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EVALUATION OF OXIDATIVE STRESS IN KIDNEY TRANSPLANT RECIPIENTS.

Biochemistry

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ABSTRACT

Kidneys are vital organs playing various functions important to sustain life. The key function of kidney is to remove waste material from body via filtration. If this ability of kidney is lost the resulting condition is called End Stage Renal Disease (ESRD).
This is life threatening condition and requires either hemodialysis or Kidney transplant. The former need to be performed on regular basis, where as latter can be done once. However, transplant needs to be taken care by life time medications.
As result of transplant and a treatment associated with it, there is an alternation in certain biochemical parameters. Oxidative stress is the state which has more burden of free radicals (oxidants) as compared with antioxidants. Antioxidants nullify the deleterious effects of free radicals. Oxidative stress has been found to play a crucial role in causation of many disease. The current study revealed how the markers of oxidative stress changes during transplant process.
The study has been conducted on 31 kidney transplant recipients and 31 sex-age matched healthy controls. MDA levels were found to be significantly high in pre-transplant as compared to control but after transplant there was significant reduction noted. Whereas Total antioxidant capacity and other antioxidant enzymes have been found to increase gradually after transplantation. Our study also revealed mild effect of immunosuppressant regimen on oxidative stress.

KEYWORDS

Oxidative stress, Glutathione Peroxidase, Antioxidants, Free radicals, MDA, Superoxide dismutase, Total antioxidant Capacity.

INTRODUCTION

Chronic kidney disease (CKD) is a progressive loss in renal function over a period of months or years. Based on creatinine levels, eGFR (estimated glomerular filtration rate) and extend of kidney damage, CKD is divided into different phases from stage I to stage V, where stage V is considered to be the severe. Stage 5 CKD is often called end stage renal disease (ESRD), end stage renal failure (ESRF), or end-stage kidney disease (ESKD) (1,2,3).

ESRD is global problem that affects considerable number of world population. These patients need life time hemodialysis which throws lot of restrictions on them. Kidney transplant is the other way to overcome the problem of ESRD. Past few decades, kidney transplantation has become the choice of treatment in the patients with ESRD.In India though transplant procedure had been in practice since last 50 years, in recent years the procedure has taken a considerable leap. It is now a second largest Programme in world after US (1). The burden of this disease in India cannot be accurately assessed. But approximately the prevalence is measured to 150-200 per million of population (1,3)

Free radicals are uncharged highly reactive unpaired valency electron molecules. These free radicals are oxygen radicals hence also called as reactive oxygen species (ROS). Oxidative stress (OS) occurs when these ROS submerge the capacity of antioxidant thereby leading to increased lipid peroxidation in the organism (4). OS is said to be a major facilitator of undesirable harmful consequences throughout the course of transplantation. Transplanted kidneys are susceptible to OS – mediated injury by pre- and post-transplant conditions that causes reperfusion injury or imbalance between oxidants and antioxidants (5). OS boosts the inflammation process which in succession of processes intensifies the effect of ROS thereby activating the immune system increasing the chances of allorejection.

ESRD patients are already burdened with oxidative stress. As the renal damage progresses the oxidative stress tend to gradually increase. (2) Assuming that the kidney dysfunction is the major reason to induce oxidative stress, this could start reversing after transplantation. CKD is associated with inflammation and many other biochemical parameters. (6) Further immunosuppressants are administrated to the kidney transplant patients which could also have its own effect on oxidative stress. Current study was therefore planned to evaluate the cumulative effect of all these factors on oxidative stress in kidney transplant recipients.

MATERIAL AND METHODS:

31 patients who were awaiting kidney transplant were selected for study. It was conventional sampling with observation and analytical study. Those who undergoing hemodialysis and having eGFR ≤15ml/min were included. Informed written consent was obtained from all the individual participants included in the study. Also, for those patients who were not in condition to provide self-consent, the consent was taken from their family head/ members. All procedures performed in the study were in accordance with the ethical standards and followed as per the norms of the Institutional Ethical Committee approval. The patients were receiving immunosuppressants (Tacrolimus) therapy as per Kidney Disease: Improving Global Outcomes (KDIGO) (7) guidelines and was part of routine patient management. 31, age and sex matched healthy controls were selected from same socioeconomic group.

Inclusion and exclusion criteria:

The patient who lost the follow-up have been excluded. Those who were having habits of smoking, tobacco chewing, alcohol have also been excluded. Subjects in both the groups were not under any medication or antioxidant treatment which could directly affect the study parameters.

Sample collection and procedure:

10 ml of blood sample was collected before transplant and then for the period of 6 months after transplant from each subject. The intervals of sample collected and analyzed has been depicted in Table. 1. Samples were processed as per requirement of the standard method used for determination of biochemical parameters.

Colorimetric Assay of serum Superoxide Dismutase (SOD) was carried out by RANSOD kit. (8) Glutathione peroxidase (GPx) activity was measured by using Biovision GPx kit. (9) Total Antioxidant Status (TAS) was done using RANDOX kit which was based on ABTS (2,2'-Azino-di [ethylbenzthiozolinesulphonate]) method. (10). The index of lipid peroxidation was estimated in the form of Malon-Dialdehyde (MDA) by using Sigma Aldrich kit which is based on Thio-Barbituric acid method. (11).

Statistical Analysis:
After collection of data the statistical analysis was done by using MS-Excel-2019 software. Values were expressed in mean±SD and were compared by using students' t test. Correlation coefficient was calculated by using Pearson's method.

RESULTS
After analysis results obtained are mentioned in following table. All the values are expressed in mean±SD. All the parameters of Group I were compared with pre transplant (Pre-Tx) samples obtained from Group II patients by using unpaired 't' test. Whereas Pre-Tx group was then compared with same patients after transplantation with regular intervals as mentioned in table by using paired 't' test. All antioxidant parameters were correlated with MDA by using Pearson's correlation test. P value ≤0.05 was considered as significant.

Study had produced following results for various parameters in Group I and II.

Table 1 : Serum SOD, GPx , TAS and MDA levels.

Parameters	Controls (Group I) (n=31)	Transplant recipients (n=31) (Group II)					
		Pre-Tx	1st day	7th day	1 Month	3 Months	6 Months
SOD (U/ml)	303±27	175.3±17*	210.3±20#	247.6±24#	264.9±25#	288.7±28#	300.3±29#
GPx (U/ml)	2.71±0.28	0.53±0.23*	1.03±0.23#	1.97±0.20#	2.53±0.30#	2.63±0.30#	2.71±0.32#
TAS(mMol)	1.78±0.21	0.68±0.16*	0.81±0.19#	0.94±0.22#	1.13±0.26#	1.37±0.32#	1.65±0.38#
MDA(nMol/ml)	1.27±0.39	7.90±1.4*	6.37±1.3#	4.29±1.2#	2.63±0.7#	2.13±0.6#	1.68±0.6#

* p value ≤ 0.05 when compared with control, # p value ≤ 0.05 when compared with Pre-Tx

Our results have shown that the patients with ESRD were burdened with oxidative stress which was reflected in the increased levels of serum MDA. These levels have found to be decreased significantly (p≤0.05) immediately after the transplant. However MDA values have returned close to normal only after the period of 1 month and then normalized after 6 months. All antioxidant parameters were found to be significantly decreased in ESRD (Pre-Tx) patients as compared with controls. All of these parameters have shown positive improvement over the period of 6 months after transplant. Negative correlation (r=-0.81) was revealed in MDA and TAS levels. Whereas positive correlation was observed with all antioxidant parameters with TAS. Similar observations were made with other antioxidant parameters as compared with MDA.

DISCUSSION
Microvascular network of kidney is the unique and essential to sustain various processes and thereby life. Intrarenal vasculature is heterogeneous and medulla resides in hypoxic milieu. This causes energy deprivation which can be controlled by various molecules such as prostaglandins, hormones, Nitric oxide etc.(6). Nitric oxide on one hand is useful molecule for signal transduction but on other hand is a potential free radical species of nitrogen and oxygen together which in excess can shift the balance towards oxidative stress.

Different mechanisms will explain why oxidative stress is existing in ESRD patients. The basic characteristics of renal patients is diabetes, hypertension and advanced age. Many literatures suggest that these diseases are already associated with oxidative stress (11). Gradual decrease in oxidative stress in our study is indicative that the functional graft can normalize abnormalities associated through oxidative stress (Figure 1). The significant improvement was observed within a month of transplant. However thereafter the improvement was found to be listless. Our results were similar to AbdulgafarVural et.al. (11), who had assessed these parameters till 28 days after transplantation.

In addition to this, ESRD causes dysregulated metabolic waste disposal which results in an environment,susceptible to increase production of OS. Hemodialysis does not improve oxidative stress, instead with every dialysis it exhausts the antioxidant capacity of the body. (12) However, after transplantation, as suggested by our study status of oxidative stress started inclining in favour of antioxidant. GPx activity increases significantly after transplant as kidney is one of the major organ which synthesizes this enzyme. (11,13)

Calcineurin inhibitors such as Tacrolimus and Cyclosporine are known to induce acute and chronic nephrotoxicity. Both drugs mediate their effect through LDL related oxidative mechanism. (14,15,16) However there are other contradictory research articles (17,18) which argued the effect of these drugs on oxidative stress. In our study we could observe mild effect of the drug. Considering the fact that transplantation reduces oxidative stress MDA levels have found to be declined in one month. The effect of drugs can be visible as the MDA levels had then showed gradual decrease with improvement of antioxidant status. We suggest that this improvement could have been achieved quite earlier with antioxidant treatment as an adjuvant therapy.

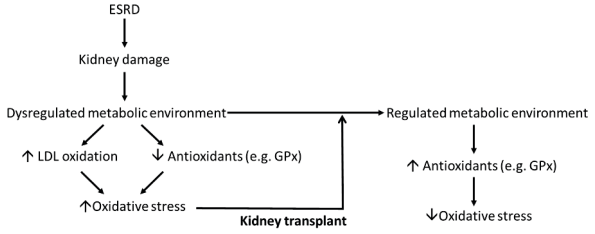

Figure 1: Overall effect of kidney transplant on oxidative stress

CONCLUSION
Our study suggests that ESRD patients are burdened with oxidative stress. Kidney transplant improves this to the maximum extend though do not entirely. This could be due to mild effect of immunosuppressants. This can be overcome by treating kidney recipients with antioxidant supplements.

Limitations:
Our study comprises small sample size. Further all of our patients have administered with Tacrolimus as an immunosuppressant hence effect of other calcineurin inhibitors remains unrevealed. Follow-up period of only six months could not catch up any graft dysfunction. Study does not include effect of antioxidant therapy as subjects are not given any antioxidants along with conventional therapy.

REFERENCES

1. Sunil Shroff Current trends in kidney transplantation in India.Indian J Urol. 2016 Jul-Sep; 32(3): 173–174.
2. Natalia Krata, RadosławZagożdżon, BartoszForoncewiczet.al.Oxidative Stress in Kidney Diseases: The Cause or the Consequence?.ArchivumImmunologiae et TherapiaeExperimentalis (2018) 66:211–220
3. Wolfe RA, Ashby VB, Milford EL, et al. Comparison of mortality in all patients on dialysis awaiting transplantation and recipients of a first cadaveric transplant. N Engl J Med. 1999; 341(23): 1725-30
4. Tucker PS, Scanlan AT and Dalbo VJ Chronic Kidney diseases influences multiple systems: describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. Oxid Med Cell Longev 2015:806358
5. Cerrillos-Gutierrez J, Miranda-Diaz A, Preciado-Rojas P, et al.The beneficial effect of Renal transplantation on altered oxidative status of ESRD patients.Oxi Med CelluLonge. 2016
6. SimonaMihai, Elena Codrici, IonelaPopescueet.al. Inflamation related mechanisms in Chronic Kidney Disease prediction, Progression and Outcome.Journal of Immunology Research Volume (2018) 1-17
7. Margaret Bia, Deborah B. Adey, Roy D. Bloom et al.KDOQI US Commentary on the 2009 KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients.American Journal Of Kidney Diseases, 2010, Vol 56 No 2: 189-218
8. Wooliams, JA, Wiener G et al RANSOD kit by RANDOX laboratories-RX MONZA SD 125Method- CH Research in Veterinary Sciences 1983, 34: 253-256
9. Miller N.J. Rice Evans , Davies et al TAS kit by RANDOX laboratories-NX2332Method- Clinical Sciences (1993) 84, 407-412
10. Lipid Peroxidation (MDA) Assay kit Sigma Aldrich -CNMAK085Method: TBA
11. AbdulgaffarVural, MahmutIlkerYilmaz, KayserCaglaret. al.Assessment of Oxidative Stress in the Early Posttransplant Period: Comparison of Cyclosporine A and Tacrolimus-Based Regimens.Am J Nephrol 2005;25:250–255
12. Natalia Krata, RadosławZagożdżonet. al. Oxidative Stress in Kidney Diseases: The Cause or the Consequence?ArchivumImmunologiae et TherapiaeExperimentalis (2018) 66:211–220
13. Bronisław A. Zachara, JolantaGromadzińskaet.al. Red blood cell and plasma glutathione peroxidase activities and selenium concentration in patients with chronic kidney disease: A review. ActaBiochemicaPolonica Vol. 53 No. 4/2006, 663–677
14. Ahmed SS, Strobel HW, Napoli KL, Grevel J Adrenochrome reaction implicates oxygen radicals in metabolism of cyclosporine A and FK-506 in rat and human liver microsomes. J PharmacolExpTher 1993; 265: 1047–1054.
15. Varghese Z, Fernando RL, Turakhia G et.al. Oxidizability of low-density lipoproteins from Neoral and tacrolimus-treated renal transplant patients. Transplant Proc 1998; 30: 2043–2046.
16. Apanay DC, Neylan JF, Ragab MS et.al. Cyclosporine increases the oxidizability of low-density lipoproteins in renal transplant recipients. Transplantation 1994; 58: 663–669.
17. McGrath LT, Treacy R, McClean E, Brown JH Oxidative stress in cyclosporin and azathioprine treated renal transplant patients. ClinChimActa 1997; 264: 1–12.
18. Morris-Stiff GJ, Oleesky DA, Smith SC, et.al. Sequential changes in plasma selenium concentration after cadaveric renal transplantation. Br J Surg 2004; 91: 339–343.