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Role of Oxidative Stress and Antioxidants in Children with IDA

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Abstract

Background: Increased oxidative stress with free radical generation in iron deficiency anemia (IDA), its aggravation at therapeutic doses of iron and reduction of oxidative stress by antioxidant supplementation are well studied in adults. Studies in this regard are scanty in children.

Objective: The co-prescription of antioxidants with iron supplementation in IDA to counter the oxidative stress is a well studied and established fact in adults .Though iron supplementation is a common clinical practice in children it is not conventional to co-prescribe anti-oxidants in children. Therefore a study was undertaken to evaluate the oxidative stress in IDA in children and the effect of antioxidants during iron supplementation and to evolve an optimal suitable therapeutic strategy to minimize oxidative stress and there by adverse clinical effects.

Methods: All the children attending the pediatrics OPD in IRT PMCH during JULY/AUGUST 2008 were randomly screened for anemia by clinical and hemoglobin evaluation. 21 children whose parents gave consent for participation in the study were included for evaluation. They were in the age group of ten months to sixteen years. Nutritional status of the study population was recorded by a twenty four hour recall survey method. After deworming, children were started with oral iron supplementation in three different groups; group I – oral iron only, group II – oral iron with vitamin C, group III – oral

iron with vitamin E. Lipid peroxides and Lipid hydroperoxides were measured as the indices of oxidative stress before initiation, tenth day (I follow up), thirtieth day (II follow up) after oral iron therapy. Serum iron profile was also studied for evaluation.

Results: There was no significant difference in serum iron profile response to oral iron therapy between the groups. Oxidative stress indices showed a decreasing trend in all the groups with no significant difference among the groups. There were no clinical adverse effects of oral iron supplementation in all the groups.

Conclusion: Unlike in adults, oxidative stress in iron deficiency anemia is not aggravated by oral iron supplementation in children. There was no significant difference between oral iron alone and oral iron with antioxidants in terms of clinical and biochemical response. Lipid hydroperoxides seems to be an early indicator of oxidative stress.

Key Words: IDA, Children, Oxidative Stress, Iron therapy

Introduction

Iron deficiency anemia is a major public health problem in many developing countries. The vulnerable segments of the population are pregnant women and children and one of the common short term measure to control Iron deficiency anemia in them is oral iron supplementation (Srigiridhar, Nair KM 2000).The prevalence of iron deficiency anemia is still on the high in developing countries like india though a lot is known about it and many treatment strategies are available. The increasing prevalence could be due to poor compliance which is attributed to the side effects that arise during oral iron supplementation.

Absorption of iron is a highly regulated process. Only 10-15% of orally administered iron gets absorbed in small intestine and in iron deficiency the percentage may even increase to a maximal of 40% (Madhavan Nair 2001). Iron absorption is increased in iron deficiency, i.e. when iron stores are low, and in anemia, i.e. when tissue oxygen supply is compromised. Conversely, iron absorption decreases when iron stores are high. There are two forms of iron namely haem and non haem iron. Haem iron gets directly absorbed into the intestinal cells. In the duodenal brush border, Dcyt b reduces ferric (trivalent) non-haem iron to the ferrous (divalent) state, which is taken up from the lumen by the ‘‘Divalent Metal Transporter 1’’ (¼ DMT-1), the expression of which is related to body iron status. The ‘‘mucosa block’’ mechanism reduces iron absorption after a preceding high iron exposure, presumably by diminishing the number of DMT-1 receptors.

Ferrous iron is the form that is mostly used for correction of iron deficiency. Acidic milieu facilitates the absorption by keeping iron in the ferrous form. Most of the ferrous iron gets converted into ferric in the basolateral membrane and enters the circulation through transferrin and the amount of iron entering the circulation is regulated by body iron demands. A part of ferrous iron is converted to ferric iron and stored in intestinal mucosal cells as ferritin .The iron which is stored in the intestinal mucosal cells as ferritin will be lost after 2-3 days. Ferrous iron is a central pro-oxidant that propagates free radical reactions through Fenton chemistry during conversion of ferrous to ferric iron both locally (in the gastrointestinal tract) and systemically. An excess of pro-oxidants over antioxidants results in oxidative stress (OS).

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^- + OH^+ \text{ (Fenton)}$$

$$Fe(III) + O_2^{2-} \rightarrow Fe(II) + O_2$$

Intake of oral iron preparations at therapeutic dose levels frequently causes nausea, vomiting and epigastric discomfort. These effects seem to be due to mucosal irritation and altered gastrointestinal motility probably caused by ROS. Iron and reactive Oxygen species (ROS) can enhance mucosal injury by several mechanisms. One is by initiating lipid peroxidation either by iron itself or by the ROS produced during the Fenton reaction. Lipid peroxidation of cell membranes, including mitochondrial membranes, can in turn compromise cell integrity

and function and affect its energy status, thereby causing further tissue injury. Iron and ROS can also amplify intestinal inflammation by such mechanisms as increasing mucosal permeability, recruiting and activating more neutrophils and activating NF-κB, thereby upregulating the production of proinflammatory cytokines (Carrier et al 2002).

Unabsorbed dietary iron enters the colon and in conjunction with intraluminal bacteria may become available for participation in a combination of Haber-Weiss and Fenton type reactions that generate hydrogen peroxide and hydroxyl radicals at the mucosal surface. Moreover continuous exposure of iron to intestine is believed to decrease iron absorption from subsequent doses (Lund et al 1999).

Oxidative stress as shown to play an important role in pathogenesis of IDA (Vives et al; 1995).The results of a recent study confirmed that antioxidant enzymes activity like GSH-Px is decreased in children with IDA. These are indicators of increased lipid peroxidation (Tekin et al 2001). Furthermore, it has been shown that the addition of synthetic antioxidants in the treatment of children with IDA results in decrease of lipid peroxidation, prevention of pathologic progression and rapid improvement of clinical manifestations (shved et al; 1995). This confirms iron deficiency anemia is a state of oxidative stress.

The effects of antioxidants with oral iron to combat the stress and side effects have been tried in both human subjects (Carier et al 2002) and animals (Srigiridhar, Madhavan Nair 2000). Studies of this kind in children are scanty (Tekin et al 2001; Panchenko et al 1979). Commonly used antioxidants are vitamin C and vitamin E. Vitamin E is the most potent liposoluble antioxidant and has the potential to improve tolerance of iron supplementation and prevent further tissue damage (Burton et al 1986). Vitamin C helps in the absorption of iron by reducing non haem ferric to ferrous iron. By facilitating iron absorption, vitamin c makes more ferrous iron available to participate in Fenton reaction leading to oxidative damage. Addition of vitamin c with iron has proved to be a toxic cocktail rather being an advantage as an antioxidant (Anna EO Fisher† and Declan P Naughton; 2004). The role of vitamin C as an antioxidant or pro-oxidant is still not clear. Therefore a study was undertaken to evaluate the oxidative stress in IDA in children and the effect of antioxidants during iron supplementation and to evolve an optimal suitable therapeutic strategy to minimize oxidative stress and there by adverse clinical effects

Methodology

Study Sample Population

All the children attending the pediatrics OPD in IRT PMCH during JULY/AUGUST 2008 were evaluated clinically for anemia.

Inclusion Criteria

Clinically suspected anemic children were subjected to hemoglobin evaluation to confirm their anemic status.

Operational Definition for Anemia

The children were termed anemic keeping the WHOs criteria

<11g/dl for children aging 6 months to 6 years

<12g/dl for children >6 years

Later a written consent form was obtained from the parents of all children. Everyone were given a brief counseling regarding iron deficiency anemia and the present study. Health education was also imparted to the parents regarding best dietary practices for good iron stores.

In order to exclude other causes for anemia a complete blood count (CBC) with ESR including hematological indices like MCV, MCH, MCHC, and RDW were done for all children. CBC and other indices were done using an automated counter.

Iron Profile

Iron profile including serum iron, feritin , percentage saturation of transferrin and TIBC were done for each subject to confirm iron deficiency anemia and included in the study. Serum iron was measured by Ferrozine method without deproteinisation (micro g/dl) and serum ferritin by fully automated bidirectionally interfaced chemiluminescent immunoassay (ng/ml). With respect to iron profile, children were graded into 3 grades namely grade 1- negative iron balance, Grade 2 – stage of iron deficient ineffective erythropoiesis, grade 3 – iron deficiency anemia.(Harrison internal medicine 17th edition; volume 1; page 630; fig 98.2)

Exclusion Criteria

Children who were already diagnosed as anemic and were on hematinics were excluded for the study. Children with other systemic illnesses were also excluded.

Nutritional Assessment

The nutritional assessment of the children was done by using a twenty four hour dietary recall survey method and a food frequency questionnaire. The survey aimed at evaluating the general dietary pattern and the number of meals consumed per day. The questionnaire included the frequency of various food items like staple foods (rice/wheat/bajra/ragi/maize), gram (green/Bengal/black/peas), greenleaves (amaranth/sirukeerai/cauliflower), oil and nuts(coconut/groundnut/cashew nuts), fruits, milk, spices and animal sources (egg/beef/crab/prawn/chicken) and iron rich snacks.

The recommended dietary allowance (RDA) for each of the nutrient in terms of calories (Kcal), Protein (g), Fat (g), iron (mg), and calcium (mg) was calculated for each child and compared to the standard RDA for each age group as laid down by the National Institute Of Nutrition (NIN), ICMR, Newdelhi, India.

The questionnaire also included demographic factors like height, weight and were expressed in terms of percentile for height (cm) by age and weight (kg) by age as specified by CDC 2000 standards.

Oxidative Stress Profile

The level of oxidative stress was assessed by measuring plasma lipid peroxides and plasma lipid hydroperoxides).Lipid peroxides were measured in plasma by the **thiobarbituric acid method** as described by **BUEGE and AUST,** 1978 (micro moles/L) and lipid hydroperoxides were measured with FOX Reagent II as described by **JIANG et al** 1990 (micro moles/L).

Sampling Techniques and Study Groups

Children in the study were assigned numbers starting form 1,2,3 and so on as they were diagnosed sequentially as anemics (first diagnosed was assigned 1, second one as 2 and so on). The children in the study were randomly assigned into three groups in the following manner. Children assigned numbers 1,4,7,10 etc were in group 1, children numbered 2,5,8,11 etc were put in group 2, children numbered 3,6,912 etc were put in group 3.The three groups were different in terms of oral iron supplementation.

Group I- oral iron only, in the form of ferric ammonium citrate (syrup ferrochelate), 6mg/kg/day in two divided doses; **Group II-** oral iron with vitamin C (tablet Celin) at the dose of 250 mg once daily; **Group III-** oral iron with vitamin E (capsule Evion) at the dose of 200 µg once daily. The parents and the biochemical analyst were blinded regarding the groups.

All the children in the study were dewormed before therapeutic intervention.

Follow-up

All the children in the study were followed up continuously up to 30 days after initiation of drug treatment. The parents were instructed to contact at any time if they face any adverse effects during the treatment. All the parents were requested to attend the OPD for 2 follow ups:

- First Follow up: 10 days after initiation of treatment
- Second Follow up: 30 days after initiation of treatment

Adverse effects (if any) were asked for during each follow ups. Indices of oxidative stress namely plasma lipid peroxides and lipid hydroperoxides were measured again during both follow up. Iron profile (serum iron and ferritin) was measured in the second follow up to look for improvement due to therapeutic intervention.

Institutional committee approval was obtained prior to the study

Statistical Analysis and Study Type

The statistical methods employed in the study were Analysis Of Variance (ANOVA) and Paired-t test. The software used for analysis is SPSS. Type of study is Randomised clinical trial.

Results

Majority of the subjects were male (16) and majority of them (12) belong to the age group of 1-5 years. Only two were less than 2 years and four in the age group of 5-10 years and three more than 10 years. Fourteen out of twenty one subjects had their weight less than third percentile, eleven had their height less than third percentile. When height and weight were put together, nine were less than third percentile. Most of the subjects were of low socioeconomic status.

With respect to the nutritional profile, majority of the subjects (17) were nonvegetarian. Majority of them (13) had three meals pattern per day. The food composition showed that inclusion of iron rich sources in their diet like green leafy vegetables, chicken, egg and fish was meager in majority of the subjects. The deficit profile comparing the RDA of each nutrient with the standard RDA showed that most of the subjects consumed less than their required RDA with respect to all nutrients.

By virtue of iron profile, subjects fall under three categories (Table I). Majority of them were in Grade III (iron deficiency anemia) whereas six subjects were in grade I (stage of negative iron balance), and four were in grade II (Stage of ineffective erythropoiesis). In terms of response to oral iron therapy as measured by serum iron and ferritin, all the subjects in three groups showed significant improvement by Paired T test(Table II & III) but there is no significant difference between the three groups by ANOVA (Table IV). Oxidative stress indices namely lipid peroxide and lipid hydroperoxides though showed a decreasing trend in all the three groups was not statistically significant by ANOVA (Table V&VI).The basal level of lipid hydroperoxide was statistically significant than lipid peroxide by ANOVA (Table V&VI). The parents of the subjects reported improvement in them during I Follow up. Adverse effects were not reported from any of the subjects during treatment.

Discussion

Iron deficiency anemia presents with a spectrum of oxidative stress and altered antioxidant activity. The view of treating IDA has been changing for past few years. The fact that the ability of iron in generating free radicals and hence the adverse effect lead to the role of antioxidant as adjuvant and also lead to modification of iron dosage particularly in adults. Lipid peroxidation is a well known example of oxidative damage in cell membranes, lipoproteins, and other lipid containing structures. Peroxidative modification of unsaturated phospholipids, glycolipids, and cholesterol can occur in reactions triggered by free radical species such as hydroxyl radicals derived from iron-mediated reduction of hydrogen peroxide**.** ROS like HO**.** generated by Fenton chemistry (H_2O_2/iron) gives rise to primary stage LOOHs (lipid hydroperoxides). These LOOHs may undergo iron-mediated one-electron reduction and oxygenation to give epoxyallylic peroxyl radicals (OLOO**.**), which trigger exacerbating rounds of free radical-mediated lipid peroxidation. These free radicals get neutralized by antioxidant defense system. When there is excess iron available as in case of maintaining the same dose of iron throughout the course of treatment for restoring the iron stores, there would be generation of more free radicals beyond the ability of antioxidant defense system. These leads to oxidative damage which could be responsible for the adverse clinical effects encountered during oral iron therapy. Studies (Neeta Kumar et al 2009; Jansson et al 1985; Chen et al 2007; Yang et al 1999; Rehema et al 2004; Schumann 2001; Srigiridhar, Nair KM 1998) also found that supplementation of iron leads to oxidative stress.

In the present study, there was no discernible clinical adverse effect in all the three groups. However, all the three groups showed good improvement in response to oral iron as evident from the iron profile. There was a decreasing trend in oxidative stress indices (lipid peroxides and lipid hydroperoxides) in all the three groups although there was no difference between the groups. So supplementation of antioxidants with iron in children has no added advantage. A significant level of lipid hydroperoxides was observed in children with the diseased state. These indicate that iron deficiency is a state of oxidative stress. As we know iron is required as a structural and functional component of various compounds (catalase, peroxidase, cytochrome oxidase, NADPH reductase, iron sulfur complex), it plays a vital role in maintaining the antioxidant defense system of our body. Normally there exists a balance between free radical production and antioxidant defense system. In iron deficiency anemia the enzymes involved in the antioxidant defense system will be functionally defective. So the balance gets tilted towards free radicals triggering oxidative damage. Further iron dependent mitochondrial oxidative phosporylation also gets affected in iron deficiency causing decreased ATP production and ultimately leading to loss of structural and functional integrity of cell. Studies (Isler et al 2002; Moriarty et al 1995, Kumerova et al 1998; Srigiridhar, Madhavan Nair 2000; King et al 2008) are on par with the fact that antioxidant status is lowered in iron deficiency.

The correction of iron deficiency with oral iron supplementation leads to rejuvenation of defective antioxidant system and brings back the balance between ROS and antioxidant system. This could explain the absence of any adverse clinical side effect seen during oral iron supplementation in the present study. Due to rejuvenation of antioxidant defense system and metabolic functions of the cell, supplementing antioxidants with iron to combat free radicals is not needed. Antioxidants could play a role once hemoglobin and serum iron

reaches normal level during later part of iron therapy where therpaeutic iron acts as a pro-oxidant.

Conclusion

Oxidative stress as in adults does exist in children with Iron deficiency. Among the indices measured (lipid peroxides and hydroperoxides), lipid hydroperoxides may be an earlier and sensitive indicator of oxidative stress. But unlike in adults, aggravation of oxidative stress and its consequent adverse effects do not occur with oral iron supplementation in children. There was no significant difference in therapeutic response between the groups- iron only, iron supplemented with antioxidants. Therefore, oral iron alone is safe and efficacious in children with iron deficiency anemia. However, results have to be confirmed by a larger sample size and follow up for a longer period of time with varying doses.

Acknowledgments

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Dr Parasuram & Dr Natesh Prabu

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Table I: IDA Profile

Grade	No. of Subjects	Iron Profile
Normal		Serum Iron: 50-150 micro g/dl Serum Ferritin: 50-200 micro g/dl TIBC: 300-360 micro g/dl % saturation: 30-50%
GRADE I Negative iron balance	6	Serum Iron: 50-150 Serum Ferritin: <20 TIBC: >360 % saturation: 30-50%
GRADE II Iron deficient erythropoiesis	4	Serum Iron: <50 Serum Ferritin: <15 TIBC: >380 % saturation: <20%
GRADE III Iron deficiency anemia	11	Serum Iron: <30 Serum Ferritin: <15 TIBC: >400 % saturation: <10%

Majority of the subjects were in grade III established iron deficiency anemia

Table II: Serum Iron Paired T Test

Category	Basal Mean before treatment (micro g/dl)	After II follow up Mean (micro g/dl)	Standard Deviation	Standard error	t value	Probability
I (Iron only)	47	74	42	25	1.0883	0.1951
II (Iron and vitamin C)	31	143	143	54	2.0635	0.0423
III (Iron and vitamin E)	28	85	45	17	3.3797	.074

There was no significant difference between groups in terms of serum iron in response to oral iron therapy.

Table III: Serum Ferritin Paired T Test

Category	Basal Mean (ng/ml)	After II follow up Mean (ng/ml)	Standard Deviation	Standard error	t value	Probability
I (Iron only)	14	59	32	19	2.4079	0.0689
II (Iron and vitamin C)	21	47	28	11	2.3877	0.0271
III (Iron and vitamin E)	16	27	11	4	2.837	0.0148

There was no significant difference between groups in terms of serum ferritin in response to oral iron therapy.

Table IV: Iron and Ferritin Response Profile (ANOVA)

Categories	Mean Value of Iron Basal (microg/dl)	Mean Value of Iron second follow up (microg/dl)	Mean Value of Ferritin Basal (ng/ml)	Mean Value of Ferritin second Follow up (ng/ml)
I (Iron only)	47	74	14	59
II (Iron and vit C)	31	143	21	47
III (Iron and vit E)	28	85	16	57
F Value	1.63	0.82	0.13	1.62

There is no significant difference between the three groups in terms of response.

Table V: Lipid Peroxide Profile (ANOVA)

CATEGORIES	Basal Mean (micromoles/L)	I Follow up Mean (micromoles/L)	II Follow up Mean (micromoles/L)
I (Iron only)	2.15	1.51	1.16
II (Iron and vit C)	2.41	1.54	1.02
III (Iron and vit E)	2.35	1.44	1.18
F Value	0.28	0.12	0.53

Oxidative stress index namely lipid peroxide though showed a decreasing trend in all the three groups was not statistically significant.

Table VI: Lipid Hydroperoxide Profile (ANOVA)

Categories	Basal Mean (micromoles/L)	I Follow up Mean (micromoles/L)	II Follow up Mean (micromoles/L)
I (Iron only)	3.99	4.25	3.14
II (Iron and vit C)	5.06	4.37	3.04
III (Iron and vit E)	3.60	4.61	3.19
F Value	2.77	0.09	0.05

Oxidative stress index namely lipid hydroperoxides though showed a decreasing trend in all the three groups was not statistically significant.
Among the indices measured lipid hydroperoxides may be an earlier and sensitive indicator of oxidative stress as the F value is high in children before treatment (basal)

Appendix A: Consent Form

I parent of have been explained in detail (Method,duration, risk involved etc.) about the research project entitled **"Role Of Oxidative Stress and Antioxidants in children with IDA"** by investigators.
I have been explained about the various investigations that will be done to my child.
I wholeheartedly give my consent to proceed with the investigations for this project.

Signature:
Date:

Note: This format was given to all the parents of the human volunteers in Tamil language (mother tongue of the volunteers) and all methods, procedures will be explained to them by the investigators.

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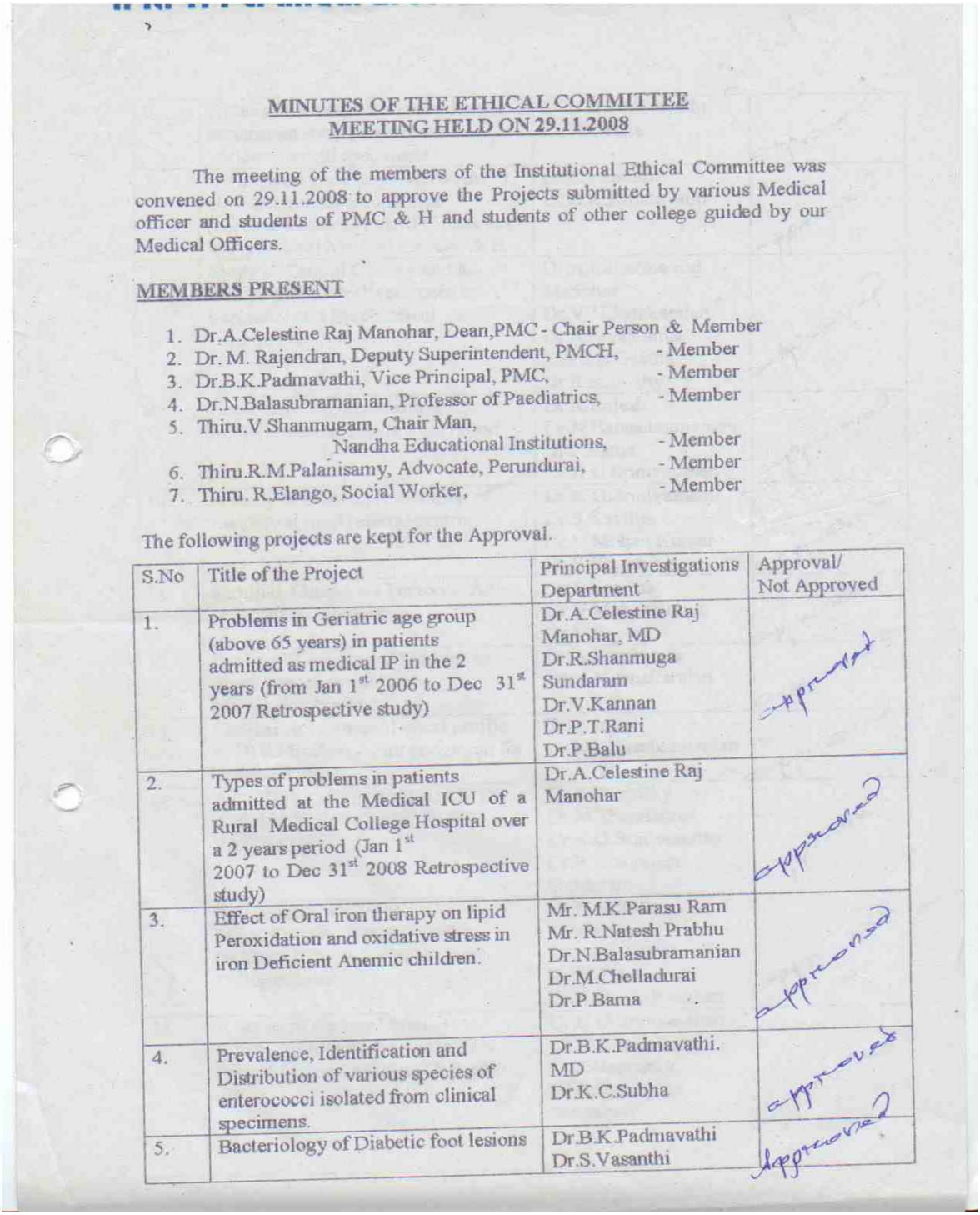
Appendix B: Institutional Ethical Committee Approval



MINUTES OF THE ETHICAL COMMITTEE MEETING HELD ON 29.11.2008

The meeting of the members of the Institutional Ethical Committee was convened on 29.11.2008 to approve the Projects submitted by various Medical officer and students of PMC & H and students of other college guided by our Medical Officers.

MEMBERS PRESENT

1. Dr.A.Celestine Raj Manohar, Dean,PMC - Chair Person & Member
2. Dr. M. Rajendran, Deputy Superintendent, PMCH, - Member
3. Dr.B.K.Padmavathi, Vice Principal, PMC, - Member
4. Dr.N.Balasubramanian, Professor of Paediatrics, - Member
5. Thiru.V.Shanmugam, Chair Man, Nandha Educational Institutions, - Member
6. Thiru.R.M.Palanisamy, Advocate, Perundurai, - Member
7. Thiru. R.Elango, Social Worker, - Member

The following projects are kept for the Approval.

S.No	Title of the Project	Principal Investigations Department	Approval/ Not Approved
1.	Problems in Geriatric age group (above 65 years) in patients admitted as medical IP in the 2 years (from Jan 1st 2006 to Dec 31st 2007 Retrospective study)	Dr.A.Celestine Raj Manohar, MD Dr.R.Shanmuga Sundaram Dr.V.Kannan Dr.P.T.Rani Dr.P.Balu	Approved
2.	Types of problems in patients admitted at the Medical ICU of a Rural Medical College Hospital over a 2 years period (Jan 1st 2007 to Dec 31st 2008 Retrospective study)	Dr.A.Celestine Raj Manohar	Approved
3.	Effect of Oral iron therapy on lipid Peroxidation and oxidative stress in iron Deficient Anemic children.	Mr. M.K.Parasu Ram Mr. R.Natesh Prabhu Dr.N.Balasubramanian Dr.M.Chelladurai Dr.P.Bama	Approved
4.	Prevalence, Identification and Distribution of various species of enterococci isolated from clinical specimens.	Dr.B.K.Padmavathi. MD Dr.K.C.Subha	Approved
5.	Bacteriology of Diabetic foot lesions	Dr.B.K.Padmavathi Dr.S.Vasanthi	Approved

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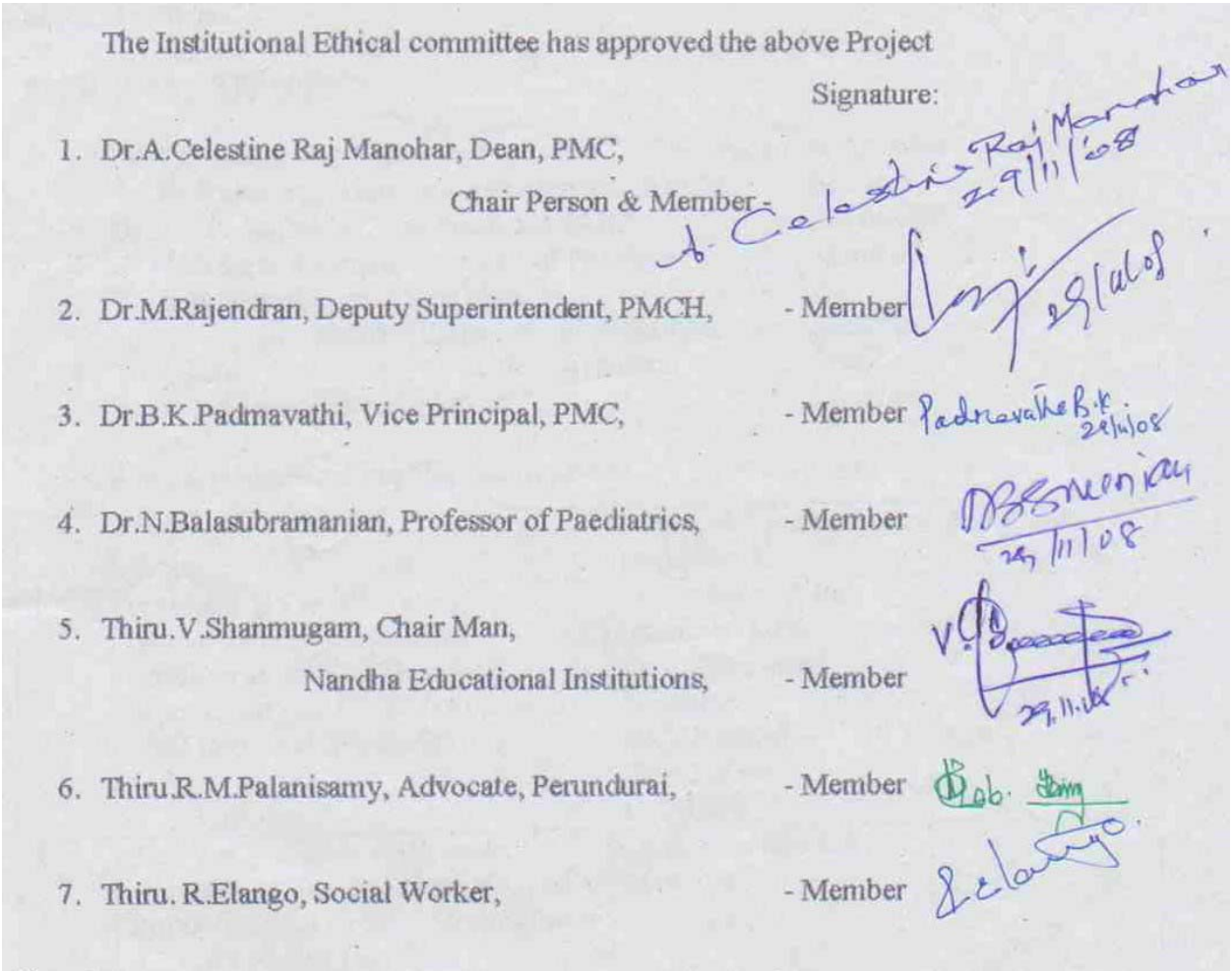

The Institutional Ethical committee has approved the above Project

Signature:

1. Dr.A.Celestine Raj Manohar, Dean, PMC,
   Chair Person & Member -
2. Dr.M.Rajendran, Deputy Superintendent, PMCH, - Member
3. Dr.B.K.Padmavathi, Vice Principal, PMC, - Member
4. Dr.N.Balasubramanian, Professor of Paediatrics, - Member
5. Thiru.V.Shanmugam, Chair Man,
   Nandha Educational Institutions, - Member
6. Thiru.R.M.Palanisamy, Advocate, Perundurai, - Member
7. Thiru. R.Elango, Social Worker, - Member