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JBB

RFP Journal of Biochemistry and Biophysics

January - June 2019
Volume 4, Number 1

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Original Article

RFP Journal of Biochemistry and Biophysics
Volume 4 Number 1, January - June 2019

Assessment of Hematological Parameters to Study the Effect of Thiamine Hydrochloride on Lead Acetate Induced Toxicity in Wistar Rats

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How to cite this article:

P. Jasmin Lena, D. Sasikala. Assessment of Hematological Parameters to Study the Effect of Thiamine Hydrochloride on Lead Acetate Induced Toxicity in Wistar Rats. RFP Journal of Biochemistry and Biophysics. 2019;4(1):5-9

Abstract

The effect of thiamine hydrochloride against lead induced acute toxicity was experimentally studied in rats. Hematological parameters such as WBC count and differential count were determined and a decrease in their levels were observed throughout study period in the lead acetate induced animals. Cholesterol level was also found to be decreased throughout the period of study, in lead acetate induced animals. Biochemical alterations were reversed on treatment with thiamine hydrochloride due to its ameliorative role in lead induced rats.

Keywords: Lead acetate; Thiamine hydrochloride and WBC Count.

Introduction

Lead is a ubiquitous environmental pollutant that has been detected in various phases of environmental and biological systems. Lead induces a broad range of physiological, biochemical, and behavioural dysfunction in research animals and humans, including central and peripheral nervous systems, haemopoietic system, cardiac system, liver, kidneys and human reproductive systems [1]. Lead has its effects on the peripheral nervous system in adults while in children the central nervous system is much more affected [2]. Encephalopathy, lack of coordination, convulsions, paralysis and coma are the effects of lead on central nervous system [3]. It inhibits various key enzymes involved in the heme synthesis and affects the hematopoietic system. It increases the fragility of cell reducing the lifespan of erythrocytes. These two processes results in anemia [4,5]. Acute and chronic nephropathy is the renal abnormality that occurs due to lead exposure [6].

Common effects of lead seen in men include: abnormal spermatogenesis, chromosomal damage, infertility, altered prostatic function and changes in serum testosterone. Infertility, premature membrane rupture, pre-eclampsia, pregnancy hypertension and premature delivery [7] are the effects of lead on women.

Lead exposure induces free radicals generation that results in the pathogenesis, which could be overcome by antioxidant supplementation, an alternative for chelation therapy [8]. This vitamin may chelate lead from the tissues. The pathogenesis of lead toxicity might be due to its direct interruption in enzyme activation, competitive inhibition of trace mineral absorption, interrupts structural

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Received: 27.05.2019 | **Accepted:** 18.06.2019

protein synthesis by binding to sulfhydryl proteins, alteration in calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body [9,10], reported that thiamine scavenges superoxide and hydroxyl radicals thereby reduces the oxidative stress. Thiamine is a water soluble sulphydryl group containing vitamin, the most recommended therapeutic agent for the lead toxicity studies. Researchers have postulated that thiamine plays role in the decrease in lead absorption and stimulates its excretion [11]. The present study aimed to investigate the ameliorative effects of using thiamine hydrochloride on lead toxicity in albino rats using blood parameters as indicators of oxidative stress.

Materials and Methods

Male Albino rats with the weights ranging from 100-160g, were purchased from Agricultural University Extension Centre, Kattupakkam, Chennai, were kept at room temperature (32 ± 2^0C) at L:D (12:12) cycles. Experiments were done in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals" [13]. Animals were categorized into four groups of six animals in each group (Group I – control, Group II – lead acetate-treated, Group III – lead acetate and thiamine hydrochloride treated, Group IV – thiamine hydrochloride treated). All animals were acclimatized to laboratory conditions before the experiment. Animals were maintained in polypropylene cages and provided with standard food pellets and *ad libitum*. [CPCSEA No – IAEC 1/2008/02]. Thiamine hydrochloride was purchased from Sisco Research Laboratories Private Limited, Mumbai India. All chemicals inclusive of lead acetate used were of analytical grade. Group I animals served as control. Group II animals were administered with lead acetate intraperitoneally (100 mg/kg) every day for 14 days [14]. Group III animals were administered with Thiamine hydrochloride (150 mg/kg) (ip) [15] and lead acetate Intraperitoneally (ip) (100 mg/kg) every day for 14 days. Group IV animals received Thiamine hydrochloride (150mg/kg) (*ip*) for 14 days. Biochemical determinations were performed after 14 days of lead acetate and/or thiamine hydrochloride administration. At the end of experimental period (14 days) animals from all groups were sacrificed by cervical dislocation. Blood samples were collected from each group of rats. In one tube blood was collected and WBC Count [16], Differential count [17] was performed. In another tube blood was collected and left aside to clot. Cholesterol estimation was done by [18].

Analysis of variance followed by Least Significant Difference test was carried out to detect the significant differences between control and the other groups.

Results

Table 1: Total WBC count of control and experimental rat (*Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Parameter	Exposure period (No. of days)	Control (cells/cu.mm)	Lead Acetate (cells/cu.mm)	Lead Acetate+ Thiamine Hydrochloride (cells/cu.mm)	Thiamine Hydrochloride (cells/cu.mm)
WBC Count (cells/cu.mm)	7th Day	2.2 ± 0.04	2.6 ± 0.02	1.2 ± 0.05	1.9 ± 0.07
	14th Day	1.6 ± 0.01	2.8 ± 0.01	1.15 ± 0.02	1.62 ± 0.05

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Table 2: Differential leucocyte Count of control and experimental rat (*Rattus norvegicus*) exposed to Lead Acetate and Thiamine Hydrochloride

Parameter	Exposure period (Days)	Control (%)	Lead acetate (%)	Lead acetate + Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Lymphocyte	7th Day	65.25 ± 3.18	43.75 ± 3.03	51.25 ± 3.04	61.25 ± 1.09
	14th Day	70.75 ± 1.02	51.75 ± 2.38	35 ± 1.2	32 ± 1.92

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental group

Table 3: Differential leukocyte count of control and experimental rat (*Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure period (Days)	Control (%)	Lead Acetate (%)	Lead acetate + Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Neutrophil	7th Day	41.5 ± 1.25	40.75± 1.16	37 ± 1.22	32 ± 0.70
	14th Day	30.5 ± 1.11	47 ± 1.22	37 ± 1.41	28.5 ± 1.11

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental groups

Table 4: Differential leukocyte count of control and experimental rat *(Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure period (Days)	Control (%)	Lead Acetate (%)	Lead acetate + Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Basophil	7th Day	5.5 ± 1.11	3 ± 1.11	3.25 ± 0.90	4.75 ± 0.70
	14th Day	6.25 ± 0.90	3.75 ± 0,24	5.5 ± 0.5	5 ± 0.70

Values are expressed as Mean ± SD.

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Table 5: Differential leukocyte count of control and experimental rat *(Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure Period (Days)	Control (%)	Lead Acetate (%)	Lead acetate +Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Eosinophil	7th Day	5.5 ± 0.65	2.75±0.43	5.5 ± 1.65	3.25 ± 0.83
	14th Day	6.75±0.43	4.25 ± 0.78	5.75 ± 0.43	5.5 ± 1.65

Values are expressed as Mean ± SD.

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Table 6: Differential leukocyte count of control and experimental rat *(Rattus norvegicus*) exposed to lead acetate and thiamine hydrochloride.

Leukocytes	Exposure Period (Days)	Control (%)	Lead Acetate (%)	Lead acetate +Thiamine Hydrochloride (%)	Thiamine Hydrochloride (%)
Monocyte	7th Day	6.75 ± 1.11	4 ± 0,70	4.25 ± 0.83	4.25± 0.83
	14th Day	5.5 ± 1.11	4.25 ± 0.82	5.5 ± 0.5	5.5 ± 0.5

Values are expressed as Mean ± SD.

Students 't' test.

p<0.05, 0.01 –significant in all experimental groups.

Table 9: Cholesterol Level in control and experimental rat exposed to lead acetate and thiamine hydrochloride

Exposure Period (Days)	Control (mg/dl)	Lead Acetate (mg/dl)	Lead acetate + Thiamine Hydrochloride (mg/dl)	Thiamine Hydrochloride (mg/dl)
7th Day	154.1 ± 0.05	137.6 ± 0.04	85.55 ± 0.02	120.1 ± 0.01
14th Day	102.6 ± 0.04	80.3 ± 0.02	111.2 ± 0.04	128.5 ± 0.03

Values are expressed as Mean ± SD

Students 't' test

p<0.05, 0.01-significant in all experimental groups.

Discussion

Lead is one of the toxic heavy metals of much significance. Exposure to heavy metals such as lead may cause chronic diseases (diabetes, renal disease, cancer, male infertility etc.) [19]. Oxidative stress represents an imbalance between the production of free radicals and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [7]. It has been reported as a major mechanism of lead induced toxicity [6]. Under the influence of lead, onset of oxidative stress occurs on account of two different pathways operative simultaneously. First, the generation of reactive oxygen species, ROS and second, the antioxidant reserves become depleted [20]. Apart from targeting the sulfhydryl groups, lead can also replace the zinc ions that serve as important cofactors for these antioxidant enzymes and inactivate them [21]. Lipid peroxidation, another indicator of oxidative stress occurs as a result of the action of ROS on lipid membranes [22]. Products of lipid peroxidation disrupts the physicochemical properties, fluidity, and integrity of cell membranes, increasing susceptibility to lipid peroxidation and cell necrosis. Many reports have showed that thiamine detoxifies lead by the formation of complexes with lead [22,23]. lead toxicity has not been clearly elucidated until now. It might be attributed to the formation of complexes between thiamine and lead followed by its excretion. Thiamine also has been found to protect against lead-induced lipid peroxidation in rat liverand kidney [11].

In our study with lead acetate, WBC Count decreased during the entire period of study. The term leucopenia describes a condition characterized by a low white blood cell count. Researchers reported low count of WBC in the disorders of liver and spleen. Hence the decreased WBC Count in the present investigation may be due to damage of the liver caused by lead toxicity. Similar results have been reported when mice were treated with lead chromate [24]. Results show that the lead acetate increased significantly the levels of cholesterol in Group II rats which might be due to oxidative stress caused by lead acetate.

Conclusion

Thus the present study showed that lead acetate induces free radical formation in rats and this condition reverted to the normal as that of control by the treatment with thiamine hydrochloride, which proved the anti protective role of thiamine against the lead toxicity.

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Original Article

RFP Journal of Biochemistry and Biophysics
Volume 4 Number 1, January - June 2019

Utility of Cystatin C in Assessing Glomerular Filtatration Rate in Pregnant Women with Preeclampsia

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How to cite this article:

Krishnamurthy U., Nirmitha Dev M., Meera K.S. Utility of Cystatin C in Assessing Glomerular Filtatration Rate in Pregnant Women with Preeclampsia. RFP Journal of Biochemistry and Biophysics. 2019;4(1):11-14.

Abstract

Background and Objectives: Preeclampsia is a hypertensive disorder of pregnancy associated in the second Trimester of the pregnancy characterized with recent-onset of hypertension and accompanying proteinuria. It affects nearly 5% of all pregnancies, producing substantial maternal and perinatal morbidity and mortality. Kidney injury is one of the complications of preeclampsia complicating the management. Therefore this study intended to find the utility of estimating cystatin C in preeclampsia patients.

Material & Methods: 42 preeclampsia patients and equal number of age and gestation age matched normal pregnant women were enrolled for the study. Blood pressures were measured and mean blood pressure values were recorded. Serum cystatin C and creatinine were estimated. Estimated GFR was calculated using MDRD equation. All the observations were tabulated and analysed statistically using software.

Results: Cystatin C in pregnant women with preeclampsia and in normal pregnant women was found to be 0.91±0.22 and 0.56±0.16 mg/dl respectively. The difference was statistically significant ($p<0.01$).Serum cystatin C levels significantly correlated with blood pressure, serum creatinine levels and eGFR values.

Conclusion: Thus, cystatin C can be utilised to assess the renal function in preeclampsia complicated pregnancy.

Keywords: cystatin c; Preeclampsia; Pregnancy; Renal marker.

Introduction

Preeclampsia is a hypertensive disorder of pregnancy developing in the second half (≥20 weeks) of the pregnancy characterized with new-onset hypertension (BP≥ 140/90 mm Hg) and proteinuria (urinary albumin ≥ 300 mg/24 hr) as the prime characteristics [1]. Preeclampsia has a prevalence of 5-8% of pregnancies worldwide and a much higher rate of prevalence in India varying with the demographic location, and is the second leading cause of direct maternal deaths [2].

Kidney injury is one of the serious complications associated with preeclampsia [3]. In preeclampsia, both glomerular filtration rate (GFR) and renal plasma flow decrease by 30% to 40% compared with normal pregnancy of the same duration [4]. Prolonged renal hypoperfusion can result in acute tubular necrosis that is seen with severe preeclampsia [5]. Presently, GFR is calculated using the serum creatinine value and urinary creatinine levels present in the 24 hour urinary sample. Alternately, estimated GFR (eGFR) is calculated using the formulas like The *Cockcroft* and

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Received: 14.06.2019 | **Accepted:** 22.06.2019

Gault *formula* and The Modification of Diet in Renal Disease (MDRD) equation. Therefore, there is a need of a parameter that can be easily estimated and quantified to identify the kidney injury in terms of the compromise on the glomerular filtration.

Cystatin C is a low molecular weight, basic neuroendocrine polypeptide encoded by CST3 gene cystatin C. It is one of the most important extracellular inhibitors of cysteine proteases and a potent inhibitor of lysosomal proteases. It is expressed ubiquitously and can be found in various biological fluids including serum [6]. It is removed from the bloodstream by glomerular filtration by the kidneys. Conditions associated with the compromise in the renal function are found to be associated with increased cystatin levels in blood [7].

Though estimation of cystatin C is practised to assess the GFR in kidney injury due to various causes like diabetic and hypertensive nephropathy, it is not used to assess GFR in preeclampsia. Also, there are limited studies on estimation of cystatin C in Indian pregnant population. Therefore, this study was undertaken to find the serum levels of cystatin C in preeclampsia patients compared to normal pregnant women.

Materials and Methods

This case control study was conducted in the MS Ramaiah Medical College, Bangalore. Cases included 42 preeclampsia patients admitted in the M S Ramaiah Hospital. Controls include 42 normotensive, healthy women attending the outpatient department at MS Ramaiah Hospital for their antenatal checkups. This sample size was calculated with an expectation to get a result with 80% power, 95% confidence and minimum detectable difference between the two groups as 0.095 mg/l. It was determined to require a minimum of 42 subjects in each group. This study was carried out after obtaining the approval from the institutional ethics committee. Informed consent was obtained after explaining the nature and purpose of the study from all the subjects. Clinically diagnosed preeclampsia patients were included i.e patients with ≥ 20 weeks of the pregnancy, pregnancy induced hypertension with blood pressure ≥ 140/90 mm Hg and urinary albumin ≥ 300 mg in a 24 hr sample. Pregnant women with bad obstetric history, pre existing disorders like diabetes, hypertension, thyroid disorders and any other chronic illness were excluded. Women who developed thyroid illness during the pregnancy and gestational diabetes were also excluded from the study.

About 5 ml of random blood sample was collected in a gel vacutainer and allowed to clot. This is later centrifuged to separate the serum. Serum is aliquoted in ependorff tubes and stored at -20°C. Serum cystatin C and creatinine were estimated on an autoanalyser (Roche – cobas ® 6000 System) by turbidimetry and modified Jaffe's method respectively. Estimated Glomerular filtration rate (eGFR) was calculated by MDRD equation using an online calculator. A serum cystatin C level was presented in terms of mean with standard deviation and descriptive statistical analysis was performed using SPSS version 20.

Results

This case-control study included 42 patients with preeclampsia and 42 normal pregnant women. Both the cases and the controls were aged between 19 to 35 years. The mean age was 25.12 ± 3.62 and 26.62 ± 3.46 years for controls and cases respectively. Seventy five percent of the controls and 66% of preeclampsia subjects were primigravida. The results obtained were tabulated and analysed. This study observed that patients with preeclampsia had significantly raised cystatin C levels ($p < 0.01$) compared to normal pregnant women (Table 1). The cystatin C levels showed correlation with mean blood pressure readings in preeclampsia subjects. It also showed significant correlation with other parameters of kidney injury namely serum creatinine and eGFR.

Table 1: Shows the Mean ± Sd of the Various Parameters

Parameters	Cases	Controls	p Value*
Age (years)	26.62 ± 3.46	25.12 ± 3.62	0.16
Gestational age (weeks)	32.33 ± 3.01	33.29 ± 4.77	0.28
Systolic Blood pressure (mmHg)	145.14 ± 13.62	114.95 ± 7.00	<0.01
Diastolic Blood pressure (mmHg)	96.62 ± 8.19	72.62 ± 6.27	<0.01
Serum Creatinine (mg/dl)	0.86 ± 0.15	0.80 ± 0.11	<0.05
Estimated GFR (Calculated)	97.00 ± 19.16	104.71 ± 16.15	<0.05
Serum Cystatin C (mg/L)	0.91 ± 0.22	0.56 ± 0.16	<0.01

*p<0.01 - Highly significant; p<0.05 – Significant; p>0.05 - Not significant

Table 2: Shows the Pearson's Correlation between Various Parameters

	Age	Gestational age	Systolic Blood pressure	Diastolic Blood pressure	Serum Creatinine	Estimated GFR	Serum Cystatin C
Age		r=0.28 p=0.07	r=0.10 p=0.52	r=0.11 p=0.48	r=0.50 p=<0.01	r=-0.60 p=<0.01	r=0.38 p=0.01
Gestational age	r=0.28 p=0.07		r=0.13 p=0.41	r=0.11 p=0.48	r=0.09 p=0.57	r=-0.11 p=0.48	r=0.25 p=0.11
Systolic Blood pressure	r=0.10 p=0.52	r=0.13 p=0.41		r=0.38 p=0.01	r=0.55 p=<0.01	r=-0.41 p=<0.01	r=0.40 p=<0.01
Diastolic Blood pressure	r=0.11 p=0.48	r=0.11 p=0.48	r=0.38 p=0.01		r=0.48 p=<0.01	r=-0.44 p=<0.01	r=0.41 p=<0.01
Serum Creatinine	r=0.50 p=<0.01	r=0.09 p=0.57	r=0.55 p=<0.01	r=0.48 p=<0.01		r=-0.97 p=<0.01	r=0.41 p=<0.01
Estimated GFR	r=-0.60 p=<0.01	r=-0.11 p=0.48	r=-0.41 p=<0.01	r=-0.44 p=<0.01	r=-0.97 p=<0.01		r=-0.40 p=<0.01
Serum Cystatin C	r=0.38 p=0.01	r=0.25 p=0.11	r=0.40 p=<0.01	r=0.45 p=<0.01	r=0.41 p=<0.01	r=-0.40 p=<0.01	

r - Pearson's correlation co-efficient; p<0.01 - Highly significant; p<0.05 – Significant; p>0.05 - Not significant

Discussion

Because of its wide spread systemic involvement preeclampsia has the potential to produce significant maternal and foetal complications. Acute kidney injury is one of the complications in preeclampsia that compounds the difficulty in treatment of the patient with preeclampsia [8]. Currently the procedures in vogue to measure GFR are not clear and there is a need for an analyte that can be estimated easily, cost effective and interpreted. Cystatin C has been established as a simple and endogenous marker for GFR in clinical nephrology [9]. Utility of cystatin C for detection of renal impairment in preeclampsia women is not well established. Therefore, present study intended to determine the serum cystatin C values in preeclampsia and normal pregnant women and to compare with serum creatinine and eGFR.

This case-control study with 42 patients with preeclampsia and 42 normal pregnant women showed significantly increased cystatin C levels ($p< 0.01$) in pregnant women with preeclampsia. In normal pregnancy, renal plasma flow increases by 40% to 60% resulting in increased GFR [10]. In preeclampsia, both GFR and renal plasma flow decrease by 30% to 40% compared with normal pregnancy of the same duration [11]. Cystatin C in the plasma is removed from the bloodstream by glomerular filtration. It accumulates in preeclampsia due to decreased renal plasma flow and compromised GFR [12]. Thus, cystatin C concentrations increase in blood. This study also showed that cystatin C levels significantly correlated with blood pressures, serum creatinine and eGFR, thus indicating that cystatin C concentrations is in accordance with the existing parameters. Franceschini N et al. not only showed elevated cystatin C levels in preeclampsia cases compared with controls but also showed 12 fold rise for the fourth quartile patients who were distributed based on adjusted odds ratios and 95% confidence intervals [13]. Strevens D et al. performed receiver operating characteristic analysis between cystatin C and serum creatinine. They demonstrated that the serum level of cystatin C had a superior diagnostic accuracy for preeclampsia compared to serum creatinine [14]. Thus cystatin C can be a useful parameter to assess the kidney injury in preeclampsia complicated pregnancy.

Conclusion

Cystatin C levels are raised in the pregnant women with preeclampsia compared to normal pregnant women. Hence, it can be a useful parameter to assess glomerular filtration rate to identify the kidney injury in pregnant women with preeclampsia.

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Original Article

RFP Journal of Biochemistry and Biophysics
Volume 4 Number 1, January - June 2019

Assessment of Health Status among Mine Workers of Maharashtra

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How to cite this article:

Umesh Dhumne, Shalvin Nimje, Sarang Dhatrak et al. Assessment of Health Status among Mine Workers of Maharashtra. RFP Journal of Biochemistry and Biophysics. 2019;4(1):15-19

Abstract

Mine workers are exposed to harsh working condition as they carry out numerous activities with variable exposure which is hazardous to their health in various ways. This study was conducted on 97 randomly selected workers from two different mines of Maharashtra for the assessment of health status. To determine the health status of each worker, weight and height was measured by standard procedure and underwent medical examination and various clinical investigations. Data analysis was performed using Microsoft excel and online Graph Pad statistical software. Majority (80.4%) of the workers belonged to the age group of 30-50 years. Comparatively underweight (22.6%) workers were found more as compared to overweight and obese (12.3%) workers. 30.9% workers had hearing loss of which 9.27% had noise induced hearing loss (NIHL). 8.24% workers had respiratory impairment. Overall prevalence of anaemia was found to be 19.5%. Electrocardiography (ECG) results showed 11.3% workers had significant ECG changes. 19.5% workers were having high blood pressure while one worker each had diabetes and hypertriglyceridemia. 6.18% workers had refractive error. Urine analysis showed that 1 (1.03%) worker was having glycosuria and 5 (5.15%) workers having urinary tract infection. On evaluation of chest X-rays no case of pneumoconiosis and other abnormality was found in the mine worker. Present study suggests that NIHL and respiratory impairment is an important health problem in these mine workers. There is need of periodical medical examination of workers and awareness programme to educate mine workers regarding occupational and non-occupational problems should be regularly arranged.

Keywords: Mining Industry; Mine worker; Health status; Respiratory impairment; NIHL.

Introduction

Mining is an old occupation, being recognized for elevated risk of injury and occupational/non-occupational diseases. In India, workers working in different mining industries are directly or indirectly exposed to various types of hazards. There are about 481.7 million workers in India as per data given in the Indian Labour Yearbook 2015 (source office of registrar general India) out of which 348.9 million workers are in rural areas and 133.1 million workers in urban areas of which unorganised sector accounts for 83% of the total work force [1]. There are about 3703 mines according to Indian Bureau of Mines [2]. However it is known that many unreported mining is also prevalent in many parts of India, which leads to many health hazards going unregistered. In India mining sector contributes approximately 4% to GDP and workers represent half the working population and are the major contributors to economic and social development [3,4].

Mine workers are exposed to various toxic or harmful materials like fuels, coal dust, silica dust,

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Received: 02.05.2019 | **Accepted:** 08.06.2019

noise etc. as a result during the course of period many disorders develop. According to mining processes mining are divided into deep and open cast mine. Deep mines are associated with the risk of higher blood pressure, heat exhaustion, myocardial infarction and nervous system disorders. Surface mining is associated with health risk related to dust exposure [5]. Mines are noisy places and noise is generated by drilling, blasting, cutting, material handling, ventilation, crushing and ore processing, due to which miners are continuously exposed to higher level of noise that leads to increased prevalence of noise induced hearing loss among mine workers [6-8].

Mining activity involves different process like drilling, blasting etc., these processes generate dust which contains free crystalline silica. Exposure to this respirable dust is an important risk factor for many respirable diseases. Long term exposure to respirable dust in mine workers may lead to silicosis, silico-tuberculosis, pulmonary tuberculosis, COPD and occupational asthma. Workers are exposed to dust for short term can cause irritation to the upper respiratory tract [9]. Therefore this study was conducted to assess the present health status of mine workers of Maharashtra.

Materials and Methods

Present health survey comprised of 97 mine workers, randomly selected from various occupations from two different mining industries in Maharashtra for the assessment of health status.

First the workers were registered and basic information was collected from them (name, age, work exposure etc.) then the mine workers underwent medical examination with various clinical investigations. Height and weight was measured using standard techniques. BMI was calculated and classified according to WHO classification [10]. Medical examination of the workers was done with the help of questionnaire including their personal history present and past occupational and medical history etc. Systolic and diastolic blood pressure was measured by using standard mercury sphygmomanometer in the seating position. Hypertension was classified based on the joint national committee seventh classification [11]. Chest radiographs (posterior view) of all the workers were taken on 300 MA X-Ray machines. The chest X-Ray was classified as per ILO classification 2000 [12]. Audiometry test for hearing loss of each worker was conducted in sound proof audiometry booth by using Labat Asia Audiometer. Audiometry test was performed for both air conduction and bone conduction and results were interpreted as per WHO guidelines [13]. ECG was recorded by using 12 Lead Maestros magic RXI machine and interpreted by expert panel. Pulmonary function test was performed with the help of RMS Helios 102 Spirometer. Three readings of spirometry test of each worker were obtained and the best result was analysed as per the American Thoracic Society guideline for assessment of respiratory impairment [14]. Blood sample were taken from the entire workers and the level of haematology and biochemistry parameters were estimated by fully automated haematology and biochemistry analyser respectively. Anaemia was diagnosed on the basis of haemoglobin concentration and severity of anaemia was categorised as per WHO criteria [15]. Routine urine examination for ten parameters was done by strip method. Visual acuity and colour blindness of each worker was measured by Snellen's chart and Ishihara chart. Collected data and findings of various parameters were entered in excel sheet and tabulated. Data analysis was performed using Microsoft excel and Graph Pad statistical software. Variables are expressed in frequency, percentage, mean and standard deviation.

Results

Table 1 shows the distribution of workers according to age group and BMI class. The workers working in this mine are mostly 30-50 years of age group with mean age of 39.3 while only 19.5% workers are 51-60 years of age group with mean age of 54.1. According to BMI, 22.6% workers were underweight with mean BMI 17.1 and 12.3% workers were having BMI more than or equal to 25 with mean BMI 27. Table 2 showed results of the

Table 1: Distribution of workers according to age group and BMI class

Variables	Number	Percentage	Mean ± S.D.
Age groups (years)			
30 - 40	48	49.4	35.9 ± 3.09
41 - 50	30	30.9	44.7 ± 3.15
51 - 60	19	19.5	54.1 ± 2.26
Total	97	100	42.2 ± 7.63
BMI (kg/m2)			
< 18.5	22	22.6	17.1 ± 1.03
18.5 – 24.9	63	64.9	21.7 ± 1.81
25.0 – 29.9	10	10.3	26.2 ± 1.23
≥ 30	2	2.06	31.2 ± 0.01
Total	97	100	21.3 ± 3.34

Table 2: Results of audiometry screening among mine workers

Findings	Number	Percentage
NIHL	9	9.27
High frequency sensorineural hearing loss	7	7.21
Mild hearing loss	9	9.27
Moderate hearing loss	1	1.03
High frequency hearing loss	4	4.12
Normal	67	69.0
Total	97	100

Table 3: Prevalence of respiratory impairment in study subjects

Findings	Number	Percentage
Mild Restriction	5	5.15
Moderate Restriction	1	1.03
Mild Obstruction	1	1.03
Mixed Impairment	1	1.03
Normal	89	91.7
Total	97	100

Table 4: Distribution of the workers according to the level of hemoglobin and age group

Level of hemoglobin (g/dl)	Age group			Total
	30 – 40	41 - 50	51 – 60	
≥ 13.0	45 (93.7)	21 (70)	12 (63.1)	78 (80.4)
11.0 – 12.9	2 (4.16)	8 (26.6)	6 (31.5)	16 (16.4)
8.0 – 10.9	1 (2.08)	1 (3.33)	1 (5.26)	3 (3.09)
> 8.0	0	0	0	0
Total	48	30	19	97

Table 5: ECG findings among mine workers

ECG findings	Number	Percentage
T wave indicating ventricular overload/ischemia	4	4.12
Bundle branch block	4	4.12
Sinus tachycardia	2	2.06
Left ventricular hypertrophy	1	1.03
Total	11	11.3

Table 6: Distribution of workers according to other clinical findings

ECG findings	Number	Percentage
Hypertension	19	19.5
Visual impairment	6	6.18
Defective colour vision	1	1.03
Pterygium	4	4.12
Conjunctivitis	1	1.03
Elevated blood sugar level	1	1.03
Elevated triglycerides level	1	1.03
Glycosuria	1	1.03
Urinary tract infection	5	5.15

audiometry screening among mine workers. 30.9 workers were having hearing loss of which 9.27% had mild hearing loss, 1.03% had moderate hearing loss and 4.12% had high frequency hearing loss. 9.27% had noise induced hearing loss while 7.21% had high frequency sensorineural hearing loss. The prevalence of respiratory impairment in mine workers is shown in table 3. The overall prevalence of respiratory impairment was seen in 8.24% workers of which 5.15% had mild restriction while one worker each had moderate restriction, mild obstruction and mixed impairment. 91.7% of mine workers showed normal pulmonary function test.

Table 4 showed distribution of anemia among workers with association of age. The overall prevalence of anemia among the workers was found to be 19.5%. Among them 16.4% workers had mild anemia while 3.09% had moderate anemia. 16.4% workers were having anemia are in 41-60 years of age group. No case of severe anemia was found.

Table 5 showed ECG findings in mine workers. 4.12% workers showed T wave indicating ventricular overload/ischemia. Various types of bundle branch block were seen in 4 workers.2.06 workers had sinus tachycardia while 1 worker had left ventricular hypertrophy. Other clinical findings present among these mine workers are shown in table 6. Hypertension was found in 19.5% and 6.18% mine workers were had refractive error, 4 (4.12%) workers were had pterygium and conjunctivitis was seen in one worker. Defective colour vision was also seen in one worker. There were one worker each had elevated level of blood sugar and triglycerides respectively. Urinary tract infection was found in 5.15% workers and Glycosuria was found in one worker.

Discussion

Workers are exposed to more adverse working condition like stress, noise, dust, heat, vibration etc that affects the health of workers in various ways. Among these mine workers near about 50% workers are in the age group of 30-40 years while 22.6% workers were underweight. On evaluation of chest x-rays no case of pneumoconiosis and other abnormality was found in present study. Development of pneumoconiosis among the mining workers depend on the chemical composition of dust, concentration of dust in the air, period of exposure and health status of the exposed worker [16]. Present study showed 6.1% workers had restrictive impairment while one worker each had mild obstruction and mixed impairment.

Overall pulmonary impairment was seen in 9.1% workers. Prevalence of pulmonary impairment in this study was lower than other studies. Study conducted in limestone mine workers in Rajasthan State showed prevalence was 15.2% while study conducted by Nandi et al. showed that 14% of the workers had pulmonary impairment [17,18]. In this study low prevalence of pulmonary impairment and no case of pneumoconiosis was observed. It might be due to low dust level in working environment.

In our study 30.9% workers were having hearing problem of which 9.27% had noise induced hearing loss. These observations were lowered when compared to study done by Dhatrak et al. which showed 23.8% mine workers had NIHL [19]. Similar findings reported among gold mining workers in Ghana [20]. Present study showed 21.6% workers were having different grade of hearing loss which is lower when compared to study conducted by Oliveira et al. [16].

In this study 11.3% mine workers had significant ECG changes. One worker each had elevated level of blood sugar and triglycerides. 5.15% workers were had urinary tract infection and one worker had Glycosuria. A history of osteoarthritis was present in 2.06% of the mine workers. Overall prevalence of anemia was seen in 19.5% workers which are slightly higher than study conducted by Giri et al. [21]. In our study 6.18% mine workers were had refractive error, 4 (4.12%) workers were had pterygium and conjunctivitis was seen in one worker. These observation were lower when compared to the studies conducted by Rajshekhar S et al showed that 22.72% mine workers had refractive error, 12.37% had conjunctivitis and 4.87% had pterygium [22]. Defective colour vision was also seen in one worker. In this study 19.5% workers had hypertension. These findings were lower than study conducted in Gypsum mine workers in India which showed hypertension in 22.6% workers [18].

Conclusion

Workers in mining industry are exposed to a number of hazards physical, chemical or biological leading to a number of health problems or diseases. Dust and noise are more common in mining as a result, risk and prevalence of respiratory impairment and hearing loss was found among these mine workers. There is a need of regular periodical medical examination of workers and should be regularly arrange awareness program and educate the mine workers regarding occupational and non occupational health problems. Workers should be encouraged for regular use of personal protective equipment to protect them from occupational disorders.

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Original Article

RFP Journal of Biochemistry and Biophysics
Volume 4 Number 1, January - June 2019

Study of Serum Calcium,Vitamin D as Bone Markers with Other Biochemical Parameters in Childhood Nephrotic Syndrome

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How to cite this article:

R.K. Padalkar, S.M. Patil, D.V. Andure et al. Study of Serum Calcium,Vitamin D as Bone Markers with Other Biochemical Parameters in Childhood Nephrotic Syndrome. RFP Journal of Biochemistry and Biophysics. 2019;4(1):21-24.

Abstract

Background: Nephrotic syndrome causes a great morbility and mortality among the children. It is a characterized by massive loss of urinary protein along with risk of altered calcium metabolism. The aim of present study was to assess the serum calcium and vitamin D level in childhood Nephrotic Syndrome and try to find out correlation of biochemical parameters as severity in same subjects.

Material and Methods: The present study was case-control study. Total 110 subjects were included and divided into two groups. Group I consisted 55 subjects of childhood Nephrotic Syndrome in the age group 1-10 years while Group II consisted of age and sex matched 55 normal healthy individuals who served as control with no history of childhood Nephrotic Syndrome. Serum levels of calcium, vitamin D were estimated in all the subjects under study. Other biochemical parameters like serum total protein, albumin, and creatinine were measured in all subjects. Values were expressed as mean ± standard deviation. SYSTAT version 12 software was used for statistical analysis. Comparisons of study groups to control groups were done by applying student t test.

Results: Serum calcium and vitamin D level were decreased a childhood Nephrotic Syndrome compared with controls. Mean values of serum total protein, albumin, and creatinine were significantly ($p<0.0001$) lower in childhood Nephrotic Syndrome compared with controls.

Conclusion: In the present study, It can be concluded that, childhood Nephrotic Syndrome is associated with abnormalities in the level of serum calcium and vitamin D. Serum total protein, albumin, and creatinine may be used as biochemical markers to determine severity of childhood Nephrotic Syndrome and it may beneficial for better management and for developing new treatment strategies.

Keywords: Childhood Nephrotic Syndrome; Calcium; Vitamin D.

Introduction

Nephrotic syndrome is a clinical entity characterized by substantial urinary protein loss primary albuminuria results in hypoproteinemia [1] (i.e. hypoalbuminemia) and edema. It may be a systematic manifestation of general kidney disease [2] and prevalence is near about 16 cases per 100,000 cases [3].

The underlying nephrotic syndrome pathophysiology is not entirely clear. Although in children with acute nephrotic syndrome, the primary mechanism may be the more intuitive under- fill edema mechanism due to decreased oncotic pressure caused by proteinuria [4].

The loss of vitamin D metabolites in urine

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Received: 13.06.2019 | **Accepted:** 22.06.2019

combined with the detrimental effect of corticosteroids often leads to disturbances in calcium and vitamin D metabolism in nephrotic children [5].

Intestinal malabsorption as well as excessive urinary losses of various vitamin D metabolites and their binding proteins which leads to decrease bone mineral density. Thus the present study was designed to study serum calcium and vitamin D along with various biochemical parameters in childhood nephrotic syndrome [6].

Materials and Methods

The present study was conducted at Department of Biochemistry and Department of Pediatrics DVVPF's Medical College Ahmednagar. The study was approved by Institutional Ethics Committee. All participants providing informed consent and utmost care was taken during experimental procedure according to the declaration of Helsinki 1975.

Study type

Case- Control study.

Study Design

Total 110 samples were enrolled in the present study.

Control group

Fifty five (55) healthy age and sex matched individuals without any evidence of Nephrotic syndrome as per clinical examination were taken as control subjects.

Patients group

The study included total 55 patients between age group 1-10 years of essential Nephrotic syndrome.

Inclusion criteria

a) Patients with Idiopathic nephrotic syndrome attending pediatrics output patients.

b) Controls are healthy individuals, age and sex matched without any major illness and not on any medication.

Exclusion criteria

Patients with Tuberculosis, HIV-AIDS, liver diseases and history of any other medical or surgical illness were excluded.

Method of collection of data

A pre-structured and pre-tested proforma was used to collect the data. Informed consent was taken from all cases and control subjects. Baseline data including age, sex, detailed medical history, clinical examinations and relevant investigations were included as part of methodology.

Collection of blood sample

About 5 ml of venous blood was drawn from subjects under aseptic precautions, using a sterile disposable syringe and collected in clot activator and fluoride EDTA vacuum evacuated tubes. After an hour, the samples were centrifuged at 3000 rpm for 10 minutes to separate serum and used for analysis of calcium, Vitamin D and other biochemical parameters.

Methods

1) Serum calcium was determined by Trinders method:

2) Estimation of Vitamin D was done by Chemiluminiscence method:

Sample antigen and purified 25-OH Vitamin D antigen competes to combine with 25-OH vitamin D monoclonal antibody to form antibody-antigen complex with starter reagent, the flash chemiluminiscent reaction is initiated. The light reaction is measured by a photomultiplier which is proportional to the concentration of vitamin D present in sample.

3) Estimation of serum total protein was measured by biuret method, Albumin by Bromocresol green method, serum creatinine by Jaffe's method.

Statistical Analysis

Statistical software SYSTAT version-12 (by Cranes software, Bangalore) was used to analyze the data. The result were expressed in Mean ± Standard Deviation (Mean ± SD) Data was analyzed by descriptive statistics as mean, SD, percentage etc. Comparisons of study group to control group by using the Students't' test. p – Values of <0.0001 was considered as statistically significant.

Results

Table 1 showed that, the mean serum calcium and serum Vitamin D levels in childhood nephrotic

syndrome were significantly decreased when compared with normal healthy controls.

Table 1 also the mean serum Total protein, albumin and serum creatinine levels in childhood nephrotic syndrome were significantly lower ($p<0.0001$) when compared with normal healthy controls.

Table 1: Comparison of various variables between cases & controls.

Variables	Cases n=55 (Mean + SD)	Controls n=55 (Mean+ SD)	p-value
Age (Years)	1 to 10	1 to 10	----
Weight (Kg)	17.69 + 3.99	19.58 + 6.8	----
Height (Cm)	105.41 + 8.4	107.17 + 12.01	----
Sr. Total protein Gm/dl	3.2 + 0.42	6.97 + 0.5	0.0001
Sr. Albumin Gm/dl	1.45 + 0.22	4.0 + 0.7	0.0001
Sr. Globulin Gm/dl	2.1 + 0.38	2.9 + 0.4	0.0001
Sr. Creatinine (mg/dl)	0.60 + 0.13	0.60 + 0.15	0.65
Sr. Calcium	9.10 + 0.62	9.72 + 0.27	0.52
Sr. vitamin D ng/ml	12.9 + 4.73	15.01 + 03.11	0.31

Discussion

Nephrotic syndrome can be caused by variety of glomerular and systemic diseases. It is estimated that, 2 to 7 new cases of Nephrotic Syndrome per 100,000 children in western hemisphere countries [7].

Kidneys most important function in blood filtration by glomeruli which enables fluid and waste products to be excrete. It retains most blood proteins and all blood cells within the vasculature. According to previuos study, hypocalcemia is common finding in patients with Nephrotic syndrome during active disease [8].

Nephrotic syndrome children are prone to biochemical derangement in vitamin D and calcium metabolism caused by the disease as well as glucocorticoid therapy [5].

In current study, the mean serum calcium and serum Vitamin D levels in childhood nephrotic syndrome were significantly decreased when compared with normal healthy controls.

Our results are exactly coordinated with Poonam Mehta et al., and Naresh Manne et al. According to both study hypocalcemia and decreased vitamin D is a common finding in children with nephrotic syndrome. It may due to urinary loss of these metabolites or their carrier proteins or secondary corticosteroid therapy, but the exact biochemical basis for these changes remains speculative [5,9].

In present study, the mean serum Total protein, albumin and serum creatinine levels in childhood nephrotic syndrome were significantly lower ($p<0.0001$) when compared with normal healthy controls. Our results are matched with previous study of David Gitlin et al. According to their study hypoalbuminemia in children with Nephrotic syndrome is due to an increase in the fractional rate of loss of albumin. Decrased total protein mainly Hypoalbuminemia in Nephrotic syndrome may be due to decreased rate of albumin synthesis, changes in albumin distribution, decreased intake of dietary protein [10,11].

Conclusion

In the present study, It can be concluded that, childhood Nephrotic Syndrome is associated with abnormalities in the level of serum calcium and vitamin D may be due to increases their urinary loss. Altered serum total protein, albumin, and creatinine might beuse as biochemical markers to determine severity of childhood Nephrotic Syndromeand along with vitamin D and calcium it may beneficial for better management and for developing new treatment strategies.

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Review Article

RFP Journal of Biochemistry and Biophysics
Volume 4 Number 1, January - June 2019

Superoxide Dismutase, Structure Function and Mechanism

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How to cite this article:

Nancy, Pushpender Kumar Sharma. Superoxide Dismutase, Structure Function and Mechanism. RFP Journal of Biochemistry and Biophysics. 2019;4(1):25-33.

Abstract

Living organisms have evolved protecting systems to protect themselves against oxidative stress and to prevent damage from these toxic agents. They have developed several enzymatic and non-enzymatic mechanisms to detoxify these very active compounds. Enzymatically, oxygen radicals are removed mainly by the action of four enzymes: superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Superoxide dismutases (SODs) are metalloenzymes that catalyze the conversion of superoxide molecules to hydrogen peroxide and molecular oxygen and therefore form one of the cell's major defense mechanisms against oxidative stress. This review will discuss superoxide dismutase, structure function and mechanism

Keywords: Superoxide dismutase; Free radicals; Oxidative stress; Enzymes; Metalloenzymes.

Introduction

Molecular oxygen (O_2) is essential for the aerobic life on Earth. All aerobic organisms undergo complete reduction of molecular oxygen (O_2) and generate energy in the form of ATP which is used to carry out biological functions. Molecular oxygen (O_2), while crucial for the life of aerobes, is potentially toxic as when it is incompletely reduced, it produces some reactive intermediates such as hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), and the highly reactive hydroxyl radicals ($^{\bullet}OH$) and these oxy-radicals are referred to as reactive oxygen species (Fridovich, 2004). Superoxide radicals and other oxy-radical intermediates are easily formed e.g. by autooxidation and this is an unavoidable event in aerobic respiration. These reactive oxygen species (ROS) are essential for various functions such as homeostasis and cell signaling but an imbalance in favour of reactive oxygen species results in oxidative stress (OS) (Kashmiri *et al.,* 2014). Oxidative stress (OS) result in interference in the functioning of biological systems that maintain levels of environmentally produced reactive oxygen species (ROS), by readily detecting and detoxifying them (Lucana *et al.*, 2012). Reactive oxygen species rapidly react with various molecules and interfere with cellular functions and causes oxygen toxicity. This toxicity is due to the tendency of O_2 for reduction by a univalent pathway. This simplistic univalent pathway of

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Received: 23.05.2019 | **Accepted:** 22.06.2019

O_2 reduction generates intermediates that lie between one O_2 and its four electron reduction products – two molecules of water – and it is the reactivity of these intermediates that is responsible for the toxicity of O_2 (Fridovich, 2004). Therefore, protection of tissues from oxygen toxicity is one of the major requisites for aerobic life. Hence, to abate oxygen toxicity, the reactive species should be scavenged effectively at the site of generation.

Living organisms have evolved protecting systems to protect themselves against oxidative stress and to prevent damage from these toxic agents. They have developed several enzymatic and non-enzymatic mechanisms to detoxify these very active compounds. Enzymatically, oxygen radicals are removed mainly by the action of four enzymes: superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Yesilkaya *et al.*, 2000). Superoxide dismutases (SODs) are metalloenzymes that catalyze the conversion of superoxide molecules to hydrogen peroxide and molecular oxygen and therefore form one of the cell's major defense mechanisms against oxidative stress.

These antioxidant enzymes are very important and are widely distributed in prokaryotic and eukaryotic cells. They catalyze the reduction of the superoxide radical to hydrogen peroxide and dioxygen in a critical reaction i.e.,

$$O_2^- + O_2^- + 2\,H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

That protects aerobic organisms against oxidative damage. Superoxide dismutases, as the name suggests dismutes superoxide. Dismutation reaction is that in which two equal but opposite reactions occur on two separate molecules. SOD takes two molecules of superoxide, removes the extra electron from one and add it to the other. At the end hydrogen peroxide (less damaging) and oxygen are formed (Fridovich, 1989). Hydrogen peroxide can then subsequently be reduced to water, or to water and molecular oxygen, by the action of other enzyme systems (Stromqvist, 1993).

Superoxide dismutases (SODs) have been classified into four families based on their different types of metal centers: copper/zinc, nickel, manganese and iron (Beyer *et al*., 1991). In prokaryotes, on the basis of metal cofactor, three types of SODs have been defined: Cu-Zn- (SodC), Fe- (SodB), or Mn-type (SodA) SODs. FeSOD and MnSOD are characteristically prokaryote enzymes, but MnSOD is also present in mitochondria of eukaryotes. Cu-ZnSOD, on the other hand, is mainly found in the cytosol of many eukaryotic organisms. However, several prokaryotes containing Cu-ZnSOD and Ni-SOD (Hammouda *et al.*,1999) have been reported.

There are many structural and chemical differences between bacterial and human SODs. Bacterial SODs do show novelties not found in eukaryotic dismutases. These novelties may suggest ways to engineer human dismutases or new ways to formulate on-going questions of clinical importance. MnSOD is present in both eukaryotic and prokaryotic cells and study of bacterial MnSOD can lead to the development of various therapies involving human MnSOD.

Oxidative stress

Aerobic organisms need molecular oxygen (O_2) for respiration process or nutrient oxidation to obtain energy but it is also a potentially very toxic agent because of its capability to form oxy radicals. Despite that it provides vast advantages, it also contains a universal toxicity (Fridovich, 1983). Numerous researches have reported oxygen toxicity in various species (Gottlieb, 1971; Wolfe and De Vries, 1975). It has been revealed to be mediated by products generated from the univalent reduction of molecular dioxygen, including the superoxide radical ($O_2^{\bullet}$), the hydroxyl radical ($OH^{\bullet}$) and the hydrogen peroxide (H_2O_2) (Fridovich, 1983; Halliwell and Gutteridge, 1984; Carlioz and Touati, 1986). Derived from molecular oxygen, these intermediates are by-products of cellular respiration that are produced continuously in cells growing aerobically, and are called reactive oxygen species (ROS). There are abundant sources of reactive oxygen intermediates which include partial reduction of oxygen during respiration, radiation exposure or exposure to redox-active compounds, and the burst of phagocytes during respiration (Yesilkaya *et al.*, 2000). These reactive oxygen species (ROS) induces the oxidative stress (OS) (Kashmiri *et al.*, 2014). Normally ATP is generated from glucose and O_2 by cytochrome oxidase and in this process of generating ATP, O_2 is usually reduced to H_2O by four electrons. Infrequently (approximately 5% of the time), O_2 is reduced by single electron, yielding superoxide ($O_2^{\bullet}$). Further reduction of O_2 occurs by one or two additional electrons yielding hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), respectively. However, there are additional sources of these oxy-radicals such as the interaction of ionizing radiation with biological tissues and other metabolic processes (James, 1994).

Molecular oxygen (dioxygen) is comparatively unreactive in its ground state, yet it has the capability to give rise to fatal reactive excited states such as free radicals and their derivatives. A complete stepwise reduction process utilizes O_2; where four electrons reduce to water and during this incompletely reduced reactive intermediates are generated (Fig. 1).

Partially reduced reactive species comprises the superoxide radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$). Thus, molecular oxygen, although crucial for the survival and existence of aerobic life, boons living organisms with a variety of biological challenges collectively called "oxidative stress." These reactive oxygen species leads to damage of proteins, nucleic acids and cell membranes as shown in Fig. 2. Increasing evidence suggests that the collective damage caused by these reactive oxygen species leads to numerous diseases.

Several enzymes are expressed constitutively by cells to suppress oxidative stress. The reactive oxygen species are detoxified by these enzymes and thus helping in the repair of damage produced by them. In addition, the different cells of bacteria, yeast and mammalian, all have adaptive responses to elevated levels of oxidative stress, showing that these cells detect the increased amount of reactive oxygen species and this signal is transduced into an enhanced expression of defensive activities (Storz and Imlay, 1999).

Superoxide dismutases

During 1970s, rapid growth and development was observed in the field of biology of free radicals and medicine. Many people considered the event of discovery of superoxide dismutase (SOD) to be responsible for this growth and development. The incident of discovery of SOD has been as often by chance and was made not by design (McCord and Fridovich, 1988). SOD (EC 1.15.1.1) was first extracted from bovine blood and was known to be a green copper protein (Mann and Keilin, 1938) and its function was thought to be of copper storage.

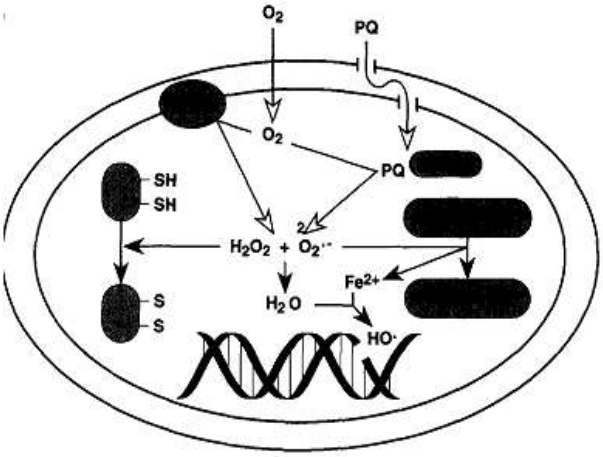
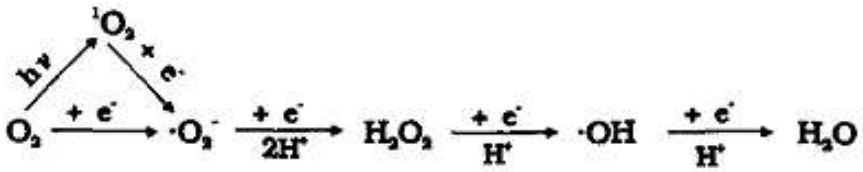


Fig. 2: Mechanism of oxidative cell damage by endogenous oxidants. Molecular oxygen passively diffuses into cells and is converted to •O2- and H2O2 by the direct oxidation of flavoproteins, including NADH dehydrogenase II (Ndh II). Redox-cycling drugs, including paraquat (PQ), accelerate the formation of these oxygen species by catalysing the transfer of electrons from redox enzymes such as sulphite reductase (SiRase) to oxygen. •O2- oxidatively destroys iron-sulfur clusters (here, from aconitase, Acn). The released iron can react with H2O2 to form hydroxyl radical •OH, which directly damages DNA (Storz and Imlay, 1999).

During several years after the discovery, the enzyme was variably known as erythrocuprein, indophenol oxidase, and tetrazolium oxidase (Scandalios, 1993). In 1968, at Duke University, Irwin Fridovich along with Joe McCord were the first to discover the enzymatic action of superoxide dismutase. Until this discovery, the actual function of SODs was not known and they were considered to be a group of proteins that have metal ions at



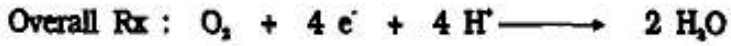


Fig. 1: Pathways showing the reduction of O_2 to water resulting in the formation of various intermediate reactive O_2 species (Scandalios, 1993).

their active centres i.e. metalloproteins. Later on, the manganese-containing (Mn)(Fridovich, 1970) and the iron-containing(Fe) (Fridovich, 1973) SODs from *E. coli* bacteria and the MnSOD (SOD_2) from mitochondria were also discovered by Fridovich and his research group. Mitochondrial MnSOD is now considered as a vital mammalian protein (Fridovich, 1988). Brewer in 1967 analyzed the proteins of starch gels using tphenazine-tetrazolium technique and identified a protein, indophenol oxidase, that later known as superoxide dismutase.

Superoxide dismutases (SODs) catalyze the dismutation reaction where conversion of O_2 into H_2O_2 and O_2 occurs, i.e.,

$$O_2^- + O_2^- + 2\,H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

and by doing so, they provide a significant defense. The product of dismutation reaction, i.e. H_2O_2, is further eliminated by other defensive enzymes that include the catalases and peroxidases. In this way, the rigorous action of the SODs together with the catalases and peroxidases inhibits the formation of the very reactive hydroxyl radical (HO**)** (Fridovich, 2004).

Cells which respire and which can thus produce O_2^- must have evolved appropriate defenses against this radical. The underpinning of such defenses is provided by enzymes, which scavenge O_2^-, by catalyzing the above reaction. These enzymes, which have been termed as superoxide dismutases, are vital for the survival of respiring organisms. This enzyme has been identified in a widespread range of living things and has been considered as an important defense against the universal potential toxicity of oxygen. This statement applies to bacteria, algae, protozoa, fungi, plants, insects, birds and mammals (Fridovich, 1975).

Virtually, there are two main types of SOD present in different organisms that catalyze the dismutation of O_2^- and this occurs within different cell organelles and other cellular compartments. The SODs can be characterized on the basis of metal ions (Mn/Fe, Cu, and Zn) present at the active site of the enzyme. The presence of the superoxide dismutase enzyme also depends on the type of cell. Prokaryotes especially bacteria normally contain one type of SOD either Mn/Fe or Cu-Zn, whereas almost all eukaryotes contain both types. Superoxide dismutase enzyme is ubiquitous in nature. Though it is widely dispersed among oxygen consuming organisms, it is also found in aerotolerant anaerobes, and some obligate anaerobes (Fridovich, 1986).

Biological function of superoxide dismutases

The biological function and importance of SODs as defensive enzymes against O_2 toxicity have been exhibited in several studies with prokaryotes, eukaryotes both lower and higher, including higher plants (Fridovich, 1986; Hassan and Scandalios, 1990; Scandalios, 1990, 1992; Bowler et al., 1992; Gralla and Kosman, 1992). When the amount of SOD enzyme in cells decreases, it results in the increased generation of oxy-radicals. This imbalanced condition has been associated with wide-ranging pathological conditions such as inflammatory tissue necrosis, formation of cataract and aging, tumor development, asthma, drug-induced liver necrosis (comprising acetaminophen damage), and many neurodegenerative disorders (James, 1994).

The cumulative action of superoxide dismutase (SOD) and catalase (CAT) reduces the production of the most lethal and highly reactive oxidant that is the hydroxyl radical ($OH^{\bullet}$) (Scandalios, 1993). SOD and other related antioxidants are localized withinand around cells of damaged tissues in a way to prevent oxidative stress produced by superoxide andits metabolites (Inoue 1994).

Classification of superoxide dismutases

There are SODs that are classified on the basis of metal ion present at the active site, i.e. Cu (II) plus Zn (II), Mn (III), Fe (III), and Ni (II). They all catalyze the dismutation of O_2 into H_2O_2 and O_2. All SODs function in a similar manner where the metal at the active site is reduced by one O_2 molecule and then reoxidized by the next O_2 molecule. Thus the active metal centre acts like a mediator that passes an electron from one molecule of oxygen to another (Fridovich, 2004). As mentioned above, based on the metal centre there are four major families of SODs as follows:

1. MN SODs - This type of SOD enzyme is contained in both prokaryotes and eukaryotes. This enzyme, whether from bacteria or from the mitochondrial matrix, showed discernible sequence similarity, revealing a close evolutionary history and showing an endosymbiotic origin for mitochondria. There is some structural difference between bacterial and mitochondrial Mn SOD. The bacterial SOD is generally a homodimer, and the corresponding enzyme from mitochondria is a homotetramer. The weight of subunit of this enzyme is 23 kDa. Some bacterial enzymes are also

tetrameric as is the case of *Cryptococcus neoformans*.

2. CU, ZN SODs – This type of SOD is usually found in chloroplasts, the periplasm of gram-negative bacteria, cytosols of eukaryotic cells and in the intermembrane space of mitochondria. The bacterial enzyme found in eukaryotic cytosols is usually a homodimeric protein with subunit weight of ~16 kDa, whereas the enzyme from periplasm of *E. Coli* is monomeric protein. In higher animals, the extracellular Cu, Zn SOD is present. The extracellular Cu, Zn SOD is usually homotetrameric in structure and is glycosylated with subunit weighing ~ 23 kDa.

3. FE SODs – Fe SODs are highly homologous to the Mn SODs and are present in bacteria and in plants. Although Fe SODs are usually homodimeric proteins, homotetrameric Fe SOD has been detected in *Mycobacterum tuberculosis* and *Rhodococcus bronchialis*. The Fe SOD enzyme of *Escherichia coli* is constitutive so it is found even in cells grown anaerobically. It can thus be viewed as a stand by defense against O_2, which is always maintained to protect in the event of a sudden exposure to O_2 (Fridovich, 2004).

4. NI SODs – These SODs are found in prokaryotic organisms. The Ni SOD enzyme is hexameric protein and comprises of right-handed 4-helix bundles. Each right-handed helix bundle comprises of N-terminal hooks and these assist in chelating a Ni ion. The Ni-hook comprises of motif His-Cys-X-X-Pro-Cys-Gly-X-Tyr. This motif is accountable for most of the interactions that are critical for binding with metal and catalysis (Barondeau *et al*., 2004).

Bacterial superoxide dismutases

Superoxide dismutase enzyme is found in a variety of bacterial sources such as *Escherichia coli* and mammalian sources and it has been confirmed that it catalyzes the dismutation reaction where univalently reduced oxygen gets disproportionated (Keele *et al*., 1970). The mammalian enzyme contains copper and zinc and due to this it imparts blue colour, whereas the corresponding enzyme in bacteria was found to contain manganese and imparting red-purple colour. This enzyme when isolated from *E. coli* bacteria were found to have a molecular weight of 39,500 daltonand this was determined by sedimentation equilibrium technique. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the protein showed that this enzyme comprises of two subunits having equal size. The protein was also analyzed chemically and by electron paramagnetic resonance spectrometry and the analysis demonstrated that one molecule of the enzyme contains atoms of manganese that numbers between 1.6 and 1.8. The enzyme contained no substantial amounts of copper or zinc (Keele *et al*., 1970).

Another difference in SODs from bacterial and mammalian sources is the detection of occurrence of reactive oxygen species by regulators that are distinctive from SoxR and OxyR. A good example of this is the regulation by PerR repressor in *Bacillus subtilis*. PerR is a metal-binding protein with Fur-like structure that suppresses the expression of catalase which is an alkyl hydroperoxidasereductase, and of Dps-like protein. It is proposed that PerP activity might be controlled by metal-catalyzed oxidation of a bound metal ion (Storz and Imlay, 1999).

Manganese superoxide dismutases

This enzyme, whether from bacteria or from the mitochondrial matrix, showed discernible sequence similarity, revealing a close evolutionary history and showing an endosymbiotic origin for mitochondria. There is some structural difference between bacterial and mitochondrial Mn SOD. The bacterial SOD is generally a homodimer, and the corresponding enzyme from mitochondria is a homotetramer. The molecular weight of subunit of this enzyme is 23 kDa. Some bacterial enzymes are also tetrameric as is the case of *Cryptococcus neoformans*. Providing the similarity in primary and three-dimensional structures of the manganese and iron SODs (MnSOD and FeSOD), it can be concluded that they have undoubtedly evolved from a common ancestor. Although they are structurally homologous, the Mn- and FeSODs have distinct functional roles. Only in exceptional cases, the endogenous Mn (or Fe) is substituted by Fe (or Mn) while retaining the catalytic activity. The Mn- and FeSODs are further distinguished in their distribution among bacterial species. Strict anaerobes contain one SOD that is FeSOD. Bacterial aerobes usually contain an MnSOD or both Mn- and FeSODs. (The MnSOD is also widely found in eukaryotes (Steinman, 1987).

In *E. coli*, the biosynthesis of Mn SOD is under the control of the soxRS regulon, which coordinately up-regulates the expression of a number of genes in response to O_2. The constitutively expressed SOX R protein is transcriptionally inactive in its reduced form. It can be oxidized by O_2 and then activates the expression of the SOX S protein, which in turn

activates all the genes in the regulon. Thus, MnSOD is not measurable in extracts of anaerobically grown *E. coli*, but exposure of cultures to aerobic conditions elicits production of MnSOD. Increasing production of O_2, by raising pO_2, or by adding compounds such as viologens, which can mediate enhanced production of O_2, increases the level of Mn SOD. It has been possible to force *E. coli* to produce Mn SOD to 7% of its soluble protein by aerobic exposure to the viologenparaquat. The nectar of tobacco flowers has been found to contain a stable Mn-protein named nectarin that appears to be anMn SOD (Fridovich, 2004).

Structure of manganese superoxide dismutase

Several crystal structures of SODs have been determined. The known SOD structures fall into two groups: the Cu/Zn SODs fold into a flattened eight-stranded Greek-key β-barrel, while the MnSODs and FeSODs fold into two-domain structures mainly composed of α-helices (Wuerges et al., 2004). Crystal structures have been reported of the MnSODs from Homo sapiens (Hsieh et al., 1998) and many bacteria, including *Escherichia coli* (Edwards et al., 1998), *Bacillus halodenitrificans* (Liao et al., 2002), *B. anthracis* (Boucher et al., 2005), *Porphyromonas gingivalis* (Sugio et al., 2000) and *Thermus thermophilus* (Ludwig et al., 1991). All MnSOD structures comprise two domains: an α-helical domain and an α/β-domain (Liu *et al*., 2007).

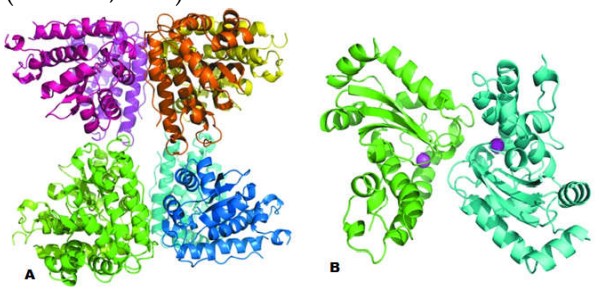


Fig. 3: Overall structure of B. subtilis MnSOD. (A) The asymmetric unit of the MnSOD structure is composed of four dimers, which are colored dark and light magenta, gold, blue and green. (B) The MnSOD dimer backbone colored by subunit with Mn atoms shown as magenta spheres (Liu et al., 2007).

Bacterial Mn/Fe SODS can be either homodimeric or tetrameric, whereas eukaryotic mitochondrial Mn SODS are tetrameric as are the cytosolic Fe SODS of plants and protists. The monomeric size of the Mn/Fe SODS is usually approximately 20-24 kDa. Mn/Fe SODS contain one metal ion per monomer. The metal is specific and replacement results in loss of activity, except for a few enzymes where Mn and Fe appear to be interchangeable. Mn/Fe SODS comprise mainly α-helices with the metal bound to three histidine residues and one aspartate (James, 1994). The combination of designed mutations and x-ray crystal structures were used to study metal site structure and function for both the cytoplasmic Cu, Zn superoxide dismutase (Cu, Zn SOD) and the mitochondrial Mn superoxide dismutase (MnSOD) (Tainer *et al*., 1993).

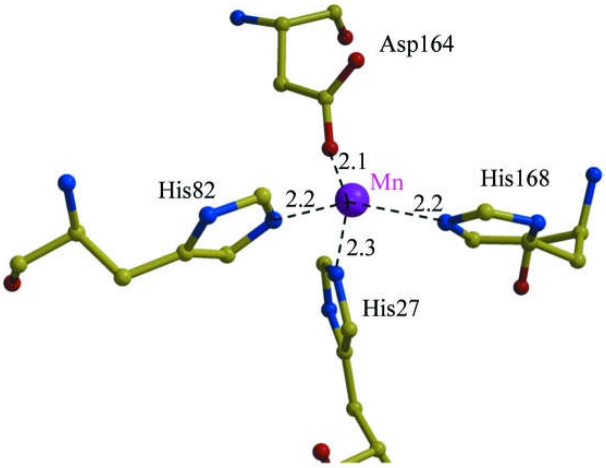


Fig. 4: Active site of MnSOD. Coordination of the Mn atom of B. subtilis MnSOD is shown. Interatomic interactions are shown as broken lines with distances in angstroms (Liu et al., 2007).

Mechanism of action of manganese superoxide dismutase

The primary function of MnSOD is to protect cells and mitochondria from free radical damage due to superoxide. Unlike the Cu/Zn- and Fe-containing enzymes, MnSOD expression in bacteria is induced under times of cellular stress as a result of exposure to a variety of elements including interleukin-1, tumor necrosis factor, paraquat, and X-ray radiation. The induction cascade is not fully understood: however, it is related to the superoxide response regulator (SoxR)-mediated pathway and correlates with both metal concentrations in the cell as well as the cell's redox environment, being activated when it is oxidative. In eukaryotes, MnSOD is targeted to the mitochondria after it is expressed in the nucleus. As over 90% of the dioxygen used by an organism is processed in the mitochondria, MnSOD primarily encounters reactive oxygen species formed as a result of mitochondrial function (Stroupe, 2011).

Superoxide dismutase (SOD) acts as the first line of defense against free radicals, it catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet}$) into hydrogen peroxide (H_2O_2) by reduction. Enzyme undergoes both oxidation and reduction therefore the metal ion present at the centre be

both oxidizable and reductable by superoxide (Fee *et al,* 1973; Sawyer *et al.,* 1979). There is an electrostatic attraction between metal centre and superoxide anion.

The enzymatic reaction comprises of two distinct half reactions, an oxidative reaction in which the substrate, O_2^- is oxidized to dioxygen and a reductive half reaction in which O_2^- is converted into H_2O_2.

O_2^- + Mn(III)SOD $\longrightarrow$ O_2 + Mn(II)SOD (oxidative reaction)

O_2^- + $2H^+$ + Mn(II)SOD $\longrightarrow$ H_2O_2 + Mn(III)SOD (reductive reaction)

The H_2O_2 formed is converted into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx). SOD converts superoxide to H_2O_2, a relatively stable molecule (Fig. 5). Although it occurs spontaneously, the role of SOD is to increase the rate of the reaction to that of a diffusion-controlled process. In the cytosol and the intermembrane space of mitochondria, superoxide is eliminated by Cu, Zn-SOD, whereas in the matrix, it is eliminated by Mn-SOD (Bayir and Kagan 2008).

Applications

Superoxide dismutases enzymes has great potential to act as an anti-aging agent, it has been demonstrated previously that with the increase in age the SOD level goes down, at the same time free radical levels increase. Another therapeutic application of Manganese superoxide dismutase (MnSOD) is that it can be used as a biomarker of different human diseases and this can help in the prevention of cancer and its treatment (Moradi *et al.,* 2015). A new form of human MnSOD is isolated from a human liposarcoma cell line (LSA) was able to kill cancer cells expressing estrogen receptors, but it did not have cytotoxic effects on normal cells (Borreilli *et al.,* 2014). Other applications of SODs in their topical forms are to help to reduce facial wrinkles, scar tissue, heal wounds and burns, lighten dark or hyperpigmentation, and protect against harmful UV rays.

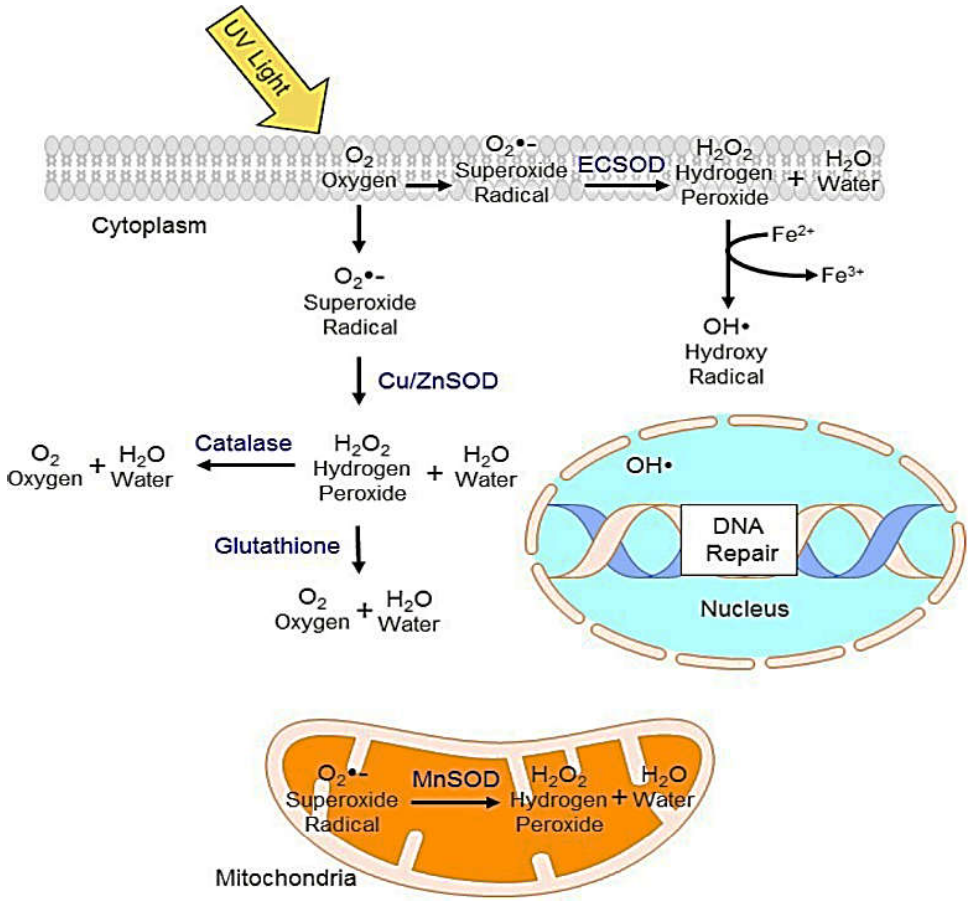


Fig. 5: Mechanism of action of SOD (Bayir and Kagan 2008)

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Review Article

RFP Journal of Biochemistry and Biophysics
Volume 4 Number 1, January - June 2019

Healthcare on the Path of Advancement in Technologies

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How to cite this article:

Sachin C. Narwadiya, Gulshan Karhade, Deepika Dixit. Healthcare on the Path of Advancement in Technologies. RFP Journal of Biochemistry and Biophysics. 2019;4(1):35-37.

Abstract

Healthcare is a sector which is highly demanded and used by the public. There is scope of better diagnosis, treatment and surgeries in healthcare. The healthcare industry is spread worldwide. Medical tourism enabling citizens of various countries to interact with each other and also save lives by opting better healthcare facilities. Healthcare saw major changes during past few decades. The invention of X-rays brought a visible change in this sector. The Magnetic Resonance Imaging-MRI, Computational Tomography-CT scan, Robotic Surgeries are some area of advancement in Healthcare. The invention of camera and its further advancement in output like High Definition-HD quality, Liquid Crystal Displays-LCDs, Light emitting diodes-LEDs are incorporated in the various medical diagnostic instruments. The present review study is focussing on the various advancements in healthcare with emphasis on robotic surgeries. The medicine and healthcare sector becoming more and more advanced in technology, since last many decades. As there is advancement in surgery and the technology the surgeons become more specific while performing surgery. Laser surgery is one of the examples of it. In this surgery laser light is used for surgical procedure. Use of machine like robots can now become possible in various areas of health care and medicine. The robots which can be used by doctors may be classified on basis of work they performed. The various categories that may include in the robotic doctors are Surgical Robots, Rehabilitation Robots, Bio-Robots, Tele-Presence Robots, Pharmacy Automation, and Disinfection Robots.

Keywords: Liquid Crystal Displays-LCDs; Light emitting diodes-LEDs; Robotic Surgeries; Tele-Presence Robots; Pharmacy Automation.

Introduction

The medicine and healthcare sector becoming more and more advanced in technology, since last many decades. As there is advancement in surgery and the technology the surgeons become more specific while performing surgery. Laser surgery is one of the examples of it. In this surgery laser light is used for surgical procedure. Use of machine like robots can now become possible in various areas of health care and medicine. The robots which can be used by doctors may be classified on basis of work they performed. The various categories that may include in the robotic doctors are Surgical Robots, Rehabilitation Robots, Bio-Robots, Tele-Presence Robots, Pharmacy Automation, and Disinfection Robots. The surgical robots either carry out whole surgery or may help doctor to do some part of surgery. This type of Robot can be operational from remote place where presence of doctor is not needed and doctor can operate

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Received: 17.04.2019 | **Accepted:** 04.05.2019

surgery from distant place. Rehabilitation robots are the robots that facilitate and support the lives of infirm, elderly people, or those with dysfunction of body parts effecting movement. These robots are also used for rehabilitation and related procedures, such as training and therapy. Bio-Robots represent a group of robots intended to replicate the cognition of humans and animals. The Tele-presence robots on the other hand can allow off-site medical professionals to move, look around, communicate, and participate from remote locations. Thus, allowing the doctor to operate from remote place. The Pharmacy automation represents robotic systems to dispense oral solids in a retail pharmacy setting in a hospital. The Disinfection robot has the capability to disinfect a whole room in mere minutes, generally using pulsed ultraviolet light.

In the medicine history first time, in April 2016 Shafi Ahmed a cancer surgeon did an operation using a virtual reality camera at the Royal London hospital. It is a big step for in the field of surgery. Others also participated in the operation in real time through the Medical Realities website and the VR-Virtual Reality in OR-Optical Reality application. In this operation promising medical student from Cape Town, an interested journalist from Seattle or a worried relative, everyone could follow through two 360 degree cameras how the surgeon removed a cancerous tissue from the bowel of the patient [4-5].

This opens new horizons for medical education as well as for the training of surgeons. VR could elevate the teaching and learning experience in medicine to a whole new level. By using VR, surgeons can stream operations globally and allow medical students to actually be there in the OR using their VR goggles. The team of The Body VR is creating educational VR content as well as simulations aiding the process of traditional medical education for radiologists, surgeons, and physicians [1].

Automation in medical diagnosis

The automation is medical diagnosis of disease open up new path of advancement in medicine field. The diagnosis involves coordination in various subject areas in medicines like Medical Biochemistry, Human Pathology, Medical Microbiology, Radiology, with many sub branches like cytology, histopathology, immunology and so on. All these branches and sub branches experience the advancement with the advancement in the camera photography. The camera is specially moulded technology in the diagnosis whether it is sono-graphy or digitized X-ray. The use of spectroscopy in Medical Biochemistry revolutionized the diagnosis in Blood and other body fluids analysis. Now the time consumption becoming lesser for analysis with increased accuracy of the diagnosis. Here are certain examples regarding automation in medical diagnosis. The example in the histopathology is important to be highlighted. In the histopathology slides and cassettes were required to process the tissue isolated as sample. Cassette markers and slide writers are now commercially available and can be utilized by all laboratories. Manual data entry laboratories would rely on manual input into these machines. Laboratories with more sophisticated systems can interface specifically with these machines, so that cassette marking and slide writing become totally automatic procedures linked to, for example, data entry and work list generation, respectively. The advent of these machines has enabled clear, concise labelling of cassettes and slides and has reduced transcription error to a minimum [2].

If we look towards the microbiology in angle of diagnosis, then it can be well observed that today, automated instruments became the essential part of many clinical microbiology laboratories. The automated equipment is also available for the detection of positive blood cultures, the antimicrobial susceptibility testing and identification of microorganisms, the screening of urine samples for bacteria, and the isolation and antimicrobial susceptibility of *Mycobacterium tuberculosis* in clinical samples. The rapid detection of microorganisms in a patient's blood is of diagnostic and prognostic importance. Blood cultures, therefore, are essential in the diagnosis and treatment of the aetiological agents of septicaemia. As septicaemia constitutes one of the most serious infectious diseases, the rapid detection and identification of blood-borne bacterial pathogens is a major function of the clinical microbiology laboratory. Consequently, automated blood culture systems have been developed and refined over the past 30 years. The first semi-automated instrument to be used, the BACTEC 460 (Johnston Laboratories Inc.), detected radioactive carbon dioxide metabolized by microorganisms growing in a liquid medium with 14C incorporated. This soon gave way to the non-radiometric BACTEC 660/730, using infra-red detection of carbon dioxide [2].

Thus, in any medical field from taking medical

history to prescribing medicines, diagnosis, pharmacy every where the technology is making its important role. Now, the doctors and surgeons job became easy due to use of technology in their job. Surgeons and doctors can have more time for their knowledge up-gradation with use of technology in their work. The telemedicine concept revolutionized the medicine delivery system. Indian villages are now connected through telemedicine with the big hospitals. The expert doctors can now able to examine patients from distant locations and prescribe treatments. Thus telemedicine is in the path to fulfill the mandate of World Health day observed on 7th April 2018 "Health for All". Healthy citizens of a country can only be the valuable assets for that country. Our deep route will become stronger, if we concentrate on Healthy Citizens of India [3].

Conclusion

In past few decades there is revolution took place in the proper use of technologies in various healthcare sectors. At present we are far behind for better healthcare to fellow citizens of this world. Many diseases still have resultant death of the patients. The diseases like Cancer, AIDS-acquired immune deficiency syndrome, Tuberculosis are pandemic diseases. Many human beings falling prey in front of these diseases. The technological advancements even not able to help critical conditions of patients suffering from these diseases. Hence, applicable research is need of the society beyond the boundaries of the countries is the present need. Nanotechnology can be a better help in curing these diseases in future.

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Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

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Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

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Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O,

Kidd EAM, editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

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