"SERUM ADENOSINE DEAMINASE ESTIMATION AFTER BCG VACCINATION AS A MARKER OF CELL MEDIATED IMMUNITY AND ITS CORRELATION WITH TUBERCULIN SKIN TEST"

Submitted by

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Dissertation submitted to the

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In partial fulfillment of the requirements for the degree of

MD

In

PEDIATRICS

Under the guidance of

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SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL &

RESEARCH CENTRE, BIJAPUR

2010

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KARNATAKA, BANGALORE

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"SERUM ADENOSINE DEAMINASE ESTIMATION AFTER BCG VACCINATION AS A MARKER OF CELL MEDIATED IMMUNITY AND ITS CORRELATION WITH TUBERCULIN SKIN TEST"** is a bonafide and genuine research work carried out by me under the guidance of **DR.R.H. GOBBUR M.D.,DCH. PROFESSOR & UNIT CHIEF, DEPARTMENT OF PEDIATRICS, SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, BIJAPUR.**

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LIST OF ABBREVIATIONS

ADA	**Adenosine Deaminase**
AMP	**Adenosine monophosphate**
ATP	**Adenosine triphosphate**
BCG	**Bacille Calmette Guerin**
CD	**Cluster of Differentiation**
CMI	**Cell Mediated Immunity**
DTH	**Delayed Type Hypersensitivity**
IAP	**Indian Academy Of Pediatrics**
ID	**Intra Dermal**
IFN	**Interferon**
IL	**Inter Leukin**
IU/L	**International units per litre**
LC	**Lymphocyte**
mm	**Millimeter**
NK	**Natural killer**
PPD	**Purified Protein Derivative**
RNTCP	**Revised national Tuberculosis control program**
SD	**Standard Deviation**
TB	**Tuberculosis**
TST	**Tuberculin Skin Test**
yrs	**Years**

ABSTRACT

Objectives

This study was designed to measure serum ADA levels 6+/-1 week after BCG vaccination and to clinically compare them with matched controls to evaluate if this test can be used to measure cell mediated immunity evoked by BCG vaccination and to clinically compare tuberculin reactivity after BCG vaccination with ADA levels.

Methods

This study is carried out in the Department of Pediatrics, BLDEA'S Shri. B. M. Patil Medical College, Bijapur from November 1 2007 to July 2009. 30 Term healthy neonates were taken up from post natal wards of the hospital (study group), 30 Infants of 6 to 8 weeks of age who were not vaccinated earlier were taken as matched controls (control group). Serum ADA (Adenosine deaminase) estimation was done in the study group with in 24 hours of birth and 6 weeks after BCG vaccination and TST(Tuberculin skin test) was done clinically to compare tuberculin reactivity and ADA levels. Serum ADA levels were estimated in control group to compare them with the study group at 6 weeks.

CONCLUSION

The present study concludes that the rise in ADA levels 6 weeks after BCG vaccination is statistically significant; concluding cell mediated immunity is provided BCG vaccination. Also the ADA levels of the study group at 6 weeks compared to matched controls who were not vaccinated earlier is significant. Tuberculin skin test is the most common measure performed to evaluate the efficacy of BCG vaccination. The

present study showed a conversion rate of 96.67% and a positive correlation with the ADA levels. **The present study is the first of its kind to measure CMI after BCG vaccination in the form of increased ADA activity and to compare the same with TST.**

Key words: BCG vaccination, Serum ADA (Adenosine deaminase) levels, TST (Tuberculin skin test).

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INTRODUCTION

Tuberculosis, a disease caused by bacterium Mycobacterium tuberculosis has affected mankind for over 5000 years and the disease continues to be a major cause of morbidity and mortality although the bacilli has been discovered over a century back (1882 Robert Koch) and drugs have been available for more than 70 years. Nearly a third of world's population is infected with TB bacilli. Poor living conditions debility and malnutrition predisposes population to disease.

The SE Asia region, with 25% of the world's population accounts for 34% of the TB burden, of the 22 high burden countries, (accounting for 80% of global TB burden), five are in the region, including India the highest TB burden country in the world. India accounts $1/5^{th}$ of the global incidence and $2/3^{rd}$ of cases in SE Asia. Nearly 40% of Indian population is infected with TB bacillus. Each year 1.9 million new cases occur, of that 0.8 million are infections new smear positive pulmonary TB.

Tuberculosis is a major cause of childhood morbidity and mortality. It is estimated that about 6 – 8% of all new causes are seen in the 1 to 4 year age group due to their low resistance to prevent progression of infection to diseases. The epidemiological data available to date demonstrate that spread of Tuberculosis (TB) is a global health emergency. The continuous spread of the TB pandemic justifies a need for the accelerated development of safe, more effective and affordable vaccines and new therapeutic strategies. The currently available vaccine the only one available against Tuberculosis was developed more than 100 years ago. Several variants of the vaccine based on different attenuated strains of M. bovis (BCG- Pasteur, Tokyo, Glaxo Smith Klin Biologicals, Tice etc.) are being used as a part of childhood immunization programs in many parts of the world supported by WHO and UNICEF.

Over the past years, BCG has been shown to be safe and inexpensive. However it has been established that BCG vaccines are not able to prevent infection with wild type M. tuberculosis in children, although they are still effective in preventing meningitis and miliary tuberculosis in 80% of vaccinated children[1]. In recent years no vaccine other than BCG has been the subject of such controversies and extensive reviews, regarding its efficacy for protection against tuberculosis[2].

For prevention of childhood TB, BCG vaccine is advocated. The mechanism of protection from BCG vaccination involves a reduction in the hematogenous spread of bacilli from the site of primary infection. The increase in the incidence of Tuberculosis in children cannot be decreased by chemotherapy alone; immunoprophylaxis needs continuous researching for availability of more efficacious BCG vaccination.

IAP subspecialty chapter of Tuberculosis was made in 1989.To tackle this global health emergency of Tuberculosis now a committee has been formed involving WHO experts, health ministry , Pediatricians of IAP who have made guidelines for management of tuberculosis in children[3].The rates of infection estimate the disease burden in the community[4].

Adenosine deaminase (ADA) is an enzyme with principal biological activity in T-Lymphocytes. It is required for lymphocyte and differentiation. The enzyme activity is known to be elevated in certain infections where immunity is cell mediated. The central and diversified role of T lymphocyte cells is well documented. T-cells are the main affector cells of cell mediated immunity. They regulate all types of response by either acting as helper or suppressor cells. As a relationship exists between ADA

activity and cell mediated immune response, it is considered as a marker of cell mediated Immunity[5].

The tuberculosis skin test has been the traditional method of diagnosis infection with mycobacterium tuberculosis. Appropriate use of the skin test requires a knowledge of antigen used (tuberculin), the immunological basis for reaction to this antigen, the technique of administering and reading the test, and the results of epidemiologic and clinical experience with the test.

Tuberculin reactivity after BCG vaccination has been the most common measure of effect of BCG vaccine. The current study is taken up to measure cell mediated immunity in the term of ADA activity induced by BCG vaccination and to correlate it clinically with tuberculin test.

AIMS AND OBJECTIVES

1. To measure serum ADA levels 6+/-1 week after BCG vaccination and to clinically compare them with matched controls to evaluate if this test can be used to measure cell mediated immunity evoked by BCG vaccination.
2. To clinically compare tuberculin reactivity after BCG vaccination to measure CMI and to compare with ADA levels.

REVIEW OF LITERATURE

In Greek literature description of Tuberculosis appears around the time of Hippocrates (460-377 BC) .The Hippocratic school considered pulmonary pthisis a hereditary rather than a infectious disease[6].

Tuberculosis is caused by a prokaryote, demonstrated by Robert Koch, a physician from Wolstein, Germany who was able to isolate pure cultures of tubercle bacilli and inoculate them into healthy mice and guinea pigs, concluding in the famous Kochs postulates. Koch published his findings on 10th April 1882 after presenting his findings on 21st march 1882. The bacterium was named mycobacterium, (Greek mykes, fungus: bacterium; small rods) in 1896 by lehmannand Neumann in reference to the mold like pellicles for med by the bacteria on liquid medium.

M. Tuberculosis is clinically most important of the M. Tuberculosis complex which includes M. Tuberculosis, M. bovis and its BCG (Bacille Calmette- Geurin) variant, M africanum and M Microti. The members of this complex are closely related as seen by DNA Homology[7,8].

Figure 1, Robert Koch.

TB HISTORY AND DATES

1851 – one person in four killed by TB in Europe and America.

1854 – Sanatorium treatment began. Treatment commenced with 24 hours confinement to their bed, with no activity allowed at all. Slowly periods of increasing activity were introduced. Fresh air was advised for patients at all times and in all weathers – many former patients of sanatoria remember being snowed on when their bed had been wheeled out into the open air – some even slept like this! A healthy diet was also promoted.

1882 – Robert Koch identifies that TB is caused by an organism, Mycobacterium tuberculosis.

1904 – the first charity seal to fundraise for tuberculosis patients introduced in Denmark. America adopted the idea in 1907.

1920 – the first human trials of the vaccine Bacille Calmette Guérin (BCG), an attenuated version of Mycobaterium bovis (Bovine TB).

1935 – Pasteurisation of milk introduced in Britain.

1944 – Drs Schatz, Bugie and Waksman announced the discovery of a drug called 'Streptomycin' and the first patient to be successfully treated with the drug.

1952 - Drs Robizek and Selikoff at Seaview Hospital, New York, use a new drug called 'Isoniazid' to treat TB patients.

1953 – BCG vaccination introduced in secondary schools in the UK, after the introduction of similar programmes in France and Scandinavia following a survey of 50,000 children which showed an 80% reduction in infection rate. At the time, those most at risk of TB in the UK were young adults in industrialised settings. The USA opts not to use BCG after their research showed contrary conclusions.

1960 - Dr John Crofton, a TB expert at the University of Edinburgh, (and now Honorary President of TB Alert) proposed that a combination of drugs, Streptomycin, PAS and Isoniazid made TB completely curable and he declared "all out war" to conquer the disease. His proposals included the pasteurization of milk, tuberculin testing of cattle, BCG vaccinations of whole populations, mass radiography for the early detection of disease, triple therapy for every infected patient, isolation of the infectious until no longer so and reduction in household overcrowding. All this would hopefully be accompanied by a general improvement in the standard of living.

1970 – first outbreak of drug resistant TB in USA.

1987 – TB figures in England and Wales at their lowest since records began - 5086 cases - following a downward trend since the beginning of the century. Since then, figures have increased almost every year

1993 – The World Health Organisation declares TB a global emergency, estimating that one third of the world's population (2 billion people) is latently infected with TB and 7-8 million cases of active TB occur each year. TB is killing more people than any previous year in history.

Directly Observed Treatment, Short-course (DOTS), locally known as the Revised National Tuberculosis Control Programme (RNTCP), introduced in India. DOTS is the only strategy which has proven effective in controlling TB on a mass basis and India carries the burden of the highest number of TB cases in any one country.

1995 – the first recorded outbreak of MDR-TB at a London hospital HIV Unit.

1999 -TB Alert, the UK's National Tuberculosis charity founded in 1998, in response to the fact that, despite the rising figures and the World Health Organisation's statement that TB is a global emergency, there was no dedicated charity in the UK working with TB.

2004 – National Action Plan, "Stopping Tuberculosis in England" published by Chief Medical Officer.

Nelson Mandela called for stepped up efforts to control tuberculosis, saying the fight against AIDS is incomplete without targeting the lung disease. "TB is too often a death sentence for people with AIDS. It does not have to be this way," said Mandela, who successfully battled tuberculosis while in prison during the apartheid era.

Tuberculosis is one of the most common diseases that attacks AIDS patients after their immune system has been destroyed by the virus. Many people with HIV die prematurely from TB because they are not treated in time. "The world has made

defeating AIDS a top priority. This is a blessing, but TB remains ignored," said Mandela, at the International AIDS Conference in Thailand.

He noted that mankind had known the cure for TB for more than 50 years. But what had been missing was the "will and the resources to quickly diagnose people with TB and get them the treatment they need. We can't fight AIDS unless we do much more to fight TB as well."

2005 – Withdrawal of the BCG school vaccination programme in the UK. The programme was replaced by a targeted, risk based strategy. This followed the example of other risk based BCG vaccination programmes in Holland and Germany. Figures from the Health Protection Agency showed that the pattern of disease in the UK was concentrated within specific groups; the homeless, people with substance misuse issues, prisoners and those coming from countries of high incidence. Research had also shown that BCG had been found to be most effective at preventing the more severe forms of TB in younger children but was not as effective at preventing pulmonary (lung) TB in adults.

The World Health Organization (WHO) Regional Committee for Africa comprising health ministers from 46 Member States declared tuberculosis an emergency in the African region - a response to an epidemic that has more than quadrupled the annual number of new TB cases in most African countries since 1990 and is continuing to rise across the continent, killing more than half a million people every year.

2006

January: The Stop TB Partnership launched its Action Plan to Stop Tuberculosis 2006-2015.

The Plan aims to cut deaths from TB in half in the next ten years and provide treatment for 50 million people. It requires $56 billion to carry out its aims – less than $1 per day of healthy life gained, with 14 million lives saved by 2015. At the launch of the Plan, which took place at the World Economic Forum in Davos, Bill Gates pledged to triple investment through the Gates Foundation, taking the amount committed from $300 million to $900 million. The total funding gap to carry out the Global Plan is estimated at $31 billion.

This is the second Global Plan from the Stop TB Partnership. The first, covering 2001-2005, led to the number of patients treated in DOTS programmes being doubled over 5 years, from 2 million in 2000 to 4 million in 2004, as well as a major improvement in case detection – both India and China which between them have 35% of the world's TB cases are now close to the target of 70%. The new plan, based on WHO's new Stop Tuberculosis strategy, builds on the first one in that, it seeks to deliver more on the ground and gives greater emphasis to the issues of HIV/TB co-infection and MDR TB through adapting the use of DOTS.

The barriers to stopping the TB are complex and vast and the Plan recognises that these need to be identified and removed. As well as increasing the accessibility of quality anti-TB drugs, the social burdens of the disease for patients also need to be addressed. Equally, health services need to be adequately resourced and committed to eliminating TB. More effective tools are also an aim of the Plan, with targets of diagnostic tests at the point of care by 2012, a safe, effective and affordable vaccine by 2015 and a treatment regime of 1-2 months shortly after 2015.

US-Danish non-profit initiative, the Aeras Global TB Vaccine Foundation, started the first laboratory dedicated to improving the TB vaccine. The current BCG vaccine was invented over 80 years ago and is frequently ineffective, especially in people with lowered immunity. Aeras has received a $82.9 million grant from the Bill and Melinda Gates Foundation for its work.

March: The National Institute for Clinical Excellence (NICE) publishes "Guidelines for the clinical diagnosis and management of tuberculosis, and measures for its prevention and control" in the UK.

EPI: Expanded Program On Immunization 1974.

UIP: Universal Immunization Program 1985.

RNTCP & DOTS: 1992 Onwards.

Despite the NTP (National TB Control Program) been in existence since 1962, no appreciable change in the epidemiological situation in the country had been observed.

The HIV –AIDS epidemic and MDR-TB were further threatening to worsen the situation .In view of this in 1992 WHO and SIDA renewed NTP TO RNTCP(Revised National TB Control Program).RNTCP adopted DOTS(Directly Observed Treatment Short Course) the most systematic and cost effective approach in INDIA to fight TB.

Taxonomy

Mycobacterium is the only genus in the family mycobacterium. The high guanine + cytosine (GC) ratio is similar to that of other mycolic acid producing bacteria- nocardia (60-69%), Rhodococcus (59-69%) and cornyebacterium (51-59%). This similarity may support the consolidation of these genera into a single family. Currently there are over 100 recognized species of mycobacterium. This genus contains obligate pathogenic, opportunistic and saprophyte species. A phylogenetic analysis based on 16s rRNA placed the fast and slow growing species into clear and separate branches of the evolutionary tree[9].

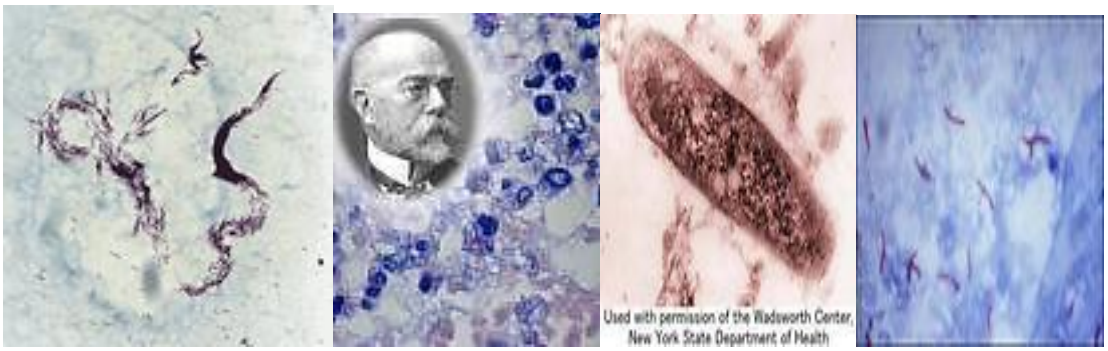


Figure 2 M.tubeculosis, Electron microscopy & ZN staining.

The mycobacteria are nonmotile, non spore forming, slightly curved or straight bacilli, 0.2 -0.6 by 1-10mm in size, sometimes branching filamentous or mycelium like may occurs, but it easily fragments into rods or coccoid elements.

My co bacteria have a unique cell wall responsible for its virulence, diagnostic staining and treatment. The cell wall has high lipid contents that includes a waxy coat made of 3 major components; mycolic acids, cord factor, wax D and sulfatids.

The mycolic acids of mycobacteria are unique alpha branched chains of hydroxyl fatty acids. These are strong hydrophobic and significant determents of

virulence, as they prevent attack on mycobacteria by cationic proteins, lysozymes and oxygen radicals in the phagocytic cells. They also protect the extra cellular organisms from complement attack. The cord factor is another constituent responsible for serpentine cording of the organism. It is toxic to mammalian cells and is also inhibitor of polymorphonuclear cell migration. The cord factor is most abundant in virulent strain of M. tuberculosis. The robust cell wall also provides resistance to killing by acidic and alkaline compounds, osmotic lysis via complement and resistance to common antibiotics.

Mycobacteria are not readily stained by gram strain. The acid fast staining technique although pioneered by Ehrlich, is now known as ziehl- Neelsen (ZN method) and has remained essentially unchanged since 1884. Decolorization with alcohol and cold staining techniques as well as the use of fluorescent dyes (e.g. auramin - 0) to visualize the mycobacteria have also been developed.

A typical mycobacteria, MOTT (mycobacteria other than tubercle) have been on the rise, mainly as a consequence of the AIDS epidemic [10].The mycobcateria are one of the slowest growing (generation time in animal tissues approximately 24 hrs) organisms, with varying colony morphology. M. tuberculosis forms rough colonies with bacilli compacted into curving strands (serpentine cords). In contrast M.avium complex usually forms smooth transparent colonies with the bacilli arranged in no definite pattern. Egg based solid media like Lowenstein – Jensen petragnani or American Thoracic society medium are very rich and contain phospholipids that tend to bind and/ or neutralize toxic products in clinical specimens. Sometimes Agar mediums are also used with low yield (middle brook 7H10 or 7H11).

Figure 3 , M.tuberculosis media (Bactec).

IMMUNITY AND TUBERCULOSIS

The two arms of the immune system are Cellular and Humoral. Cellular immunity is more important in dealing with intracellular organisms like mycobacteria, leishmania, listeria etc. in tuberculosis, alveolar macro phages, activated T lymphocytes, activated NK (natural killer) cells and their induced cytokines and chemokines provide the initial defense against infection. While macrophages are the principle effecter cells that kill bacteria, T lymphocytes are the inducers of protection.

Further, the cell mediated Immunity is of two types –

1. Cell –mediated immunity (CMI): Refers to a clonally – expanded, thymus derived T lymphocyte, population – specific for the antigens of the tubercle bacillus. This is a beneficial response to the host.
2. Delayed type hyper sensitivity (DTH): It is an adverse immunologic reaction to components of the tubercle bacillus that is also mediated by T lymphocytes and some cytokines produced by them.

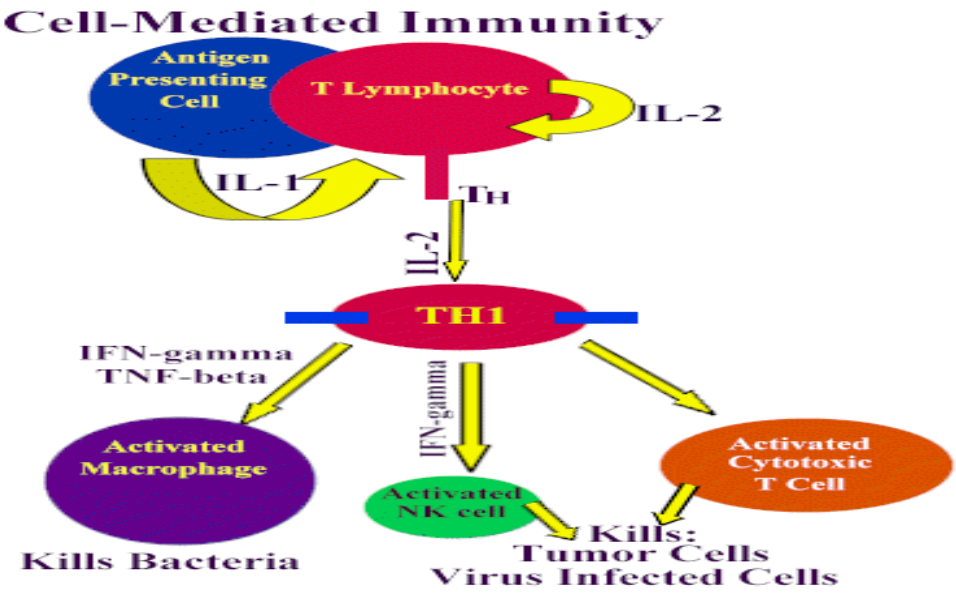


Figure 4 , Immunity Mechanisms.

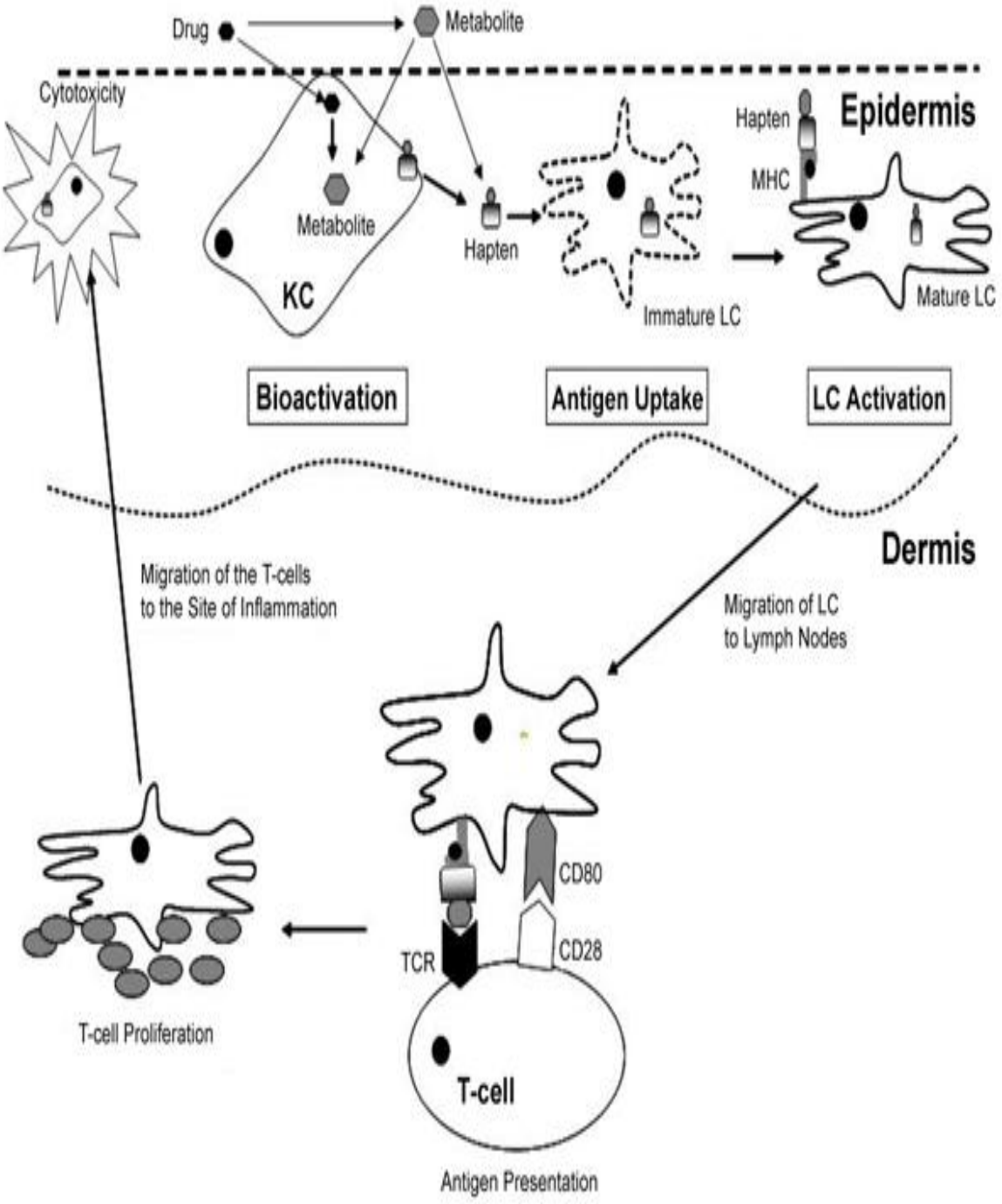


Figure 5, Delayed Type Hypersensitivity.

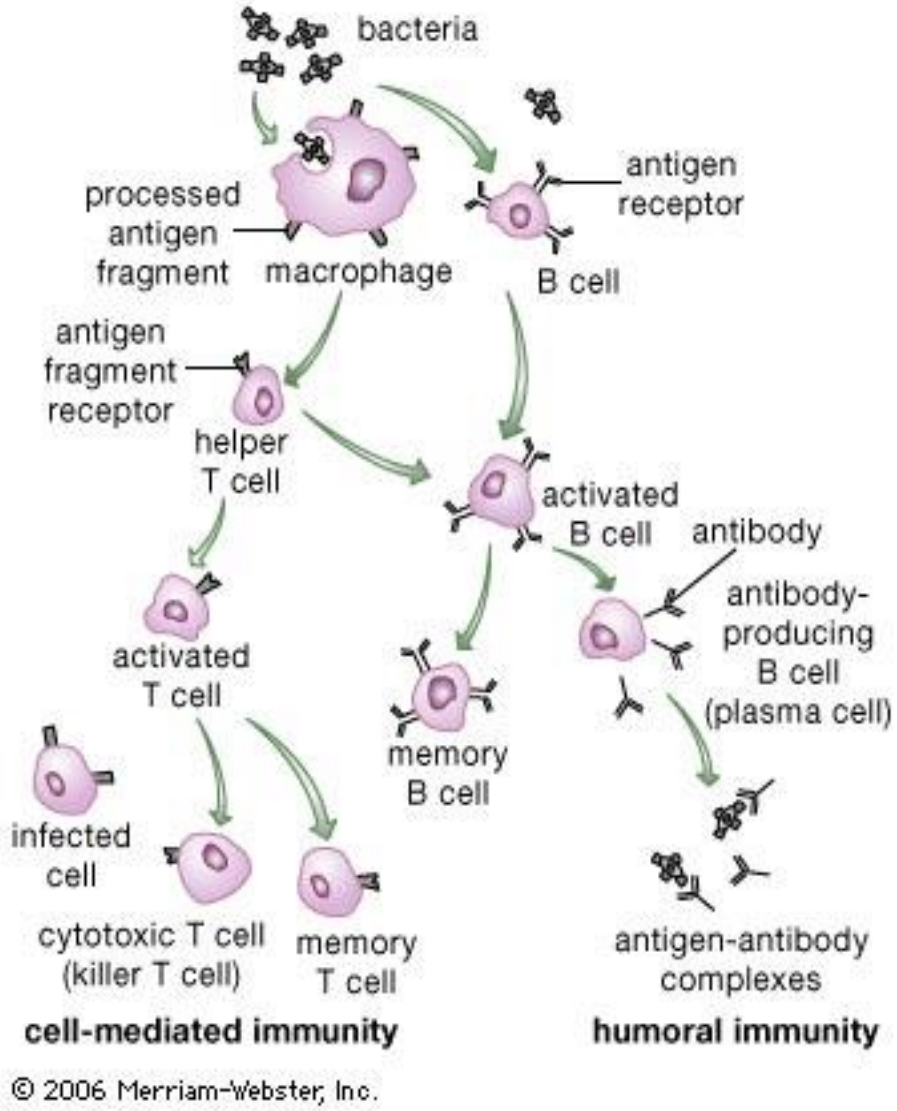


Figure 6, Arms of Immunity.

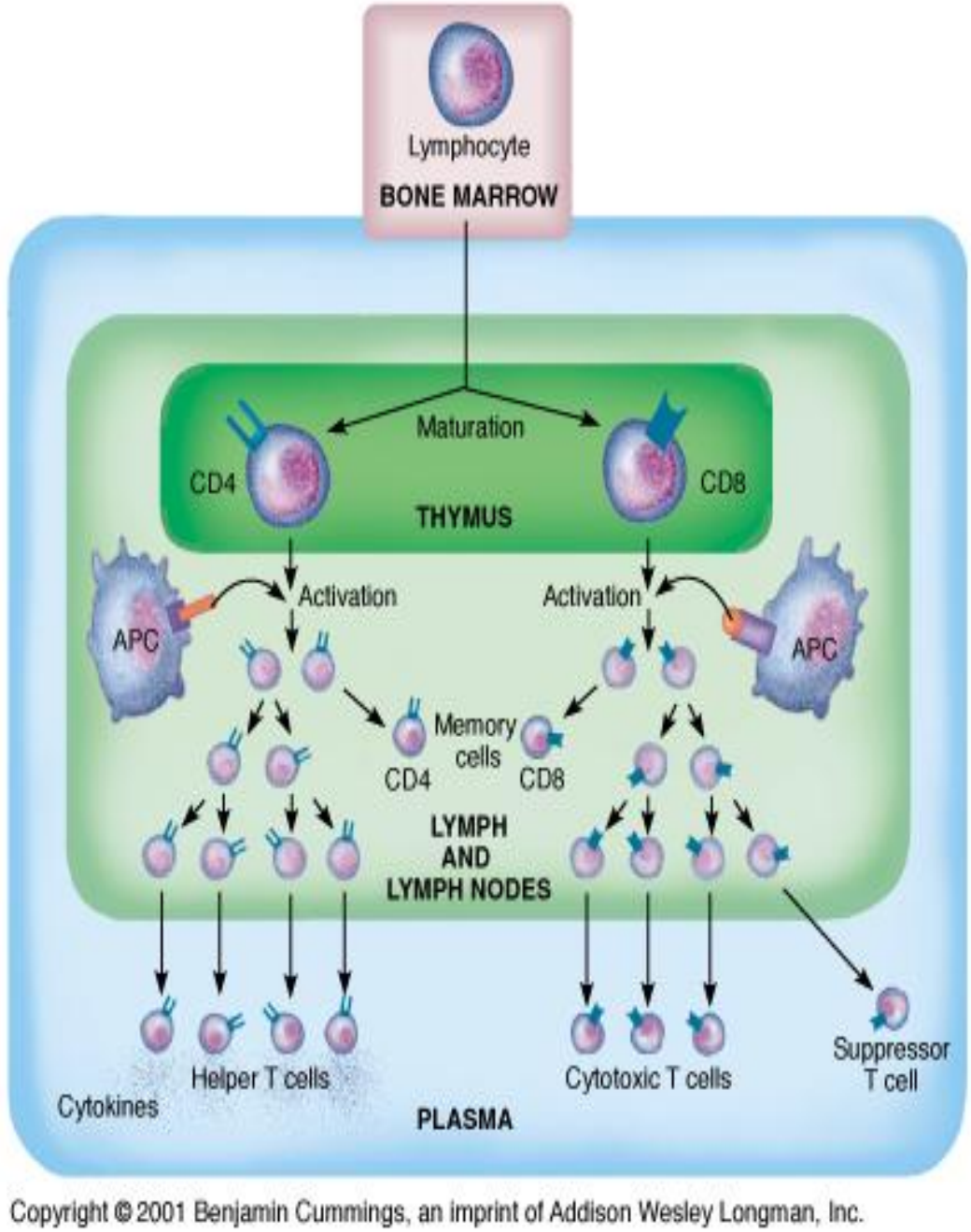


Figure 7 ,T Lymphocytes.

The tubercle bacilli engulfed by alveolar macrophages which attempt to kill the bacteria within the phagolysosome. Macrophages produce certain specific cytokines or mediators including interleukins IL-1, IL-6, IL-10, TNF-α and TGF β[11]. these cytokines have immunoregulatory effects and also mediate some of the clinical manifestations of tuberculosis for example: IL-1 contributes to fever, IL-6 may

mediate hyperglobulinemia and TNF- α essential for granuloma formation. IL-10 inhibits cytokine production and TGF- β suppresses T-cell proliferation. These two inhibitory cytokines prevent excessive inflammation and tissue damage.

Cluster of Differentiation (CD antigens)

Numerous glycoprotein's, known as CD antigens are present on the surface of lymphocytes and on other cells of immune system and can be used as a phenotypic surface markers to further sub categories immuno competent cells.

Cell Type	**CD Antigen**
All T Cells	CD2, CD3
T helper cells	CD4
Helper Induced T Cell	CD4+4B,4+
Suppressor induced T Cell	CD4+2H,4+
T Suppressor cell	CD4- CD8+
T Cytotoxic cell	CD8+ CD4-
Natural killer cell	CD16, CD56
All B Cell	CD19 Sig M

Table 1, CD markers.

The T cell activation in the reticulo endothelial system of lymphocytes is initiated by the interaction of TCR-CD3 complex with the antigen. T cell are activated by IL-1 secreted by the alveolar macrophages. Primary lymphocytes IL-2 and INF-γ initiate clonal expansion of T cells

Cytokines produced by macrophage and by Th1 and Th2 cells

Cytokine	Produced by		
	Macrophage	**Th1**	**Th2**
IL – 2	-	+	-
IFN – gamma	-	+	-
Lymph toxin	-	+	-
IL – 4	-	-	+
IL – 5	-	-	+
IL – 6	+	-	+
IL – 10	+	+	+
IL - 3	-	+	+
GM – CSF	+	+	+
TNF	+	+	+
IL – 1	+	-	-
TFG – beta	+	-	-
Elicits DTH	-	+	-
IgE Production & Eosinophilia	-	-	+

Table 2, Cytokines.

Th1 cells are considered important in mediating immunity against intracellular pathogens and Th2 play a role in atopic and parasite diseases[12].

CMI response Versus DTH

There are basically two types of responses that the body can mount against M. tuberculosis one is tissue damaging (DTH) and the other macrophage activation (CMI). DTH implies a harmful effect while CMI implies a beneficial effect, when bacillary antigens are progression of lesions by rapid activation of the immune

response. However when tubercle bacilli and their antigens are present in high concentrations, the CMI-DTH responses cause necrosis and destruction of tissues[13]. Vaccination cannot prevent establishment of an infection with tubercle bacillus, it can only prevent the disease.

The term CMI implies that a clinically expanded T lymphocyte population exists in the host which is specific for the antigens of tubercle bacillus. The specifically of the T-lymphocyte decides the CMI and not the macrophages. Vaccination with BCG may initially increase the microbicidal power of alveolar macrophages, destruction of bacilli by macrophages occurs in both vaccinated and non vaccinated individuals.

A few years after vaccination, the BCG organism has been eliminated and the initial response to intake tubercle bacilli would be the same in vaccinated and non vaccinated individuals. However once the bacilli begin to multiply and sufficient antigen is produced to be recognized by lymphocytes, vaccinated hosts show an accelerated response. The rapid local accumulation of activated lymphocytes and macrophages arrests the focus of infection and prevents clinical disease.

The essential to effective tuberculosis immunity are functioning macrophages, dendritic cells, strong Th-1 type cell mediated immunity and a relative absence of th-2 type T cell immunity[14]. When lymphocytes are exposed to the antigen they are sensitized and secrete a wide variety of soluble substances having a number of biological actions, including attraction and immobilization of macrophages.

The most important lymphokine is MIF (macrophage migration inhibitory factor). The interaction of MIF and sensitized lymphocytes (exposed to antigen)

provides in vitro proof of delayed hypersensitivity.T- lymphocytes are produced in bone marrow and processed in thymus. They proliferate into lymphoblast and secrete lymphokines. T cells are associated with cell mediated immunity.

Adenosine Deaminase

Adenosine Deaminase is a member in the metabolism of adenine nucleotides[15].

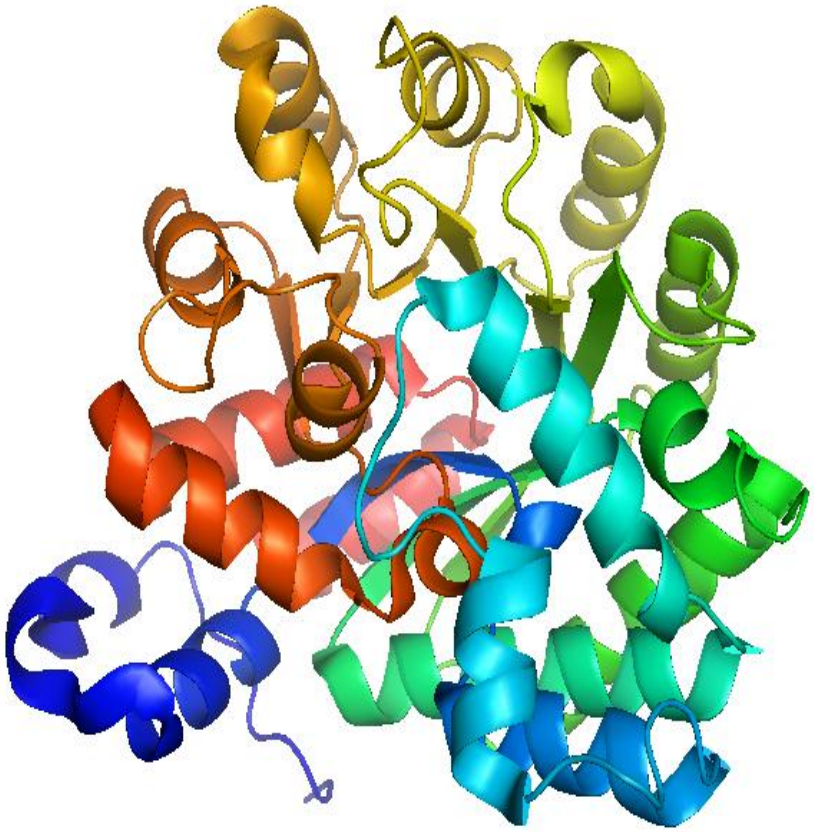


Figure 8 , Structure of ADA.

ADA is expressed in the cytosol of all cells but, in humans, lymphocytes have the highest activity of this enzyme. The deficiency of this enzyme causes a type of severe combined lmmuno deficiency (SCID), involving T-cell and B-cell depletion. Untreated ADA-deficient children die by 2years of age by over whelming infection.ADA is an enzyme predominantly of T-lymphocyte origin and has been shown to be essential for the differentiation of the lymphoid cells.

There are two iso forms of ADA; ADA1 & ADA2

ADA1 is found in most body cells, particularly lymphocytes and macrophages where it is present not only in cytosol and nucleus but also as the ecto form on the cell membrane attached to dipeptidylpeptidase-4

ADA2 was first identified in human thymus. It was subsequently found in other tissues including the macrophage where it co-exists with ADA1. the two isoforms regulate the ratio of adenosine to deoxyadenosine potentiating the killing of parasite.

Chopra RK, studied ADA and T- lymphocyte levels in patients with pleural effusions. In the group of patients with tuberculosis pleural effusions, mean ADA values both in serum as well as in pleural fluid were significantly higher compared to other groups with malignancy and being cellular transudates. The percentage of T-lymphocyte levels also correlated with ADA levels in pleural fluids of the three groups. ADA is a sensitive and specific parameter in diagnosing tuberculosis effusions[16].

There is a relation between ADA activity and cell mediated immune response, it is considered as a marker of cell mediated immunity. Kavitha K estimated cord blood ADA levels in 30 neonates of low birth weight(< 2.5 kg) and 30 neonates with normal (birth weight > 2.5 kg) .The results of the study showed that ADA levels in LBW neonates are significantly low compared to normal neonates. So study of ADA helps in early identification of such neonates for taking necessary precautions and prophylactic measures. It can be used as a marker of cell mediated immunity.

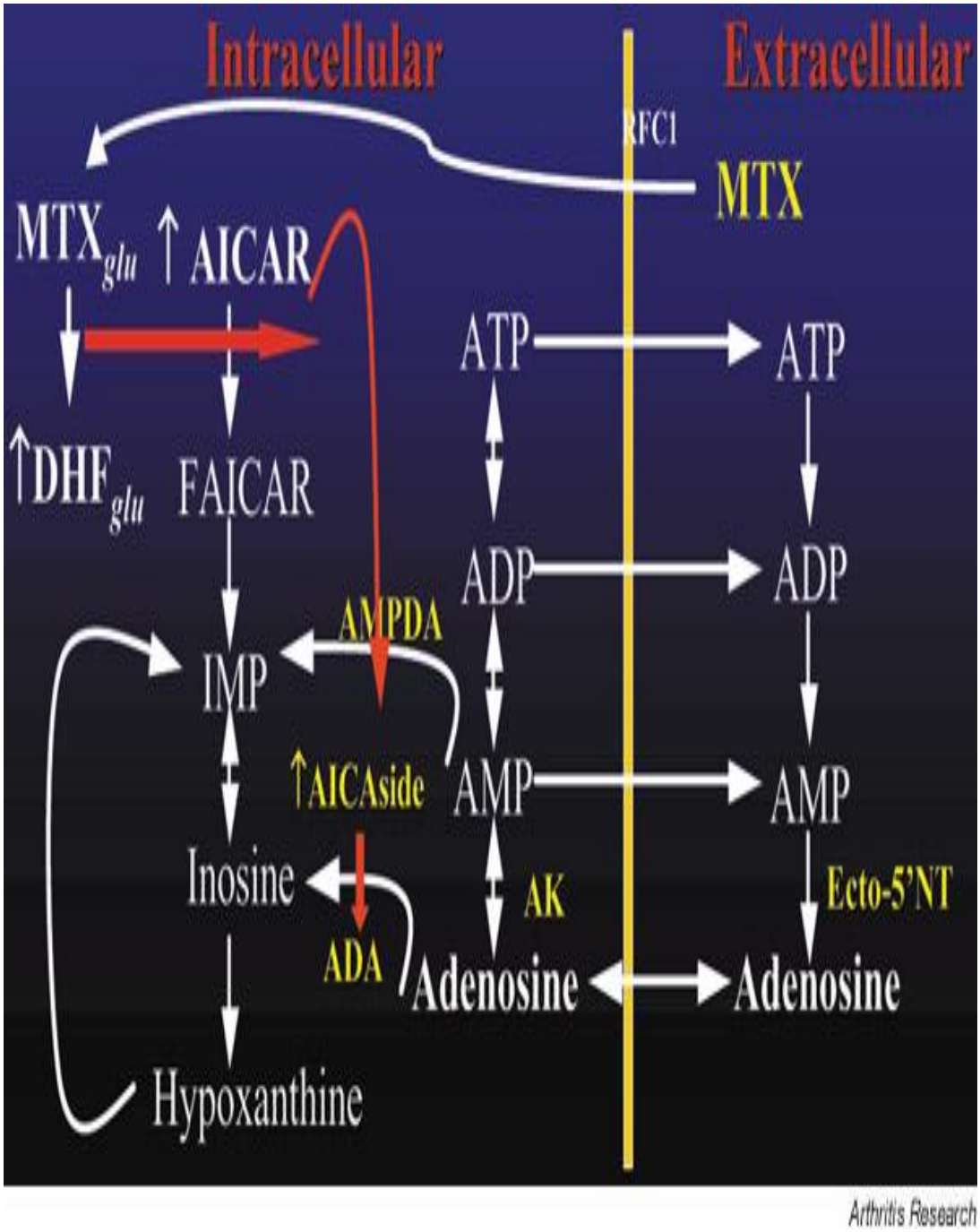


Figure 9 ,ADA Metabolism.

BCG VACCINE AND VACCINATION

Ever since Kochs discovering of the bacillus in 1882, numerous workers tried to attenuate the bacillus in the hope of producing a vaccine for the prevention of tuberculosis. In lille, Albert cal mette and camille guerin were also trying to attenuate a highly virulent strain of tubercle bacillus of bovine origin isolated by Nocard of Alfort from tuberculous mastitiss in a heifer.

The bacillus isolated was inoculated into a suitable culture medium (incorporating pieces of potato cooked at 10^0c in beef bile containing 5 percent glycerin). As the bacilli multiplied in this medium, they were repeatedly sub cultured every three weeks. In 1921, after a total of 231 transplants, the strain proved to be completely harmless even in highly susceptible guinea pigs, yet its antigenicity was un impaired. This strain was named Bacillus Calmette and Guerin (BCG).

The freeze-dried French strain (1949) from the Pasteur institute in Paris was strain 1173-P2, from which the Glaxo and Danish strains descended. BCG strains used today are not identical. Three parent strains (Glaxo, Tokyo and Copenhagen) account for more than 90 percent of the vaccine used in the world today[17]. In 1966, a WHO Expert committee on Biological standardization adopted a series of recommendations for the production of BCG vaccine. It states that vaccine should be freeze-dried and the vaccine strain should be maintained by the seed lot system whereby no vaccine is produced from a seed more than 12 passages removed from a primary freeze dried lot.

In India BCG vaccine laboratory was started in Chennai in 1948. Since 1966, Danish strain 1331 is being used for preparation of both liquid and freeze-dried vaccine. However now only freeze-dried vaccine is available in all countries. The attenuated calmette guerin strain of bovine M. Tuberculosis is present in a concentration of 0.1 to 0.4 million viable bacilli per dose of vaccine.

BCG is stored at sub-zero temperatures (-20^0c) the vaccine remains potent for 2 years. The undiluted vaccine can be stored in the middle compartment of the refrigerator (2^0-4^0c) without loss of potency up to 6 months. At the peripheral level at 2^0 to 8^0c it is good enough for use up to one week. Strict attention should be paid to maintenance of cold chain. As vaccine deteriorates on exposure to light it is usually supplied in dark colored ampoules and wrapped in black paper/ cloth[18].

Ampoules of freeze dried BCG vaccine are long and seated under vacuum. The vaccine is then reconstituted by dissolving it in normal saline as distilled water gets as a irritant. The diluents should be kept with vaccine in the cold chain system. Reconstituted BCG vaccine should be used within 3 hours. It can be prevented from contamination by proper hand washing and sterilization of the equipment and using separate needles for each child.

The standard dose of BCG vaccine is 0.1mg in 0.1 ml volume. The same dose is given at all ages (Term/ Preterm)[19]. The vaccine is ammonization. Most studies have shown good sensitization when BCG was given at birth, which shows an adequate cell mediated immune response (CMIR)[20].

Mussi – Pinhata et al[21] determined the effect of intrauterine growth retardation (IGUR) on the response of BCG vaccination. The infants were evaluated using post-vaccination skin tests to PPD and tuberculin lymphocyte transfer motion tests. Similar rate of response was found in term babies as well as IUGR. Thus the presence of IUGR should not be a reason for delaying BCG vaccination.

Conventionally BCG is given by intra dermal route in the left upper arm region. Cleaning with sterile water at the site of application is enough. BCG is administered as a single dose. When 0.1ml of vaccine is injected by intra dermal route, a wheal of 8mm diameter is raised, which is absorbed in 20-30 min. hair follicles are seen as small pits on the wheal produced. Nothing is visible at the site of injection for some days. By 3rd or 4th week indurations is felt at the vaccination site that becomes a lump of 6 to 10 mm by the 6th week. This lump would soften with pus formation and discharge, leaving a tiny ulcer heals by itself.

Figure 10 ,BCG vaccine and vaccination.

This cycle of ulceration and healing may repeat 2-3 times over a period of 2-3 months. Healing is usually complete by 10-12 weeks and the site is marked by a small pigmented scar of 5 to 7 mm in size.

Case control studies on the efficiency of BCG vaccinated of the newborn.

Country (Vaccine used)	**Age group observed months**	**No. of Cases**	**No. of Control**	**Efficiency (%)**
Brazil (Rio de Janeiro Strain)	0-12	45	90	82
Brazil (Rio de Janeiro Strain)	0-5	73	604	82-84
Burma (Japan BCG lab)	0-5	311	1536	38
Canada (Connaught lab)	0-18	71	213	60
England (Glaxo lab)	0-1	111	555	49
Chennai[22] (Japan BCG Lab)	0-5	107	321	7

Table 3,Case Control studies of efficacy of BCG vaccination.

Contact studies on efficacy of BCG vaccination against tuberculosis in children.

Country (Vaccine need)	**No. of contacts**		**No. of cases among contacts**		**Efficiency (%)**
	Vaccinated	**Un vaccinated**	**vaccinated**	**Un vaccinated**	
Thailand (Merieux)	1253	253	218	86	53
Togo (Glaxo lab)	875	546	62	113	62
Korea (Paris seed lot)	806	417	45	84	75

Table 4,Contact studies on efficacy of BCG vaccination against Tuberculosis.

It has been seen by Seth et al[23] by in vitro estimation of CMIR after BCG vaccination that almost 12-15 percent of neonates do not develop scar but have positive CMIR.BCG vaccine has also been tried for prevention of leprosy, also as an immune – modulating agent in disease like Nephrotic syndrome and bladder cancer.

TUBERCULIN SKIN TEST

In 1890, Robert Koch announced a cure for tuberculosis. This consisted of giving patients subcutaneous doses of a filtrate of heat killed culture of tubercle bacillus. This was known as Koch's lymph or Koch's remedy. There was quick heating at the site of second injection of viable organisms (Koch's phenomenon). Within a year of announcement of this therapy, it was disapproved as cure for tuberculosis. However, this discovery became the most widely used diagnostic test ever developed. Bleaker a polish researcher named Koch's remedy as 'tuberculin' in 1891 and thus the Tuberculin test was born.

In 1941 Seibert and Glenn separated a product with predictable activities and called it purified protection derivative (PPD). This is now injected intradermally for tuberculin test. The tuberculin skin test is based on the fact that infection with M. tuberculosis results in sensitivity to certain antigens of this organism that are also contained in the culture extracts called "Tuberculosis."

The potency of PPD is expressed as Tuberculin units (TU) instead of international units. The standard 5 TU dose of PPD-S is defined as the delayed skin test activity contained in a 0.1mg/0.1ml dose of PPD-S. one TU of PPD RT 23 has been defined as 0.02 mg in 0.1 ml of phosphate buffered saline with 0.005 percent Tween 80 (poly oxyethylene sorbiton monocleate).

The standard dose of commercial PPD preparation is defined as the dose of that product which is biologically equivalent to that contained in 5 TU of PPD-S (i.e., it elicits skin reactions of equivalent size ± 20%)[24].One TU of PPD RT 23 (Tween 80) corresponds fairly well to 5 TU of RT 19-20-21 and to 6 or 7 TU of RT 22. one TU of PPD RT 23 (with Tween 80) corresponds to 3 TU of PPD-S. therefore 2 TU of PPD RT-23 is used for diagnosis or surveys[25].

There are two techniques of applying the tuberculin test the intra cutaneous mantoux test and the percutaneous multiple puncture skin test. The mantoux test is performed by the intracutaneous injection of 0.1ml of PPD containing 5 TU PPD-S into the volar (ventral) surface of the forearm, in the axis of the forearm. This raises a wheal 6 to 10mm in diameter. The test should be read 48-72 hours later, as the delayed hypersensitivity reaction is maximal at 48-72 hours[26].

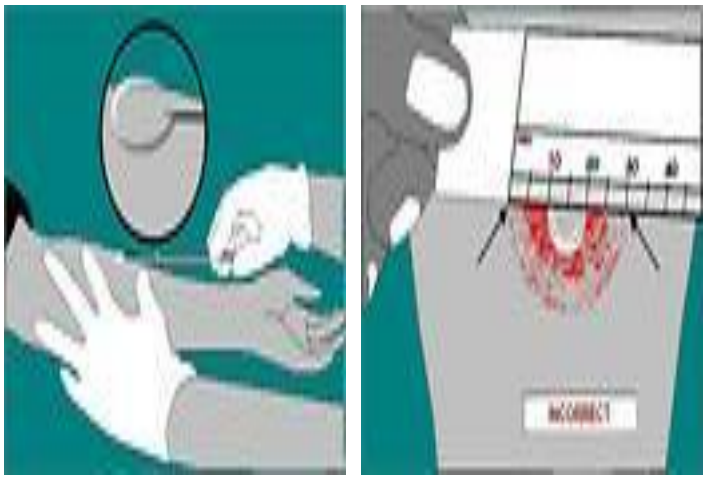


Figure 11 ,TST.

The tuberculin skin test is based on the fact that infection with M. tuberculosis results in sensitivity to certain antigens of the organism that are also contained in the culture extracts called tuberculosis. There are two main types of tuberculosis: Old

tuberculosis (0T) and purified protein derivative (PPD) old Tuberculin (0T) is a filtrate obtained from heat – sterilized concentrated broth culture of tubercle bacilli.

Florence seibert coined the term “purified protein derivative” (PPD) in 1934 for the product from heat- concentrated synthetic medium OT by precipitating the protein initially with trichloroacetic acid[27]. The PPD resulting from these experiments was called “SOTT” (Synthetic Medium Old Tuberculin trichloroacetic acid) precipitate. In 1941 seibert prepared a large batch of PPD in which ammonium surface was used for precipitation to obtain a highly purified preparation. This material lot 49608 was designated the standard tuberculin “PPD-S”, and became the International standard for all tuberculin’s.

Another commonly used tuberculin, PPD RT 23, is a large batch of purified tuberculin produced by Statens serum institute, Copenhagen and issued since July 1, 1958. Recently in view of short supply of PPD-S, a new standard has been manufactured and tested[28]. In India, currently there is shortage of PPD. The BCG laboratory, Guindy has stopped manufacturing PPD RT 23 as it has exhausted the stock of PPD RT 23. at present, span diagnostics limited is marketing a tuberculin that has been calibrated against PPD RT 23 in 3 strengths: 2U, 5U and 10 TU. Tuberculin preparations retain their potency for nearly 6 months if stored in cool (2 to 20^0c) dark place. The optimum temperature for storage is 2 to 8^0c. Opened vials of tuberculin should not be kept for more than 2 days. When diluted PPD is absorbed on glass and plastic surfaces. To minimize this, a small amount of detergent, Tween 80, is added to the diluents for PPD and solution is refrigerated in the dark.

Initial infection with tubercle bacilli is followed by the development of allergy to tubercle protein approximately after 4 to 6 weeks. This is in the form of a cell mediated, delayed hypersensitivity reaction (Type IV). The mantoux tuberculin reaction is a classic example of delayed hyper sensitivity (DTH). Infection with M tuberculosis sensitizes the person to antigenic components contained in tuberculin.

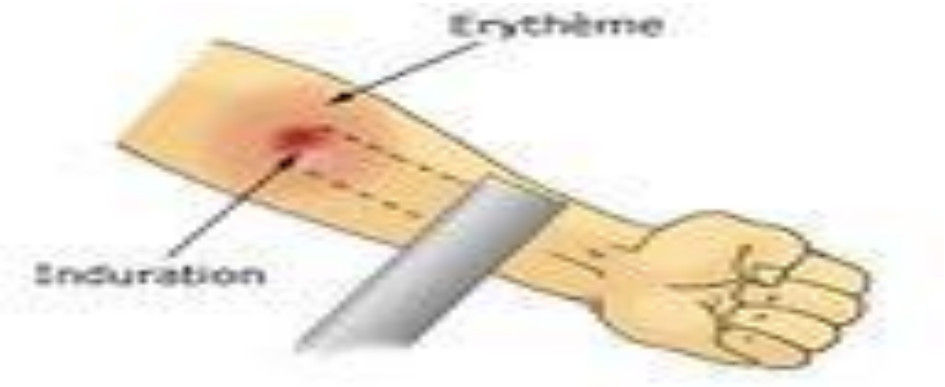


Figure 12, Measuring TST.

The Mantoux test is performed by the instantaneous projection of 0.1ml of PPD containing 5 TU PPD-S or an equivalent dose of other PPD into the volar (ventral) aspect of the forearm. The skin of the arm is lightly stretched length wise and pointer of the needle is inserted length wise, with bevel upward, into the superficial layer of the skin (intradermal,ID).

The Process of sensitization is by T-lymphocytes, reaches a level adequate to produce a detectable DTH response to 2 to 10 weeks after the initial infection with M tuberculosis. Their subsequent restimulation with the same or a similar antigen, such as an intracutaneous injection of tuberculin elicits a characteristicc delayed

hypersensitivity reaction with induration and erythema that peaks at 48 – 72 hours and subsides over a period of 5 to 7 days.

Historically, the earliest phase of the reaction is seen as perivascular cuffing with mononuclear cells followed by a more extensive exudation of mono and polymorph – nuclear cells. The sensitized lymphocytes recruit non sensitized lymphocytes to the site of inflammation, thus amplifying the immune response and giving rise to the characteristics type IV hypersensitivity response. In fact the usual clinical method of determining tuberculin sensitivity relies more on the secondary effect of oedema rather than cell infiltration[29].

The syringe is held by the barrel only and the plunger is not touched until the point of the needle has been satisfactorily inserted. After injection 0.1ml of the PPD, the finger is removed from the end of the plunger before the needle is withdrawn. The test should be read 48 to 72 hours. An immediate hypersensitivity response is of no significance[30]. The reading should be made in good light, the fore arm slightly flexed at the Elbow. It is based on the presence or absence of indurations which can be determined by either palpation or the pen method[31]. The diameter of indurations should be measured transversely to the long axis of the forearm and recorded in millimeters.

Size of indurations with 5 TU PPD-S/ 1TU PPD with RT Tween 80

< 10mm – Negative

10mm – Borderline

≥10mm – Positive

The effect of BCG vaccination on the subsequent mantoux test is highly variable. In BCG vaccinated children, the reaction to tuberculin ranges from 3 to 10 mm.

tuberculin skin reactivity due to BCG vaccination wanes with time and is unlikely to persist beyond 10 years after vaccination. Seth et al has demonstrated that, there is 50 to 60 percent waning in the first year itself[32].

Al- Kassimi et al have described the significance of positive mantoux reactions in BCG vaccinated children. They demonstrated that tuberculin sensitivity rises more steeply with age in the BCG vaccinated than the unvaccinated children[33].

MATERIALS AND METHODS

SOURCE OF DATA

This study is carried out in the Department of Pediatrics, BLDEA'S Shri .B.M. Patil Medical College , Bijapur from November 1 2007 to July 2009.

METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA

- Term healthy neonates taken up from post natal wards of the hospital.
- Healthy Infants of 6 to 8 weeks of age who were not vaccinated earlier were taken as matched controls.

EXCLUSION CRITERIA

- Neonates with Intrauterine infection, Septicemia, Birth Asphyxia, Sick due to other conditions- congenital anomalies, Neural tube defects, systemic illness.
- ADA Deficiency, (history & clinical examination, lab values less than 1 IU/L).
- Neonates born to mothers with infections in third trimester.
- Mothers who are HIV Positive / HBsAg Positive/ with Tuberculosis.

PROCEDURE

After taking written informed consent, address and fulfilling inclusion criteria, Term healthy neonates are included in the study. As per proforma, detailed clinical history comprising of age, sex, birth order, perinatal infections, perinatal complications if any are recorded. 2 ml of cord/venous blood is collected before BCG

vaccination and within 24 hours of birth and sample is sent for ADA estimation.0.1 ml of BCG vaccine is given intradermally on the left upper arm at the site of insertion of deltoid by the same person. Parents are asked to get the same babies to the immunization clinic after 6 weeks. 2 ml of venous blood is taken from the same babies for ADA estimation.

Tuberculin skin test is done by injecting 0.1 ml of PPD (5 TU) , on the outer flexor aspect of the fore arm at the time of collection of the blood sample and results read between 48 to 72 hours. A measurement of 10 mm is taken as positive tuberculin reactivity.

2 ml of plain blood is collected from Healthy infants of 6 to 8 weeks of age who were not vaccinated earlier, attending the immunization outpatient department (matched controls).

LABORATORY EVALUATION

2 ml of venous/cord blood is collected in a plain vial for ADA estimation. ADA Estimation is done by MICROXPRESS ADA-MTB enzyme kit and auto analyzer using manufacturer's protocol.

PRINCIPLE

Adenosine Deaminase hydrolyses adenosine to ammonia and inosine.The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form blue indophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue colored indophenol complex is directly proportional to amount of

ADA present in the sample[34].The sensitivity and specificity of the test is more than 90 %.

STATISTICAL ANALYSIS

6 weeks after BCG vaccination, the mean serum ADA levels are 13.8361 +/- 3.8750(σ) with an allowable error of +/- 1.5 a sample size of Total 60 with 30 study and 30 controls is calculated.

$$n = 4\sigma^2 / l^2$$

n = sample size (total 60, study group 30, control group 30)

σ = standard deviation

l = Allowable error

- Data collected is analyzed by statistical tests student t – test, unpaired t – test, correlation analysis.
- Diagrammatic representation.

Figure 13, ADA KIT.

Figure 14, ADA Constituents.

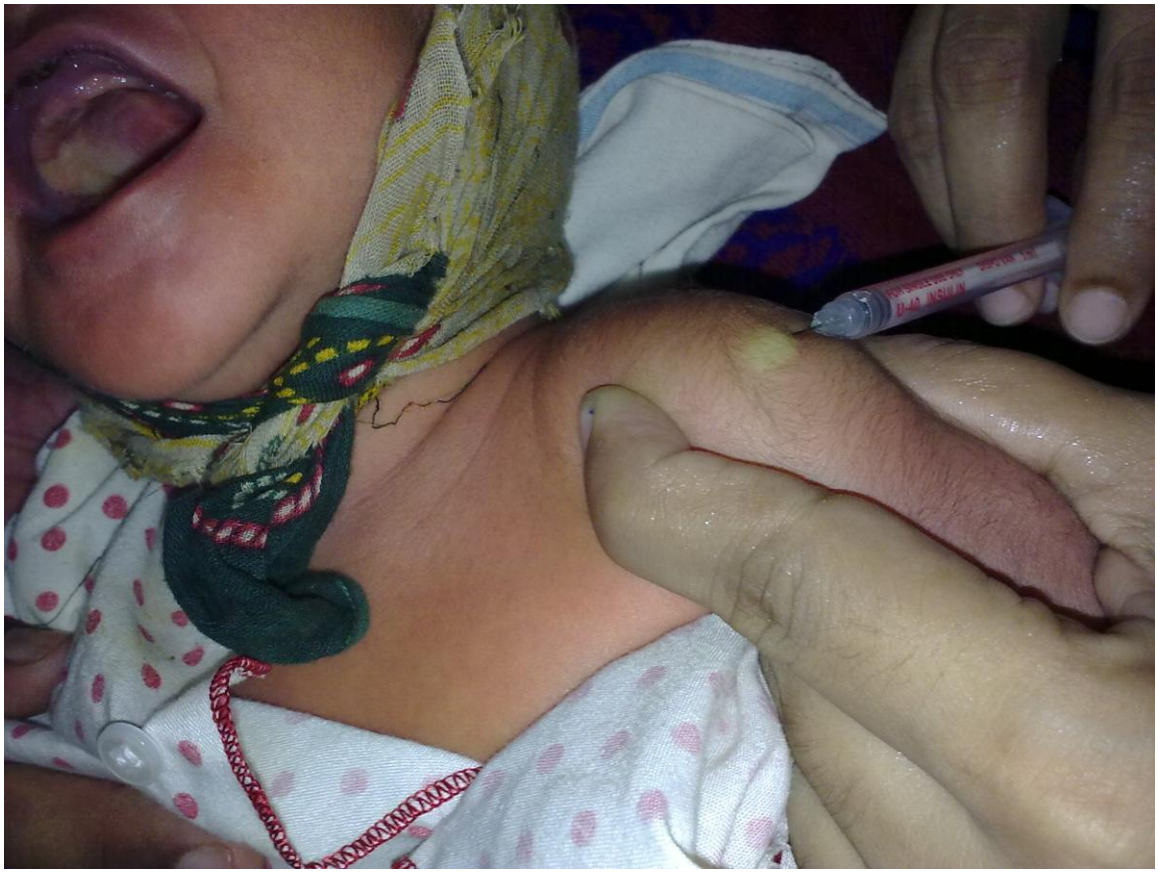


Figure 15,BCG vaccination .

Figure 16, Study hospital.

RESULTS

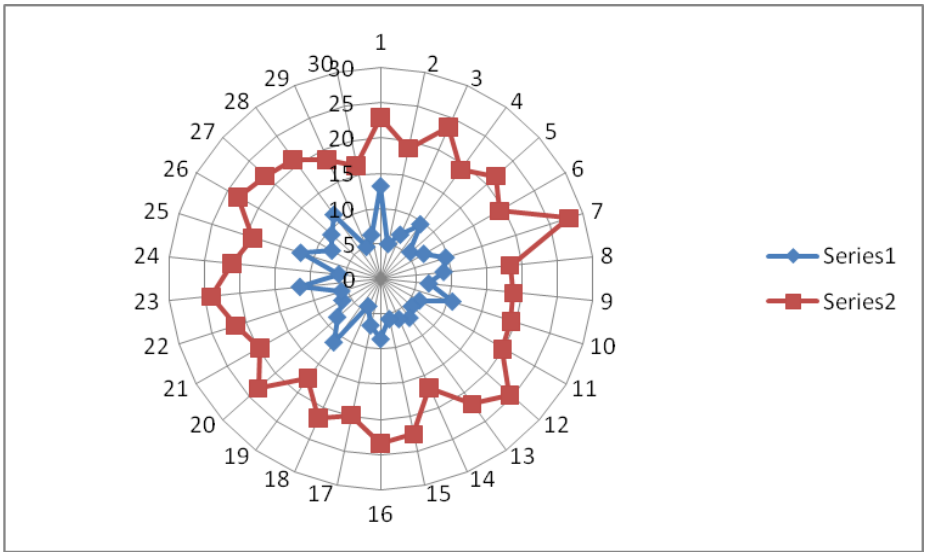
STUDY GROUP (CASES)

The statistical analysis performed concluded that in the study group the mean value of serum ADA at birth(before BCG vaccination) is 7.8 with a standard deviation of 2.351375 and at 6 weeks after vaccination was 20.94333333(mean) and 2.56699(standard deviation). The calculated p value is 1.911015 E – 28 i.e. < 0.05 which is statistically significant.

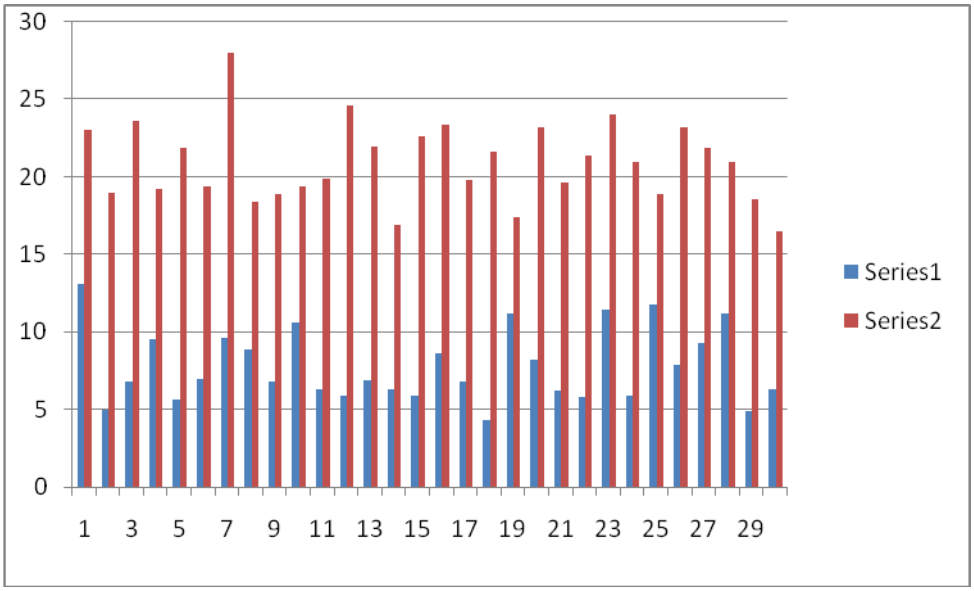
t-Test: Two-Sample Assuming Unequal Variances				
	before	after	std dev	
Mean	7.8	20.94333333	before	after
Variance	5.528965517	6.589436782	2.351375	2.56699
Observations	30	30		
Hypothesized Mean Difference	0			
df	58			
t Stat	-20.67966346			
P(T<=t) one-tail	9.55076E-29			
t Critical one-tail	1.671552763			
P(T<=t) two-tail	1.91015E-28	<.05	Significant	
t Critical two-tail	2.001717468			

Table 5, t – Test(a).

Graphical representation(Study Group)



Graph 1, Mean ADA levels, series 1 – before vaccination, series 2 – after vaccination, study group.



Graph 2, Mean ADA levels, series 1 – before vaccination, series 2 – after vaccination, study group.

STUDY(CASES) GROUP VS CONTROL GROUP

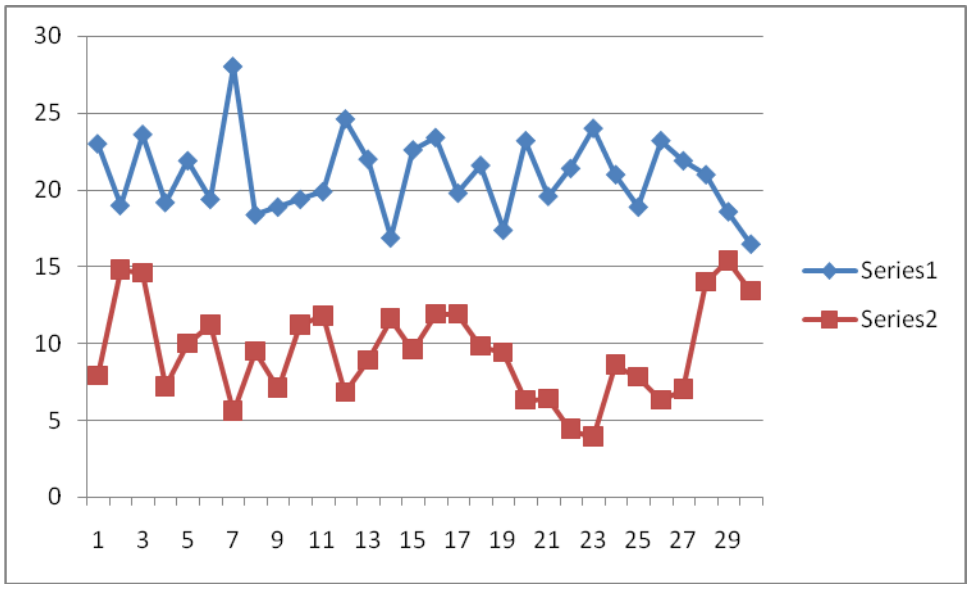
The calculated statistics for the study group (at 6 wks after vaccination) and control group are as follows,

t-Test: Two-Sample Assuming Unequal Variances				
	After vacc.	*control*	std dev	
Mean	20.94333333	9.476666667	After vacc	control
Variance	6.589436782	9.848057471	2.56699	3.138161
Observations	30	30		
Hypothesized Mean Difference	0			
df	56			
t Stat	15.49101977			
P(T<=t) one-tail	3.33389E-22			
t Critical one-tail	1.672522304			
P(T<=t) two-tail	6.66778E-22	<.05	Significant	
t Critical two-tail	2.003240704			

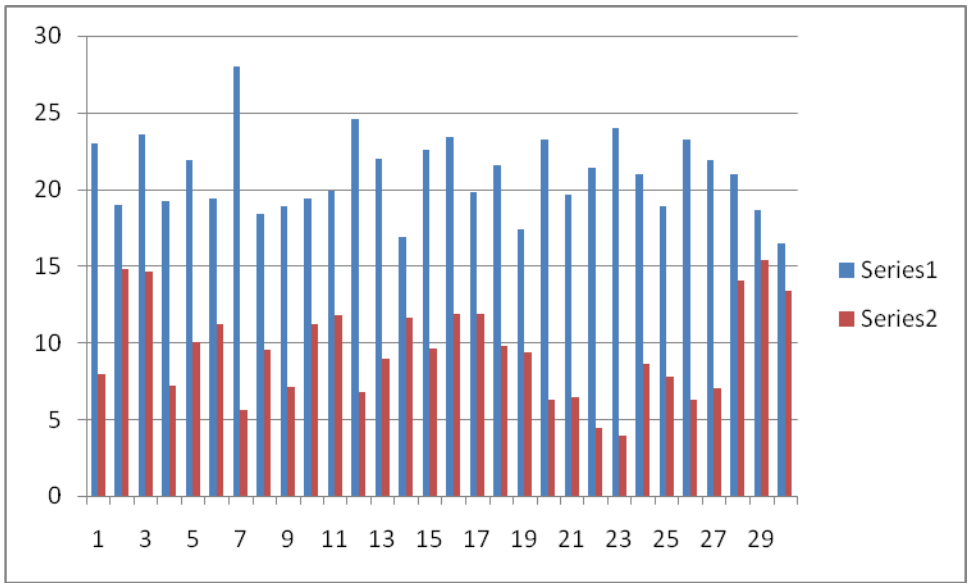
Table 6,t-Test(b).

Inference- statistically significant.

Graphical representation



Graph 3, series 1- study group (at 6 wks after vaccination),series 2 – control group.

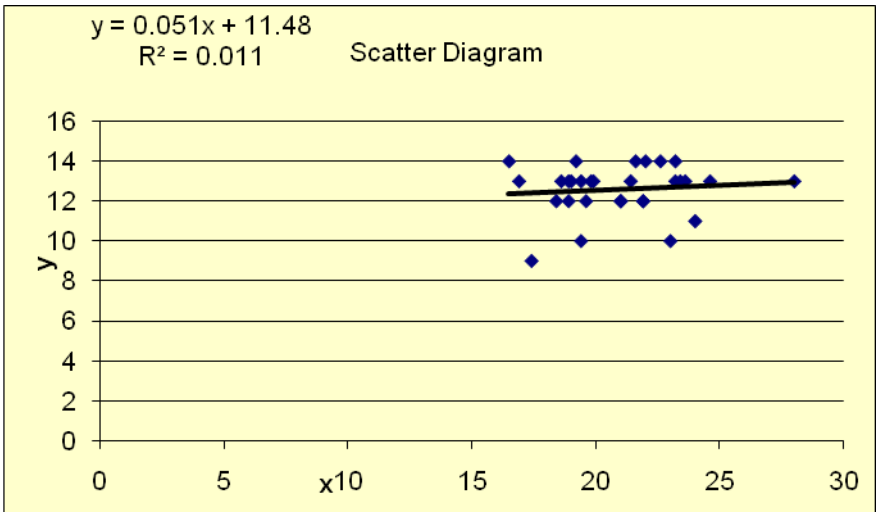


Graph 4 , series 1- study group (at 6 wks after vaccination),series 2 – control group.

TST & ADA OF STUDY GROUP(AT 6 WKS AFTER VACCINATION)

Correlation Coefficient	r	0.105933209
Coefficient of Determination	R2	0.011221845
Regression Coefficients For Line y = a + b x		
(y is dependent Variable and x is independent Variable	a b	11.48567282 0.051615177
Estimated value of y for given value of x	xi	45 13.80835576

Table 7 ,TST&ADA Correlation.

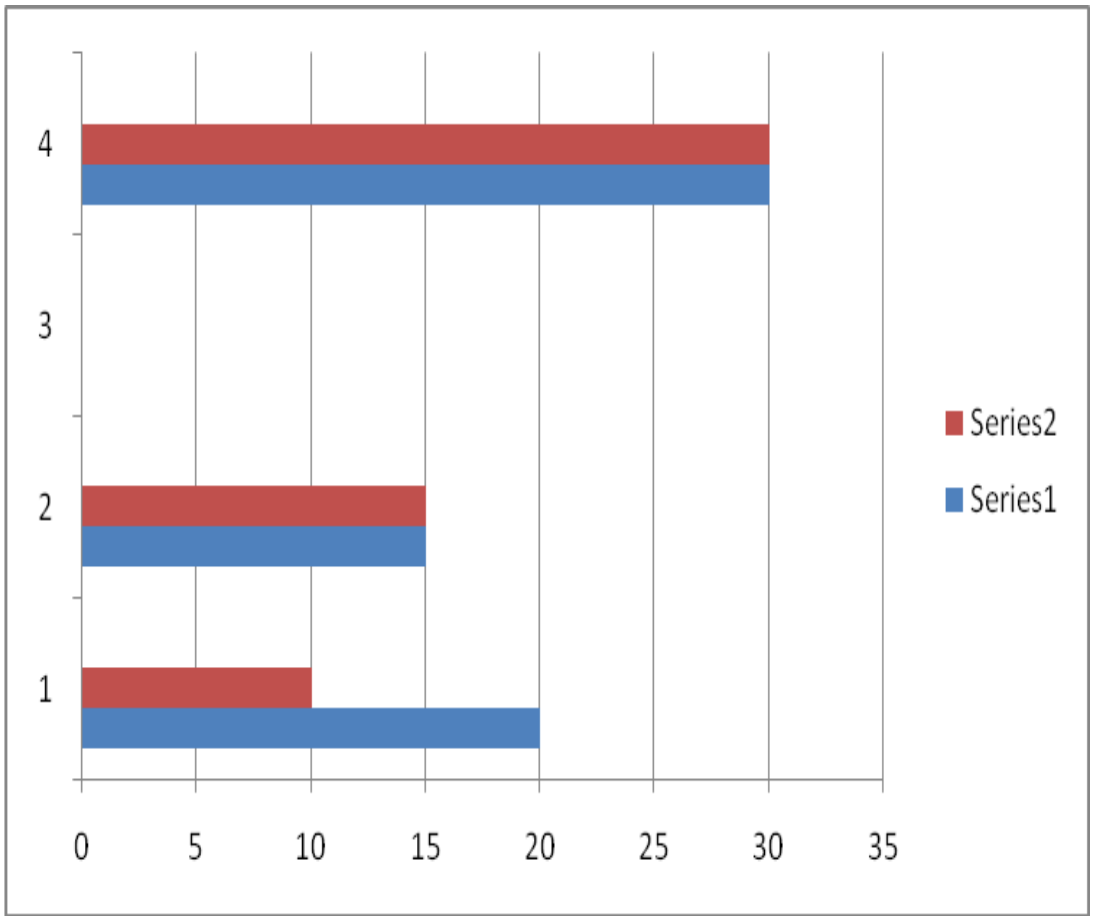


Graph 5 , correlation of ADA (X axis), TST (Y axis).

Positive correlation has been established between TST & ADA levels.

	MALES	FEMALES	TOTAL
CASES	20	10	30
CONTROLS	15	15	30

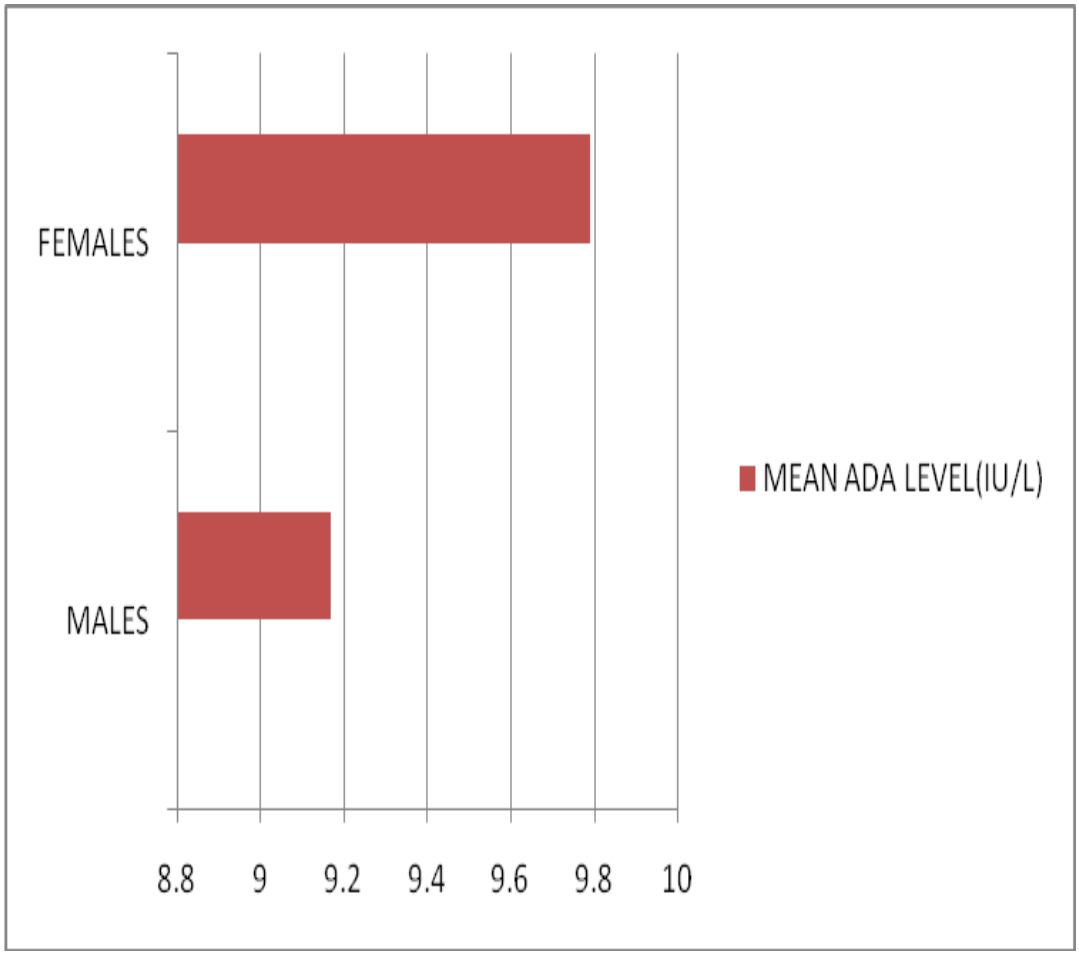
Table 8, sex wise distribution cases & controls.



Graph 6, sex wise distribution of cases and controls, series 1 – males, series 2- females.

CONTROLS	MALES	FEMALES
MEAN ADA LEVEL(IU/L)	9.17	9.79

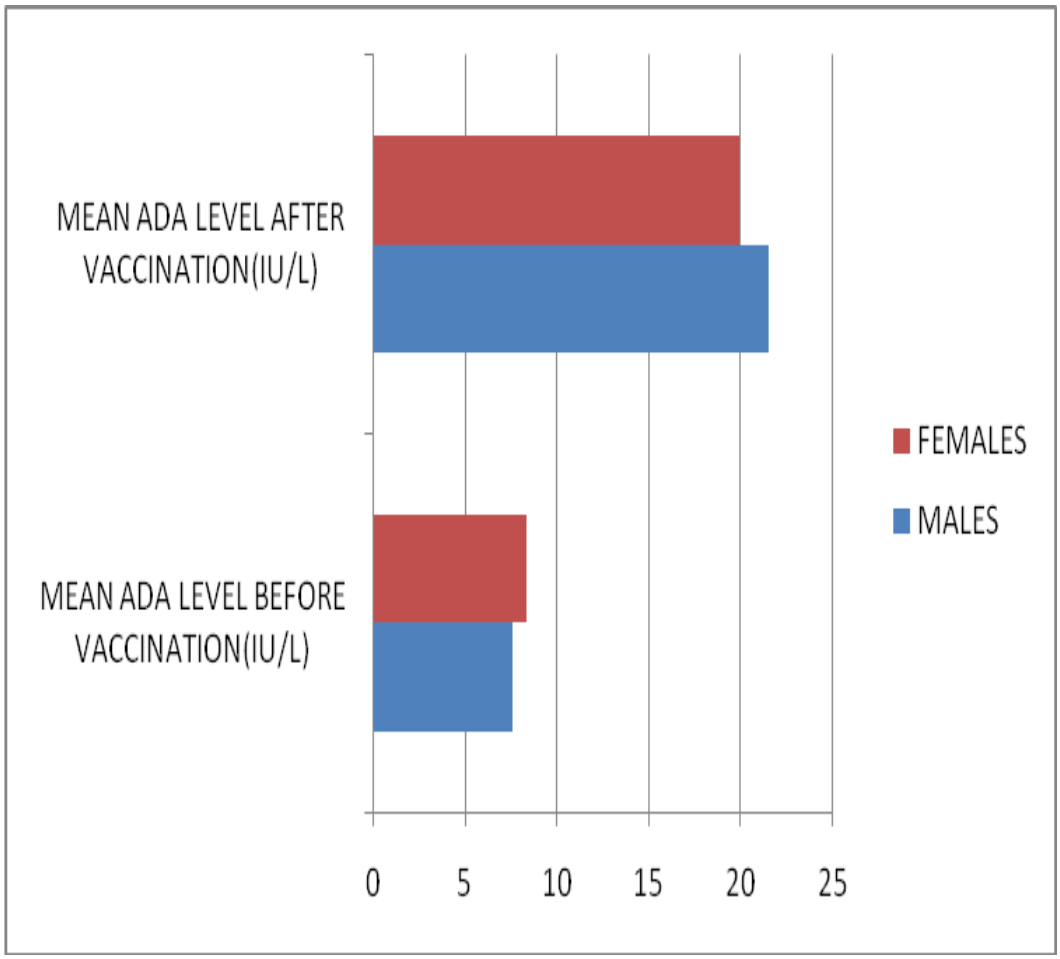
Table 9, sex wise distribution controls.



Graph 7 , Mean ADA Levels of controls.

CASES	MEAN ADA LEVEL BEFORE VACCINATION(IU/L)	MEAN ADA LEVEL AFTER VACCINATION(IU/L)
MALES	7.55	21.48
FEMALES	8.29	19.87

Table 10, Mean ADA levels before and after vaccination, cases.



Graph 8, ADA Levels before & after vaccination, cases, sex wise distribution..

DISCUSSION

Serum ADA estimation has been done in diseases which evoke cell mediated immune response like Tuberculosis, enteric fever especially in adults. The present study shows that there is significant rise in serum ADA levels after BCG vaccination and this can be used to judge cell mediated immune response that occurs after BCG vaccination.

The protection against childhood tuberculosis by BCG vaccination is attained 4 to 6 weeks after vaccination and is mainly due to cell mediated immune response. Adenosine deaminase is an enzyme of the purine salvage pathway secreted by activated T- Lymphocytes and macrophages and is raised when cellular immunity is stimulated. Thora S, estimated serum ADA weeks after BCG vaccination in a one year two month study period[35].

ADA(IU/L)	PRESENT STUDY	THORA S
CASES		
BIRTH	7.8	6.69
6 WEEKS	20.94	13.83
CONTROLS	9.47	6.45

Table 11, ADA Study

The present study concludes that the raise in ADA levels at birth and 6 weeks after BCG vaccination is statistically significant. Also the ADA levels of the study group at 6 weeks compared to matched controls who were not vaccinated earlier is significant.

Aggarwal A studied BCG induced tuberculin reactivity in newborns.0.05 ml BCG was given to neonates at birth (group 1) , 0.1 ml BCG was given at birth (group 2), 0.1 ml BCG was given to infants of 4 to 6 weeks of age group (group 3). Tuberculin skin test was done at 10 to 12 weeks which showed positive tuberculin reactivity (induration greater than equal to 5 mm) of 50.98%, 74.07%, and 76% in groups 1, 2, 3 respectively[36].

80 to 90 % protection is provided by neonatal BCG vaccination against tuberculous meningitis. Thayyil-Sudhan S, conducted a study on safety and effectiveness of BCG vaccination in preterm babies .Tuberculin skin tests were done 6 to 8 weeks after vaccination. 80% of the babies showed a positive conversion rate (induration more than 5 mm)[37].

Esqueda N, compared the delayed response to BCG immunization in preterm infants (31.4 to 31.6 weeks, group 1) with full term infants (group 2) with BCG given at birth. Tuberculin skin test was done 16 weeks later which showed positive reactivity of 81 % and 86 % in groups 1 & 2 respectively[38].

TST	**POSTIVE TUBERCULIN REACTIVITY(CONVERSION RATES)%**
PRESENT STUDY	96.67
AGGARWAL A	76
THAYYIL-SUDHAN S	80
ESQUEDA N	86

Table 12, TST Study

Kavitha k, estimated cord blood serum ADA levels in 30 neonates with low birth weight and 30 neonates of normal birth weight. The results showed that the CMI was low in the form of low ADA activity in LBW infants compared to normal neonates indicating ADA can be used as an immunoenzyme marker for measuring CMI[39].

The present study is the first of its kind to measure CMI after BCG vaccination in the form of increased ADA activity and to compare the same with TST.

CONCLUSION

The present study concludes that the raise in ADA levels at 6 weeks after BCG vaccination is statistically significant. Also the ADA levels of the study group at 6 weeks compared to matched controls who were not vaccinated earlier is significant.

The present study is the first of its kind to measure CMI after BCG vaccination in the form of increased ADA activity and to compare the same with TST.

The present study also concludes that the rise in ADA activity is induced by BCG vaccination thus ADA is a marker of cell mediated immunity and the practice of BCG vaccination at birth is evidence based medicine.

Tuberculin skin test is the most common measure performed to evaluate the efficacy of BCG vaccination. The present study showed a conversion rate of 96.67% and a positive correlation with the ADA levels. Other studies have taken an induration as 5 mm as positive.

However the infants with positive tuberculin test had no symptoms of Tuberculosis or contact history this might explain that cell mediated immunity and there by positive tuberculin test is induced by BCG vaccination is most likely.

The Study reinforces that Tuberculin skin test showing an induration of 10 mm after BCG vaccination indicates good CMI as confirmed by increased ADA levels.

Also study of ADA helps in early identification of infants with depressed cell mediated immunity and there by necessary precautions and prophylactic measures can be initiated. It can be used as a marker of cell mediated immunity.

SUMMARY

This study is carried out in the Department of Pediatrics, BLDEA'S Shri .B.M. Patil Medical College, Bijapur from November 1 2007 to July 2009.

Term healthy neonates taken up from post natal wards of the hospital.(cases/study group).Healthy Infants of 6 to 8 weeks of age who were not vaccinated earlier were taken as matched controls.

After taking written informed consent, address and fulfilling inclusion criteria, Term healthy neonates are included in the study. As per proforma, detailed clinical history comprising of age, sex, birth order, perinatal infections, perinatal complications if any are recorded. 2 ml of cord/venous blood is collected before BCG vaccination and within 24 hours of birth and sample is sent for ADA estimation.0.1 ml of BCG vaccine is given intradermally on the left upper arm at the site of insertion of deltoid by the same person. Parents are asked to get the same babies to the immunization clinic after 6 weeks. 2 ml of venous blood is taken from the same babies for ADA estimation.

2 ml of venous/cord blood is collected in a plain vial for ADA estimation. ADA Estimation is done by MICROXPRESS ADA-MTB enzyme kit and auto analyzer using manufacturer's protocol.

Tuberculin skin test is done by injecting 0.1 ml of PPD (5 TU), on the outer flexor aspect of the fore arm at the time of collection of the blood sample and results read between 48 to 72 hours. A measurement of 10 mm will be taken as will be taken as positive tuberculin reactivity.

The statistical analysis performed concluded that in the study group the mean value of serum ADA at birth(before BCG vaccination) is 7.8 with a standard deviation of 2.351375 and at 6 weeks after vaccination was 20.94333333(mean) and 2.56699(standard deviation). The calculated p value is 1.911015 E – 28 i.e. < 0.05 which is statistically significant.

The present study concludes that the raise in ADA levels 6 weeks after BCG vaccination is statistically significant. Also the ADA levels of the study group at 6 weeks compared to matched controls who were not vaccinated earlier is significant.

Tuberculin skin test is the most common measure performed to evaluate the efficacy of BCG vaccination. The present study showed a conversion rate of 96.67% and a positive correlation with the ADA levels.

The present study is the first of its kind to measure CMI after BCG vaccination in the form of increased ADA activity and to compare the same with TST.Also study of ADA helps in early identification of infants with depressed cell mediated immunity and there by necessary precautions and prophylactic measures can be initiated. It can be used as a marker of cell mediated immunity.

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ANNEXURE I

PROFORMA FOR DATA COLLECTION

SINO.

CASE/CONTROL:

INFANT OF/NAME:

AGE:

SEX:

HOSPITAL NO.

FATHERS NAME:

ADDRESS:

DATE OF BIRTH:

TIME OF BIRRTH:

MATERNAL HISTORY:

GRAVIDA PARA LIVING ABORTION

BLOOD GROUP & RH TYPE:

TYPE OF DELIVERY:

NORMAL/C – SECTION

INDICATION FOR C –SECTION

RISK FACTORS

BIRTH ASPHYXIA	YES/NO
INTRAUTERINE INFECTION	YES/NO
SEPTICEMIA	YES/NO

CONGENITAL ANOMALIES YES/NO

HIV POSITIVE MOTHER YES/NO

HBsAg POSITIVE MOTHER YES/NO

TUBERCULOSIS INFECTION OF MOTHER YES/NO

ADA DEFICIENCY (HISTORY & CLINICAL EXAMINATION) YES/NO

PHYSICAL EXAMINATION

SINGLE/TWIN:

LENGTH:

HEAD CIRCUMFERENCE:

CHEST CIRCUMFERENCE:

BIRTH WEIGHT/WEIGHT:

VITAL SIGNS:

TEMPERATURE HEART RATE RESIRATORY RATE

SKIN NORMAL/ABNORMAL

CRANIOFACIAL NORMAL/ABNORMAL

CHEST NORMAL/ABNORMAL

CVS NORMAL/ABNORMAL

LUNGS NORMAL/ABNORMAL

ABDOMEN NORMAL/ABNORMAL

GENITALIA NORMAL/ABNORMAL

EXTREMITIES NORMAL/ABNORMAL

BACK NORMAL/ABNORMAL

CNS NORMAL/ABNORMAL

NEWBORN MATURITY RATING AND CLASSIFICATION

ESTIMATION OF GESTATIONAL AGE BY MATURITY RATING

Symbols : X - 1st Exam O- 2nd Exam

NEUROMUSCULAR MATURITY

	0	1	2	3	4	5
Posture						
Square Window (Wrist)	 90°	 60°	 45°	 30°	 0°	
Arm Recoil	 180°		 100°-180°	 90°-100°	 <90°	
Popliteal Angle	 180°	 160°	 130°	 110°	 90°	 <90°
Scarf Sign						
Heal to Ear						

Gestation by Dates _____wks

Birth Date _____Hour _____ am pm

APGAR _____1 min _____ 5 min

SCORING SECTION

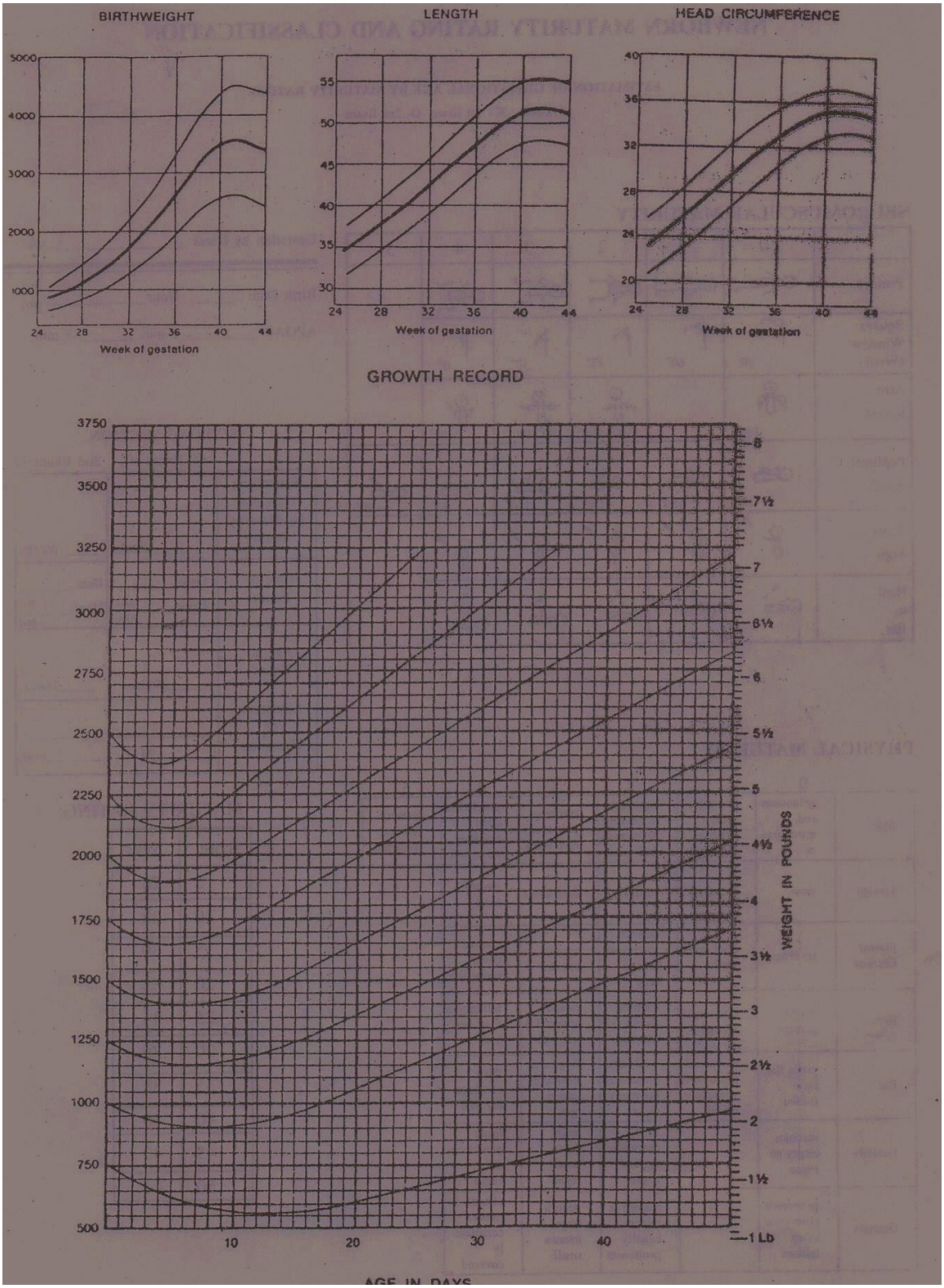
	1st Exam =X	2nd Exam=O
Estimating Gest Age by Maturity Rating	_____Weeks	____ Weeks
Time of Exam	Date_____ Hour____ am pm	Date_____ Hour____ am pm
Age at Exam	_____Hours	_____Hours
Signature of Examiner	_____ _____M.D.	_____ _____M.D.

PHYSICAL MATURITY

	0	1	2	3	4	5
Skin	gelatinous red, transpare-nt	Smooth pink, visiblle veins	superfici-al peeling & /or rash few veins	cracking pale area rare veins	parchment deep cracking no vessels	leathery cracked wrinkled
Lanugo	none	abundant	thinning	bald areas	mostly bald	
plantar Creases	no crease	faint red marks	anterior transverse crease only	creases ant. 2/3	creases cover entire sole	
Breast	barely percept.	flat areola no bud	stippled areola 1-2 mm bud	raised areola 3-4 mm bud	full areola 5-10 mm bud	
Ear	pinna flat, stays folded	sl. curved pinna; soft with slow recoil	well-curv. pinna; soft but ready recoil	formed & firm with instant recoil	thick cartilage ear stiff	
Genitals	scrotum empty no rugae		testes descendi-ng, few rugae	testes down, good rugae	testes pendulous deep rugae	
Genitals	prominent clitoris & labia minora		majora & minora equally prominent	majora large, minora small	clitoris & minora complete-ly covered	

MATURITY RATING

Score	Wks
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44


BIRTHWEIGHT
LENGTH
HEAD CIRCUMFERENCE
Week of gestation
Week of gestation
Week of gestation
GROWTH RECORD
WEIGHT IN POUNDS
AGE IN DAYS

IMPRESSION

TERM HEALTHY NEONATE YES/NO

DATE & TIME OF BLOOD SAMPLE COLLECTION

ADA LEVEL AT BIRTH

DATE OF BCG VACCINATION

REVIEW DATE

ADA LEVEL

TUBERCULIN SKIN TEST DATE

REVIEW DATE

TUBERCULIN RECTIVITY MEASUREMENT

DATE AND TIME OF SAMPLE COLLECTION OF CONTROL

ADA LEVEL OF THE CONTROL

ANNEXURE II

RESEARCH INFORMED CONSENT FORM

Title of Project : **"SERUM ADENOSINE DEAMINASE ESTIMATION AFTER BCG VACCINATION AS A MARKER OF CELL MEDIATED IMMUNITY AND ITS CORRELATION WITH TUBERCULIN SKIN TEST."**

Guide **: DrR.H.GOBBUR.MD .DCH .PROF.**

P.G. Student **: Dr. NAREN SANDEEP.D.**

PURPOSE OF RESEARCH:

I have been informed that the present study will help in thorough investigation of cell mediated immunity after BCG vaccination and its role in prevention of childhood Tuberculosis.

PROCEDURE:

I understand that after having obtained a detailed clinical history, thorough clinical examination and relevant investigations a final workup for clinical and laboratory significance of the study is planned.

RISKS & DISCOMFORTS:

I understand that my child may experience some pain and discomforts during the examination or the procedure. The procedures of this study are not expected to exaggerate these feelings, which are associated with the usual course of procedure.

BENEFITS:

I understand that my participation in this study will have no direct benefit to me or my child other than the potential benefit from evaluation of cell mediated immunity evoked by BCG vaccination in the form of ADA activity in the protection of Tuberculosis.

CONFIDENTIALITY:

I understand the medical information produced by this study will become part of my hospital record and will be subject to the confidentiality. Information of sensitive and personal nature will not be part of the medical record, but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or for teaching purpose, no names will be used and other identifiers, such as photographs will be used only with my special written permission. I understand that I may see the photographs before giving the permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time. Dr. Naren Sandeep.D. at the department of Pediatrics, is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr. Naren Sandeep.D. may terminate my participation in this study at any time after he has explained the reasons for doing so.

ANNEXURE III

INJURY STATEMENT

I understand that in the unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly then appropriate treatment would be available to me. But no further compensation would be provided by the hospital. I understand that by my agreement to participate in this study and not waiving any of my legal rights.

I have explained to _____ the purpose of the research, the procedures required and the possible risks and benefits to the best of my ability.

_____ _____

DR.NAREN SANDEEP.D. Date

(Investigator)

I confirm that Dr. Naren Sandeep.D has explained to me the purpose of the research, the study procedure that I my child will undergo and the possible risks and discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore I agree to give my consent to participate as a subject in this research project

_____ _____

Participant Date

_____ _____

Witness to signature Date

ANNEXURE IV

MASTER CHART CASES

SI.NO	A	B	C	D	E	F	G
1	B/O SHRIDEVI	1 D M	1822	13.1	23	14/2/2008	10
2	B/O JYOTHI	1 D M	1886	5	19	14/2/2008	13
3	B/O PRABHAVATHI	1 D M	128938	6.8	23.6	3/7/2008	13
4	B/O MEENAXI	1 D M	128957	9.5	19.2	3/7/2008	14
5	B/O VANITHA	1 D M	134793	5.6	21.9	10/7/2008	12
6	B/O GURUBAI	1 D M	140874	7	19.4	17/7/2008	10
7	B/O SUMITRA	1 D M	140923	9.6	28	17/7/2008	13
8	B/O SUNANDA	1 D M	163459	8.9	18.4	14/8/2008	12
9	B/O GOURAMMA	1 D M	168202	6.8	18.9	21/8/2008	12
10	B/O SANGEETA	1 D F	173955	10.6	19.4	28/8/2008	13
11	B/O LAXMI	1 D M	191157	6.3	19.9	18/9/2008	13
12	B/O GIRIJA	1 D M	168184	5.9	24.6	11/9/2008	13
13	B/O SHEELA	1 D M	173935	6.9	22	4/9/2008	14
14	B/O JAYASHREE	1D F	123773	6.3	16.9	4/12/2008	13
15	B/O ROHINI	1 D F	250863	5.9	22.6	4/12/2008	14
16	B/O ROOPA	1 D F	813	8.6	23.4	1/1/2009	13
17	B/O SOWMYA	1 D M	810	6.8	19.8	1/1/2009	13
18	B/O GEETHA	1 D M	10954	4.3	21.6	15/1/2009	14
19	B/O VAISHNAVI	1 D F	32726	11.2	17.4	12/2/2009	9
20	B/0 SHARANAWWA	1 D M	43911	8.2	23.2	26/2/2009	13
21	B/O SUMA	1 D M	67016	6.2	19.6	26/3/2009	12
22	B/0 AMRUTHA	1 D M	66958	5.8	21.4	26/3/2009	13
23	B/OVANI	1 D F	61774	11.4	24	26/3/2009	11
24	B/O VIJAYALAXMI	1 D F	83284	5.9	21	16/4/2009	12
25	B/O JAYASHREE	1 D F	83233	11.8	18.9	16/4/2009	13
26	B/O BASAMMA	1 D M	83279	7.9	23.2	16/4/2009	14
27	B/O RENUKA	1 D M	83297	9.3	21.9	16/4/2009	12
28	B/O SANGEETA	1 D M	55501	11.2	21	23/4/2009	12
29	B/O DANESHWARI	1 D F	99193	4.9	18.6	14/5/2009	13
30	B/O PRIYA	1 D F	99084	6.3	16.5	28/5/2009	14

CONTROLS

SI.NO	A	B	C	D
1	AMBIKA	2M F	143066	7.9
2	KOMAL	2M F	142920	14.8
3	YALLAMMA	2M F	725921	14.6
4	BASAVARAJ	2M M	45909	7.2
5	PRITHAM	1.5M M	36427	10
6	MAYA	1.5M F	51161	11.2
7	B/OSIDAAMMA	1.5M M	52362	5.6
8	KARTHIK	2M M	90848	9.5
9	PRATIKSHA	1.5M F	65317	7.1
10	BASAVARAJ	2M M	140939	11.2
11	VINOD	2M M	191119	11.8
12	PRAPTI	1.5M F	196697	6.8
13	ARUN	1.5M M	213068	8.9
14	NASIMA	2M F	212942	11.6
15	PRAGATI	1.5M F	191125	9.6
16	ADITYA	2M M	173978	11.9
17	RASHMI	1.5M F	245456	11.9
18	LAXMI	1.5M F	2507214	9.8
19	PREMA	1.5M F	16698	9.4
20	PRAJWAL	1.5 M M	21766	6.3
21	GANESH	1.5 M M	38493	6.4
22	AMBRISH	1.5M M	77900	4.4
23	SAKSHI	2M F	78030	3.9
24	GAUSHAY	2M M	104944	8.6
25	AKSHARA	2M F	93695	7.8
26	CHETANYA	1.5M M	121917	6.3
27	SHABANA	1.5M F	127782	7
28	GIRISH	2M M	185258	14
29	GAUTAM	2M M	152450	15.4
30	SOUNDARYA	1.5M F	261334	13.4

ANNEXURE V

KEY TO MASTER CHART

CASES

A NAME

B AGE/SEX

C IP/OP NO

D ADA LEVEL AT BIRTH(IU/L)

E ADA LEVEL AT 6 WEEKS (IU/L)

F DATE OF BCG VACCINATION

G TUBERCULIN SKIN TEST READING (MM)

CONTROLS

A NAME

B AGE/SEX

C OPNO

D ADA LEVEL (IU/L)