancreatic cells by H2O2 with resultant inhibition of insulin secretion and hyperglycemia[32] Also it was found that ROS is associated with increased incidence of cancer especially of the lung, larynx, oral, esophagus, stomach, pancreas, cervix, colorectal &breast and with rapid aging process[33]

In this study we found that the maximum catalase enzyme activity occurs in the first 15 seconds of the reaction for both groups as shown in table 2, this reflects that catalase enzyme is highly reactive and can neutralize the free radicals librated in the body within seconds of their formation and prevent their damaging effect. This may be due to their weak binding sites which aid in the transfer of substrate to the active site of the enzyme and hence increase the rate of *the* reaction, this result corroborate with other studies as Agarwal A(1) & James Grook[34] that enforce and support our findings.

In addition this study found that cases who have the lowest average Hb concentrations which is between 1.5-1.9 mg/dl have the lowest amount of decomposed H2O2 umol which is equal to l0 umol ,while cases who have the highest average Hb concentrations which is between 5-5.4 mg/dl have the highest amount of decomposed H2O2 umol which is equal to 100 umol as shown in table 3. This means chat there's a significant relationship between the Hb concentration and the enzyme activity r=0.9526,p<0.05. This result could confirm the fact that catalase enzyme is a heme containing enzyme(15) with emphasis on the relationship between catalase enzyme activity and iron deficiency anemia . There were no available studies that compare the activity of catalase enzyme in different hemoglobin concentrations .

On the other hand there was a relation between the parity and enzyme activity. Our observable notice clarified that hish paritv have higher enzymatic activity as shown in table 4, p<0.0i. There is no such study available till now to compare it with our study.

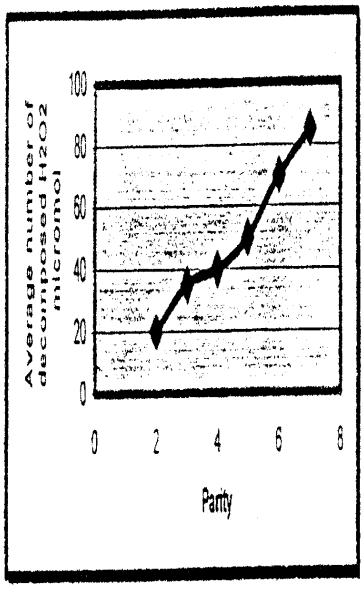
This study shows also that maximum catalase enzyme activity was found in the age groups between 26-29 years old and that minimum activity was found in the age groups above 36 years old as shown in table 5. There is also no available data that correlate erythrocytic catalase enzyme activity with the age of the human beings.

Lastly this study revealed that there is no relationship between period of infertility and catalase enzyme activity, p>0.05, this could be explained by laboratory error or due to other causes of unexplained infertility or it can be attributed to deficiency of other enzymatic antioxidants(l) . Also no data have been found to demonstrate the correlation between years of infertility and catalase enzyme activity.

Conclusion! There is a significant relationship between oxidative stress as a decrease catalase enzyme activity and unexplained infertility.

Table 4-figure 4: Demonstrate the relationship between parity and catalase enz. activity

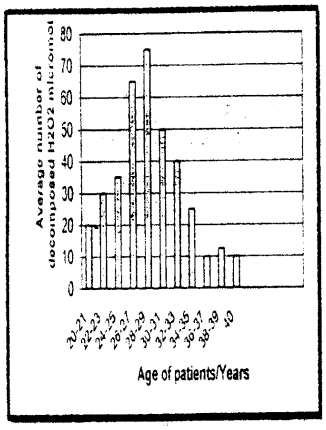
Parity	Number of patients	Average Δabsorbance	Average number of decomposed H2O2 µmol
2	8	0.4	20
3	5	0.7	35
4	6	0.8	40
5	4	1.0	50
6	4	1.4	70
7	3	1.7	85
Total	30		



r=0.9589 p<0.05

Table 5-figure 5 : Show a correlation between the age and the activity of catalase enzyme

Age of the patients (years)	Number of patients	Average Δ absorbance	Average number of decomposed H2O2µmol
20-21	8	0.4	20
22-23	7	0.6	30
24-25	9	0.7	35
26-27	6	1.5	35
28-29	5	1.7	75
30-31	5	1.0	50
32-33	4	0.8	40
34-35	7	0.5	25
36-37	4	0.2	10
38-39	3	0.25	12.5
39-40	2	0.2	10
TOTAL	60		



r=0.9589 p<0.05

Table 6-: Denotes correlation between years of infertility and catalase enzyme activity

Periods of infertility(Years)	Number of patients	Average Δ absorbance	Average number of decomposedH2O2 µmol
2-<4	8	0.3	15
4-<6	13	0.2	10
6-<8	3	0.5	25
8-<10	2	0.2	10
10-<14	3	0.3	15
14	1	1.2	60

p>0.05

Table (2)/figure(2): Demonstrate comparison between the amounts of decomposed H2O2 µmol as indicator of catalase enzyme activity in relation to time factor

Time (seconds)	Decomposed H2O2µmol in the control case	Decomposed H2O2µmol in the test case
0	0	0
15	50	15
30	70	20
45	90	20
60	100	20
75	100	20

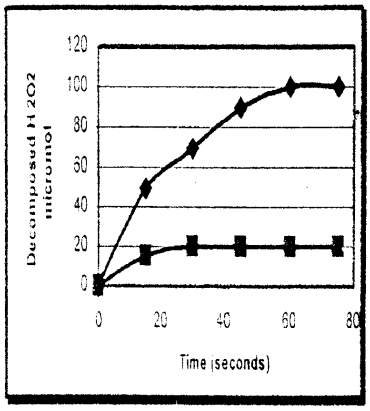
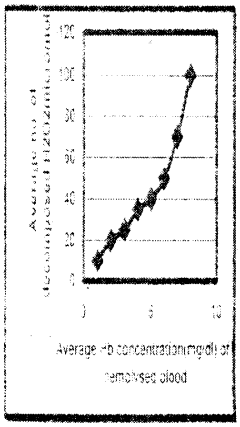


Table3-figure 3 : Denotes the relationship between the average Hb concentrations of the haemolysed blood of the whole cases (control and test groups) and catalase enzyme activity predicted by average number of decomposed H2O2 µmol .

Average Hb concentration(mg/dl)	No. of patients	Average Δabsorbance	Average number of decomposed H2O2 µmol
1.5-<3	4	0.2	10
3-<5	9	0.4	20
5-<7	4	0.5	25
7-<9	12	0.7	35
9-<11	11	0.8	40
11-<13	11	1	50
13-<15	6	1.4	70
15-<17	3	2.0	100



r=0.95 p<0.05

.Table 1: Demonstrates comparison of normal & abnormal catalase enzyme activity in both test & control groups.

	Total	Normal	Abnormal
Test group	30	7(23%)	23(77%)
control group	30	16(53%)	14(47%)

X2= 5.71 , P<0.05

Recommendations:

1. Correct iron deficiency anemia.
2. Improve nutritional status
3. Correct antioxidant deficiency by taking supplementations like: Vitamin E, Ascorbic Acid , Pyridoxine, Vitamin 86, Co-Enzyme Q10,, Acetyi Carnitine.
4. Change life style to minimize risk factors: Stop cigarette smoking, alcohol consumption, chronic stress, heavy exercise, exposure to pesticides, herbicides & other polluters, avoid excessive intake of drugs that affect antioxidants of the body as: Indomethacin

and metronidazole or taking antioxidant supplementations with them & ingestion of some drugs that act as free radical scavengers like famotidine, cemitidine . ranitidine and aspirin

Prospectively we hope to assess the activity of other antioxidant enzymes with increasing number of samples and also to assess their activity in places other than erythrocytes, more advanced devices can be used to measure ROS directly such as chemiluminesence.

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