Curr Pediatr Res 2012; 16 (2): 167-174 ISSN 0971-9032
Scientific Publishers of India

A correlation of antioxidants and lipid peroxidation between maternal and cord blood in full term and preterm deliveries.

Soumitra Chakravarty[a], Alka N. Sontakke[b]

[a]Department of Biochemistry,Dhanalakshmi Srinivasan Medical College and Hospital, Siruvachur, Perambalur , Tamilnadu , India.
[b]Department of Biochemistry,Maharashtra Institute of Medical Education & Research,Talegaon, Pune, India.

Abstract

Free radicals are implicated in several diseases of prematurity. The study aimed to correlate plasma antioxidants levels and lipid peroxidation between maternal and cord blood in normal and preterm deliveries. Superoxide dismutase (SOD), Reduced Glutathione (GSH) and Uric acid were the measured antioxidants while Malondialdehyde (MDA) was an index of lipid peroxidation. 32 mothers who delivered preterm and their babies were cases and 32 full term mothers and their babies were controls. Malondialdehyde levels in maternal blood were higher in preterm pregnancies *(7.47±1.13/ml) than full term pregnancies *(3.97±0.0.86nmol/ml). Mean Malondialdehyde in preterm babies *(7.38±1.06nmol/ml) were higher than full term babies *(4.46±0.86nmol/ml). Mean maternal SOD and GSH values were lower in preterm *(SOD;2.57±0.49units/ml and GSH; 3.84±0.55µmol/g Hb) than full term deliveries * (SOD; 3.77±0.76 units/ml and GSH; 5.38±0.77µmol/g Hb). Mean cord blood SOD and GSH values were lower in preterm * (SOD 2.40±0.44nmol/ml & GSH 3.00± 0.63µmol/g Hb) than full term deliveries * (SOD 3.90 ±0.90 units /ml and GSH 4.80 ± 1.20 µmol/ g Hb).Uric Acid levels showed no significant change in both groups. Positive correlation was established with MDA, between maternal and cord blood in both full term and preterm deliveries .Positive correlation was established with SOD levels and GSH levels between maternal and cord blood in both groups. Lipid peroxidation is accelerated in prematurity. Fetal antioxidants may decrease if maternal antioxidants are low. Free radicals damage the tissues of the newborn. Antioxidant supplementation to mothers at high risk category for delivering premature, may be beneficial. (* Mean ± SD)

Keywords: *Antioxiants, superoxide dismutase, glutathione, malondialdehyde, preterm, cord- blood*

Accepted May 21 2012

Introduction

A neonate whose calculated gestational age from the last menstrual period is less than 37 completed weeks is a preterm (premature). [1]

Oxidative stress is a term used to describe any condition that results in an accumulation of free radicals in the tissues .Free radicals can be extremely damaging to many tissues in the human body. Lipids undergo peroxidation in all tissues as part of the normal cellular function. The site of the process is the cell membrane where poly unsaturated fats are peroxidized. Excessive free radical activity damages cell membranes .This occurs both locally and at a distance because plasma lipoproteins may undergo peroxidation and be transported to vulnerable tissues within minutes [2].Oxidative stress affects a complex array of genes involved in inflammation, coagulation, fibrinolysis, the cell cycle, signal transduction and programmed cell death. It quickly becomes clear that a single pathway may be insufficient to provide clarification of oxidative stress action in the pathogenesis of the so called free radical diseases of prematurity [3].

Prematurity is an important cause of neonatal deaths and long-term morbidity such as development delays, blindness and cerebral palsy. Oxidative stress has plays a role in many illnesses affecting preterm infants, including bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) and ventricular hemorrhage. [1, 4]

In India, we routinely supplement antioxidants to the patients for various diseases and other conditions. But in prematurity, it is still not clear whether supplementation would actually benefit or not. For the same reasons we are yet to start an antioxidant supplementation program.

The current study tried to correlate the maternal and child status in terms of levels of various antioxidants and lipid peroxidation in full term and preterm deliveries.

Review of Literature

The main enzymatic antioxidants are superoxide dismutase, catalase, and glutathione peroxidase and glutathione reductase. Superoxide dismutase converts superoxide to hydrogen peroxide and oxygen. Catalase converts hydrogen peroxide to water and oxygen, and glutathione peroxidase uses glutathione to reduce hydrogen peroxide to water. Glutathione reductase reduces oxidized glutathione. Non-enzymatic antioxidants are less specific in their actions, but are characterized mainly by their large molecular size and availability of double bonds in their structure. Vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (α-tocopherol), glutathione, uric acid may absorb the effects of ODFRs (oxygen derived free radicals).

Premature infants may have lower levels of antioxidants [5]. Vitamin E is low in preterm babies [6]Vitamin C and A, glutathione , sulphur containing amino acids , ceruloplasmin and transferrin are normally transferred from maternal to fetal circulation only in the later part of third trimester. Vitamin E functions as an antioxidant to prevent peroxidation of excessive polyunsaturated fatty acids in red blood cell membranes and membranes of most other cells like cells of alveoli, intestine .Its need may be increased because of the increased membrane content of these fatty acids in the developing newborn tissues.

Uric acid concentration is dependent upon the balance in production by catabolism by purines and its excretion. However, the immature infant, unlike older babies has limited tubular resorption capacity for uric acid and hence there is a tendency to lower concentration in preterm. At birth, plasma uric acid concentration in term infant might be higher than in adults but in following weeks it declines to low levels at least in preterm. [6]

Thus the intracellular oxidative defenses seems to be lowered in preterm; the more premature the infant, lower the defense.

In 2002, a study found a positive correlation between maternal and cord blood levels of vitamins E and A. Preterm babies had fewer lipid-soluble antioxidant vitamins in their serum compared to term infants. [7]

In 2003, a study confirmed that low plasma selenium and alpha-tocopherol levels in premature infants (≤ 30 weeks' gestational age or lower) were significantly associated with an increased respiratory morbidity. [8]

In 2004, a study found that the RBCs of term and preterm babies showed higher reduced glutathione (GSH), oxidized glutathione (GSSG), glucose-6-phosphate dehydrogenase (G-6-PDH), Glutathione reductase (GR), and hexokinase (HK levels/activities) and lower GSH/GSSG ratios and higher GSH-recycling rates than those of adults. In preterm babies significant correlations were found between G-6-PDH and Catalase(CAT), GSH, GSH/GSSG ratio, and GSSG. It was speculated that preterm babies have prompter involvement of antioxidant defenses than term babies. [9]

In 2004 , a study assessed the status of oxidative stress in term small for gestational age (SGA) newborn infants born to undernourished mothers by estimating levels of erythrocyte superoxide dismutase (SOD), catalase, reduced glutathione, and serum malondialdehyde (MDA) in cord blood and comparing them to healthy appropriate for gestational age (AGA) controls. The activity of MDA was increased while levels of superoxide dismutase, catalase and reduced glutathione were decreased in term SGA born to undernourished mothers as compared to term AGA born to healthy mothers. On univariate analysis, all the markers of oxidative stress correlated significantly with maternal parameters. It concluded that intrauterine malnutrition is associated with significant oxidative stress in small for gestational age neonates born at term to malnourished mothers. [10]

In 2006, a study found out that antioxidant supplementation was associated with better maternal and perinatal outcome in pregnant women with low antioxidant status than control supplementation with iron and folate alone. [11]

In 2009, researchers in Italy tried to find out the status of Anti-oxidant enzymes and related elements in term and preterm newborns. Throughout the study period urinary 8-hydroxydeoxyguanosine (8-OHdG), taken as a marker of oxidative stress, was significantly higher in the preterm than in the term group. Until 100 days, preterm infants had significantly reduced SOD levels that appeared to reflect a shortage of the elements needed for this enzyme's activity, notably copper, the plasma concentrations of which were constantly and significantly below the control values. [12]

In 2009, a study found that the level of GSH decreased in IUGR (intra uterine growth retardation) and preterm placentas in comparison with the control group. [13]

Rationale

Looking at some of the important research work done in the last few years, the cause of increased lipid peroxidation is unclear. We have to know that whether the prema-

turity itself was a cause of the oxidative stress or the lower levels of antioxidants or both. Some studies found even better antioxidant defenses in prematurity [9]We also need to find out whether lower antioxidants can be a risk factor for prematurity itself and its associated disorders.

Materials and Methods

The study was conducted in Department Of Biochemistry, Pad.Dr.D.Y.Patil Medical College, Pune, Maharashtra, India, with approval of Institutional Ethical Committee.

Study Type
This was a case control study.

Study Duration
2 years

Sample size
The present study comprised of two groups :- 32 mothers who were delivering preterm were chosen as cases and the controls were 32 mothers who were delivering full term .Informed consent was taken from all mothers in both groups. The sample size was chosen after detailed discussion with the Statistician of Pad.Dr.D.Y.Patil Medical College.

Inclusion criteria
Women who delivered prematurely were included irrespective of parity and method of delivery.

Exclusion criteria
Past history of diabetes mellitus, gestational diabetes, smoking, fetal distress as a cause of prematurity.

Sampling Technique
About 10 ml of blood sample was collected by venepuncture from the mothers prior to onset of labor . 8ml sample was collected in plain bulb and 2ml sample was collected in ACD bulb for estimation of reduced glutathione. Immediately on delivery of infant, a segment of cord approximately 15-30cm long was doubly clamped. To ensure good vessel filling, the clamp was placed on the cord close to the infant and then the cord was milked from placenta towards the first clamp. Subsequently a second clamp is placed 10-35 cm distal to the first clamp. About 10 ml of cord blood was collected at the time of delivery after cutting of cord following premature delivery or during caesarean section in cases of prematurely terminated pregnancies whether planned or emergency. 8ml sample was collected in plain bulb and 2ml sample was also collected in ACD bulb for estimation of reduced glutathione. The samples were taken to the laboratory for prompt analysis.

Estimation of Superoxide dismutase was done by method of Marklund and Marklund [14]. Estimation of Erythrocyte Glutathione concentration was done by method of Buetler E, Duron and O Kelly. [15]. Estimation of serum malondialdehyde (MDA) was done by the method of Wilbur K.M. et al, Bernheim F. Shipiro O [16].Estimation of uric acid was done by uricase method(end-point) [17]

Results

The mean maternal age at full term delivery was 25.4±2.7 years(Mean ± SD) (Table 1)

The mean maternal age at preterm delivery was 27.8±2.6 years(Mean ± SD) (Table 2)

Mean Superoxide dismutase (SOD) levels in full term deliveries (Table 3) in maternal blood was *3.77±0.76units/ml and in cord blood it was *3.90 ±0.90 units /ml. In case of prematurity, the mean values were *2.57±0.49units/ml in maternal blood and in cord blood it was *2.40±0.44nmol/ml (Table 3). So, the levels of Superoxide dismutase levels were lower in preterm deliveries than in full term deliveries in both maternal and cord blood.*(mean ± SD)

Mean Reduced Glutathione (GSH) in erythrocytes in full term deliveries in maternal blood was *5.38±0.77µmol/g Hb (Table 1) and in cord blood it was *4.80 ± 1.20 µmol/g Hb (Table 3). In case of prematurity, the mean was*3.84±0.55µmol/ g Hb(Table 3) in maternal blood and in cord blood it was *3.00± 0.63µmol/g Hb(Table 3). So, the levels of Reduced Glutathione levels in erythrocytes were lower in preterm deliveries than in full term deliveries in both maternal and cord blood. *(mean ± SD)

Mean MDA levels in full term deliveries in maternal blood was *3.97±0.92nmol/ml(Table 3) and in cord blood it was *4.46±0.86nmol/ml. In case of prematurity, the mean was *7.47±1.13nmol/ml(Table 3) in maternal blood and in cord blood it was *7.38±1.06nmol/m(Table 3) . So, the levels of Serum Malondialdehyde levels were higher in preterm deliveries than in full term deliveries in both maternal and cord blood. *(mean ± SD)

Mean Uric acid levels in full term mothers was *6.32±1.47/dl(Table 3) and in cord blood it was *7.38±1.40mg/dl. In case of prematurity, the maternal mean level was *4.96±1.24mg/dl(Table 3) and in cord blood it was *7.38±1.06mg/dl. *(mean ± SD)

The data was analyzed for normality before calculating correlation .It had normal distribution. Pearson's correlation was hence applied.

The Pearson's correlation factor 'r' for full term deliveries in maternal and cord blood was 0.62 for MDA, 0.52 for

SOD, 0.53 for GSH and 0.28 for Uric acid.(Table 4).The Pearson's correlation factor 'r' for preterm deliveries in maternal and cord blood was 0.72 for MDA, 0.81 for SOD, 0.57 for GSH and -0.29 for Uric acid (Table 4).

Thus, for Malondialdehyde, Superoxide dismutase and Reduced Glutathione, there was positive correlation both in full term deliveries and preterm deliveries. The correlation coefficients of these parameters in preterm deliveries were higher than those of full term pregnancies.
There was insignificant correlation between maternal and cord blood Uric Acid levels in full term deliveries and in preterm pregnancies there was insignificant negative correlation of the same.

Table 1. *Sociodemographic factors for full term deliveries*

Age wise distribution of full term mothers

Age group	No of mothers(out of 32)	% of Total
15-20 years	0	0
21-25 years	15	46.87
26-30years	15	46.87
31-35years	2	6.25
>35 years	0	0

Mean maternal age at delivery :- 25.4±2.7 (Mean ± S D)

Educational status of full term mothers

Education level	No of mothers(out of 32)	% of Total
Primary school	8	25
Secondary school	10	31.25
Senior secondary school	1	3.12
Graduation	10	31.25
Post –graduation	3	9.37

Employment status of full term mothers

Employment status	No of mothers(out of 32)	% of Total
Employed	9	28.12
Unemployed	23	71.87

Maternity status of full term mothers

Gravida	No of mothers(out of 32)	% of Total
1^{ST} gravid	23	71.87
2^{ND} gravid	9	28.12
3^{RD} gravid	0	0
4^{TH} gravid	0	0

Table 2. *Sociodemographic factors for preterm deliveries*

Age wise distribution of preterm mothers		
Age group	**No of mothers(out of 32)**	**% of Total**
15-20 years	0	0
21-25 years	6	18.75
26-30years	16	50.00
31-35years	10	31.25
>35 years	0	0
Mean maternal age at delivery :- 27.8 ±2.6 (Mean ± SD)		
Educational status of preterm mothers		
Education level	No of mothers(out of 32)	% of Total
Primary school	9	28.12
Secondary school	5	15.62
Senior secondary school	1	3.12
Graduation	10	31.25
Post –graduation	7	21.87
Employment status of preterm mothers		
Employment status	No of mothers(out of 32)	% of Total
Employed	13	40.62
Unemployed	19	59.37
Maternity status of preterm mothers		
Gravida	No of mothers(out of 32)	% of Total
1ST gravid	18	56.25
2ND gravid	7	21.87
3RD gravid	5	15.62
4TH gravid	2	6.25
Causes of preterm deliveries		
Cause of preterm delivery	No of mothers(out of 32)	% of Total
Twin pregnancy	9	28.12
Placenta praevia	6	18.75
Premature rupture of membranes	10	31.25
Eclampsia	6	18.75
Abruptio placentae	1	3.12

Table 3. *Levels of MDA reduced Glutathione, SOD and Uric Acid in full term and preterm deliveries.*

Type of Sample	Sample size (n)	MDA (nmol/ml) Mean ± SD	Superoxide Dismutase (units/ml) Mean ± SD	Reduced Glutathione (μmol/gm Hb) Mean ± SD	Uric Acid (mg/dl) Mean ±SD
Full term deliveries					
Maternal Blood	32	3.97±0.92	3.77±0.76	5.38±0.77	6.32±1.47
Cord Blood	32	4.46±0.86	3.90 ±0.90	4.80 ± 1.20	7.38±1.40
Preterm deliveries					
Maternal Blood	32	7.47±1.13	2.57±0.49	3.84±0.55	4.96±1.24
Cord Blood	32	7.38±1.06	2.40±0.44	3.00± 0.63	7.08±1.1

Inference :-

- *Mean Malondialdehyde levels in maternal blood were higher in preterm pregnancies than full term pregnancies .Mean Malondialdehyde in preterm babies was higher than full term babies.*
- *Mean maternal SOD and GSH values were lower in preterm than full term deliveries.*
- *Mean cord blood SOD and GSH values were lower in preterm than full term deliveries.*
- *Uric Acid levels showed no significant change in both groups.*

Table 4*. Correlation between maternal blood and cord blood parameters in full term and preterm term pregnancies*

Parameter	Pearson's correlation (r)	p-value
Full term deliveries		
Malondialdehyde	0.62	p<0.001
Superoxide Dismutase	0.52	P=0.002
Reduced Glutathione	0.53	p<0.001
Uric Acid	0.28	p=0.09
Preterm deliveries		
Malondialdehyde	0.72	p<0.001
Superoxide Dismutase	0.81	p<0.001
Reduced Glutathione	0.57	P=0.001
Uric Acid	-0.29	p=0.17

Inference :-

- *There was positive correlation between maternal and cord blood with Malondialdehyde, Superoxide dismutase and Reduced Glutathione, both in full term deliveries and preterm deliveries.*
- *There was insignificant correlation between maternal and cord blood Uric Acid levels in full term deliveries and in preterm pregnancies there was insignificant negative correlation of the same.*

Discussion

In this study, the mean values of Superoxide dismutase and Reduced Glutathione were found to be lower in preterm than in full term deliveries for both cord and maternal blood .There is positive correlation of both parameters between maternal and cord blood in full term as well as in preterm .This suggests a relatively immature and deficient antioxidant defense mechanism in the preterm babies. This also reflects that wherever antioxidant levels are less in mothers, the levels are lower in cord blood as well. The results are in agreement with those of G.Baydas et al [7], Falciglia Horacio S. et al [8], Nassi N et al [12]and Zadrozna M. et al[13].

Serum Uric Acid levels showed no significant change in full term and preterm in both maternal and cord blood.

There is no correlation between maternal and cord blood in full term and in preterm deliveries.

In this study the levels of Serum Malondialdehyde levels were significantly higher in preterm deliveries than in full term deliveries in both maternal and cord blood. There is positive correlation between maternal and cord bloods in full term as well as preterm deliveries, though the values in full term are within normal range. This indicates an increased oxidative stress leading to increased rate of lipid peroxidation in preterm deliveries which might contribute to prematurity and its complications. The results are in agreement with Nassi N [12].

It should be remembered that a strong correlation exists between both premature births and low socioeconomic status[1]. In families of low socioeconomic status there are relatively high incidences of maternal undernutrition, anaemia, inadequate prenatal care, drug addiction and high rates of maternal obstetric complications like recurrent abortions, stillbirths and premature deliveries. Supplementation of antioxidants in such groups may be beneficial.

In 2003, a meta-analysis by Darlow BA and Austin NC [18] found that supplementation of Se to very preterm infants was associated with benefit in terms of reduction in one or more episodes of sepsis. Rumiris D et al [11] also found lower rates of preeclampsia after antioxidant supplementation in pregnant women.

However more research is needed to demonstrate actual benefits and harm if any, to the growing fetus, if antioxidant supplementation is done in pregnancy. It also remains to be seen if the supplementation may minimize or prevent preterm births and prevent the occurrence of the diseases of prematurity.

Strengths of the Study

Maternal blood was collected before onset of labor pains so that maternal stress during delivery would not affect the outcomes. Smokers and Diabetics were excluded .Samples from babies with fetal distress ending up in premature deliveries were not taken.

Limitations of the Study

The process of child birth itself could have caused an oxidative stress in the fetus .This could have affected the outcomes in cord blood samples. Several independent risk factors for oxidative stress like eclampsia, twin-pregnancy coexisted with the premature deliveries.

Conclusion

The study found that the levels of antioxidants like Superoxide dismutase and reduced glutathione are lower in preterm mother and children and there is a positive correlation between maternal and fetal levels. Lipid peroxidation was also accelerated in preterm labour in both mother and child and there was a positive correlation between them.

Acknowledgement

The study was conducted in Department Of Biochemistry, Pad.Dr.D.Y.Patil Medical College, Pune, Maharashtra, India, with approval of Institutional Ethical Committee.

We are thankful to all the staff members of Department of Biochemistry , Pad.Dr.D.Y.Patil Medical College , Pune , India and to the Department of Gynaecology and Obstetrics , Pad.Dr.D.Y.Patil Medical College , Pune , India for their valuable assistance and cooperation in this research work.

References

1. Barbara J.Stoll, Robert M.Kliegman, Prematurity and intra uterine growth retardation.In:Richard.E.Behrman, Robert M.Kliegman, Hal B.Jenson, Robert M.Kliegman,editors. Nelson's textbook of paediatrics .17th edition. Pennsylvania: Elselvier; 2004.p.550-65.
2. Fiona.B.Pipkin , Maternal Physiology in Pregnancy.In: Geoffrey Chamberlain, Philip J. Steer, editors.Turn Bull's Obstetrics. 3rd edition. Churchill Livingstone, 2002.p.77-78.
3. Deborah Schofield , Diseases of infancy and childhood. In: Ramzi S. Cotran, V.Kumar, Tucker Collins, editors. Robbins pathological basic of disease .6th edition. Philadelphia:W.B. Saunders Company;1999.p.473 – 86.
4. Elizabeth H.Thio, Adam A. Rosenberg, The Newborn Infant .In: William W.Hay, Anthony R. Hayward, editors. Current Paediatric diagnosis and treatment. 16th edition. NewYork:Mc Graw Hill; 2003.p.30 -41.
5. Richard D.Bland ,The Newborn Infant. In: Colin D. Rudolph, Abraham M. Rudolph, Margaret K. Hostetter, George Lister, Norman J. Siegel ,editors. Rudolph's paediatrics 21st edition. New York:Mc Graw Hill.2003: p.110.
6. Ola Dirik Sangstad, Hypoxia induced free radical disease in the neonatal period .In: S Arulkumaran and HML Jenkins,editors.Obstetrics and Gynaecology in Perspective.Hyderabad: Orientlongman;2000.p.229-34.
7. G.Baydas.Antioxidant Vitamin Levels in Term and Preterm Infants and Their Relation to Maternal Vitamin Status.Arch Med Res. 2002; 33(3):276-80.
8. Falciglia HS, Johnson JR, Sullivan J ,Hall CF. , Miller J , Riechmann GC, et al. Role of antioxidant nutrients and lipid peroxidation in premature infants with respiratory distress syndrome and bronchopulmonary dysplasia.American journal of perinatology. 2003, 20(2):97-107

9. Frosali S, Di Simplicio P, Perrone S, Di Giuseppe D, Longini M, Tanganelli D et al. Recycling and antioxidant enzyme activities in erythrocytes of term and preterm newborns at birth. Biol Neonate. 2004;85(3):188-94
10. Gupta P, Narang M, Banerjee BD and Basu S.Oxidative stress in term small for gestational age neonates born to undernourished mothers: a case control study.BMC Pediatr. 2004; 4:14
11. Rumiris D, Purwosunu Y, Wibowo N, Farina A, Sekizawa A. Lower rate of preeclampsia after antioxidant supplementation in pregnant women with low antioxidant status.Hypertens Pregnancy. 2006;25(3):241-53.
12. Nassi N, Ponziani V, Becatti M, Galvan P, Donzelli G.Anti-oxidant enzymes and related elements in term and preterm newborns.Pediatr Int. 2009 ; 51(2):183-7.
13. Zadrozna M, Gawlik M, Nowak B, Marcinek A, Mrowiec H, Walas S, Wietecha- Posłuszny R, Zagrodzki P. Antioxidants activities and concentration of selenium, zinc and copper in preterm and IUGR human placentas. J Trace Elem Med Biol. 2009; 23(2):144-8.
14. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase.Eur J Biochem. 1974 ;47(3):469-74
15. .Buetler E, Duron, O Kelly. Improved method for the determination of blood Glutathione .B M J Lab Clin Med. 1963, 61: 882-888.
16. Wilbur KM, Bernheim F, Shapiro OW. The thiobarbituric acid method for malondialdehyde estimation.Arch Biochem Biophys 1943; 250:305-13
17. Fossati, P., Prencipe, L. Chromogenic System for Measuring Hydrogen Peroxide: The Enzymatic Uric Acid Assay.Clin Chem 1980;26: 227–31.
18. Darlow BA, Austin NC. Selenium supplementation to prevent short-term morbidity in preterm neonates.Cochrane Database Syst Rev. 2003; (4): CD003312.

Correspondence to:

Soumitra Chakravarty
Department of Biochemistry
Dhanalakshmi Srinivasan Medical College and Hospital
Siruvachur, Perambalur, Tamilnadu , India.
Email: dr.soumitra@gmail.com