NUTRITIONAL STATUS, IgE LEVELS AND FCϵR1β POLYMORPHISM IN ASCARIASIS AND ASTHMA

THESIS SUBMITTED TO THE
GOA UNIVERSITY
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BIOTECHNOLOGY

By

VAIBHAV G. CHINDARKAR

GUIDE

Professor U.M.X.SANGODKAR

All the corrections and elaborations suggested by examiners are corrected and Certified

14/9/09

U.M.X. Sangodkar 14/09/2009

GOA UNIVERRSITY
DEPARTMENT OF BIOTECHNOLOGY

JULY 2008

GOA UNIVERRSITY

DEPARTMENT OF BIOTECHNOLOGY

I hereby state that this thesis for the Ph.D degree on "**NUTRITIONAL STATUS, IgE LEVELS AND FCϵR1β POLYMORPHISM IN ASCARIASIS AND ASTHMA**" in partial fulfillment of the requirements for the degree of Doctor of Philosophy is my original contribution and that the thesis and any part thereof has not been previously submitted for the award of any degree/diploma of any University or Institute. To the best of my knowledge, the present study is the first comprehensive study of its kind from this area. The literature pertaining to the problem investigated has been duly cited. Facilities availed from other sources are duly acknowledged .

Dated: July 2008

Vaibhav G. Chindarkar
Department of Biotechnology
Taleigao Plateau
Goa University

GOA UNIVERRSITY

Date: **July 2008**

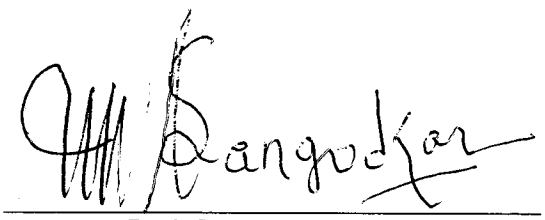
Author: **VAIBHAV G. CHINDARKAR**

Title: **NUTRITIONAL STATUS, IgE LEVELS AND FCϵR1β POLYMORPHISM IN ASCARIASIS AND ASTHMA**

Department: **Biotechnology**

Degree: **Ph.D** Year: **2009**

This is to certify that this thesis submitted by Mr. Vaibhav G. Chindarkar for the award of the degree of Doctor of Philosophy in Biotechnology is based on the results of investigations carried out by the candidate under my supervision. The thesis or any part thereof has not been previously submitted for any other degree or diploma of any University or Institute. The material obtained from other sources has been duly acknowledged in the thesis.



Prof. U.M.X. Sangodkar
Department of Biotechnology
Taleigao Plateau
Goa University

To the "never say die" attitude of my Guide

and

my Parents.

Table of Contents

List of Tables

List of Figures

Acknowledgement

During this entire journey there were many who have rendered their assistance to make this endeavor a success.

A guide is a guide, but for me Professor U.M.X Sangodkar has been a friend and a philosopher as well. If not for his tireless efforts, enthusiasm and the courage to take up something new and challenging and above all his faith in me this work would not have been possible. I am grateful to him for his guidance and advice throughout.

All the school children and their parents who volunteered for this study are to be thanked specially. I will cherish the enriching experience of interacting with so many children, teachers and village folks, throughout my life. Vishant Gaonkar, DMLT, was the one whose expertise in drawing blood with syringe made even the most frail child comfortable. Not only me but all the children involved with the study also must be thankful to him.

Dr. S.B Barbuddhe, Sr. Scientist at ICAR stands out in my mind as being one of the most helpful and selfless persons i met during this entire journey. He was very kind in providing me all the facilities to

work in his laboratory. I am also thankful to the Director, ICAR, Goa center for giving me the necessary permission to work at the center. Dr. Rajiv Kamat, pediatrician and the physicians of Sanquelim Health Center were very kind and supportive in giving me the details of the asthmatic children.

My colleagues in college Ms. Preethi Pednekar, Mahendra Pednekar, Dr. Joydeep Bhattacharjee, Ms. Lucy James, Sanjay Jhagirdhar, Nilesh Natekar, Avinash Patil, Dr. Suphala Pujari, Ms. Suman Gad and others were always very keen in knowing my progress. Dr Santhosh George of Mathematics department in our college who introduced me to the "Latex" deserves a special word of appreciation. The layout of the thesis would not have been the same had I not typed it in "Latex".

Dr. M.S Kulkarni, lecturer in statistics and demography, Goa Medical College, was very kind in helping me with statistical analysis and Sharath Jamkhandi of English department for proof-reading the text of my thesis.

I thank the laboratory staff of my college, Ms. Trupti mazgaonkar, Mahadev Parab and Anant Naik for their help and assistance during the study.

My teachers of Zoology department at Goa University, Prof A.B Shanbhag, Dr. I.K Pai, Dr. S.K Shyama and Dr. R.R Roy were always encouraging and supportive. My visits to the Biotechnology department, Goa University were always made pleasant by the warmth and affection of the office staff there.

My students have always been very special towards me, their good wishes and help have always been in my favour. Some of them, now my colleagues were around for help and advice whenever needed. I would like to thank Ms. Sugandha Pal in a very special way for helping me with data management.

Lastly, i wish to express my deep gratitude towards my parents, sisters and Rashmila, my wife for their unconditional support and showing enormous patience through out this endeavour of mine.

There are many whose mere good wishes have made the difference. Though it is not possible to remember all of them at this moment. I would like to express my gratitude and indebtedness to all those who have helped me directly or indirectly in accomplishing this study.

Prelude

Mixed emotions of happiness, fear and pride run through my mind as I am in the process of writing the thesis for my highest degree. I recollect those days when I decided to take the plunge to pursue this all important academic and perhaps social event of my life. Ever since my college days as a student I had nurtured the thought of doing my Ph.D on human health and today I see that dream being realized. The work being presented in this compilation is a humble effort to know a little more about the health of our children - the future hope of our esteemed nation and the world at large.

Like the teleological approach in ethics, where the focus is on ends,

for most of us the end result matters the most but for many others, the process of reaching the end is as important as the end result. The entire process of the study, from choosing the topic to the completion of the study, was full of interesting events, challenges and of-course, learning which made the entire journey a lifetime experience. It may be that in science as well, the end is the most important thing, perhaps because of this not many of us write about the processes of achieving the end. Here is an attempt to recollect a few of the many thoughts and experiences and to introduce some of the key elements of this compilation-the thesis.

All along the study, language played a critical role. In fact, language is a very important aspect of communication and all along it was indeed a challenge to communicate to people in their "own language" and that made me realize how important it is to be simple in communicating with general public. Perhaps that is one of the biggest reasons why this entire write-up is in very simple language.

The US novelist and sailor Herman Melville (1819-1891) has said:

> **"A man thinks that by mouthing hard words he understands hard things"**;

but at times, it becomes more difficult to use simple words to make others understand difficult things.

Most of our scientific write-ups are full of jargons and difficult vocabulary which make it awfully difficult for a commoner to understand it or create a phobia against such documents. The value of a scientific document like thesis will perhaps increase many folds if it is made common to public for their reading and the message of the entire study can be communicated in an easy language. This was an important underlying thought while writing the present thesis. All the thesis writing norms though have been followed religiously, the text has been kept fairly simple for a common man to understand and comprehend.

The entire study was conceptualised in such a way that at the macro

level it involved interacting with people and at the micro level with the genetic material with application of various levels of knowledge and skills. The study encompassed anthropometric analysis - taking physical measurements, routine hematological examination, biochemical analysis involving ELISA and molecular biological analysis comprising gene amplification and its enzymatic digestion. Thus, an attempt to look at the problem holistically has been made within the limitations of time and money.

There was an intense debate throughout the study about using Indian growth standards over the international standards for anthropometric analysis. Though there are many studies in India itself, where WHO/NCHS/CDC (National Center for Health Statistics, United States/ Center for Disease Control) standards have been used yet there are others who are not convinced about the use of these international standards. Finally, the matter was put to rest by the release of new 2007 WHO growth standards. Contacts with some of the experts in the

field of anthropometry also confirmed the suitability of using the international standards, especially the new WHO 2007 reference values for school children and adolescents which have been developed in continuation with the recently released WHO standards for children up to 5 years and which were developed by considering data from various ethnic backgrounds and cultures including India. The example given by one of the experts in the subject pointed out towards the attainment of same growth patterns by Indian expatriate children. One of the experts commented that it has been proven beyond doubt that if fed properly children grow to same size. This indeed was very relieving, though in the present study all the three major growth references, namely WHO/CDC 1978, CDC 2000 and WHO 2007 standards have been used. Moreover, the growth standards released by ICMR dates back to 1984 and the reference values are not continuous for all the ages.

Harvard bibliography style has been followed in citing the up to-date

references in the present report. A section on glossary of important terms used in the report has also been added.

This thesis was typeset using the program LATEX2$_\varepsilon$ in double line spacing. The text was written with WinEdt version 5, and the LATEX distribution used was MiKTEX version 2.3. The LATEX source was converted into a PDF file using pdfLATEX. The report has 183 typed pages starting with the title page and ending with glossary, with 20,738 words excluding bibliography and glossary.

Chapter 1

Ascaris Infection and Nutritional Status

1.1 Introduction

1.1.1 Helminth Infection

Five billion years old earth has carried and witnessed the existence of many life-forms. Origin of life on earth has been a subject of intense speculation. According to an estimate, about 10 million species have

inhabited the earth so far and among these human has arrived comparatively recently. During their 100,000 years of journey from southern or eastern part of Africa to other parts (**Paul L Hancock et al.**, 2000), humans have acquired almost unbelievable number of parasites, over 342 species of helminths (**Horton J**, 2003) and more than 70 species of protozoa (**Cox FEG**, 2002). In fact, some of these parasites have been carried by humans right from the beginning of their existence. Thus, the history of parasitic infection is as old as human himself. It has been suggested that some species of helminths probably co-evolved with primates and humans (**Brown M et al.**, 2006).

Our environment is filled with millions of potential disease causing agents (Roitt, 1988). Of the hundreds of diseases which humans suffer from, the diseases caused by the parasites inflict immense miseries and affect millions. The term "parasite" has been derived from a classical Greek word used to refer to "a guest who comes to dinner and doesn't leave" or "a class of priests who had meals at public expense"

(**McKerrow JH**, 2005). Parasites are known to reside in humans since time immemorial (**Cox FEG**, 2002). The tropical region, owing to its humid climate and higher temperature supports an array of life forms including a large number of parasites. Evolution of parasitic forms is in itself an interesting field. Many of the parasitic forms inflict humans right from early childhood.

Many of the tropical parasitic diseases are formally referred to as 'neglected tropical diseases' or NTDs which afflict mostly the poor. All together 13 diseases are classified under NTDs. Hookworm, Trichuris, *Ascaris*, *Schistosoma*, Dracanculus, Onchoceros, Filaria are the seven major helminths which cause much of the damage (**Jeffrey SD**, 2007). In developing countries, these parasites are especially abundant due to poor sanitation, open sewers and lack of personal hygiene (**Kang G et al.**, 1998).

Helminth infections, such as ascariasis and trichuriasis, represent a significant public health burden, particularly for children aged five to

fourteen (Strategic Directions for Improving the Health and Development of Children and Adolescents. WHO 2003). It is estimated that as much as 60% of the world's population is infested with gut parasites which are mostly helminth (**Kang G et al.**, 1998), and infectious and parasitic slum diseases would rank at the top of all categories of diseases (**Campbell T et al.**, 2007). More than a quarter of the world's population is infected with the most common parasites-roundworms (**Awasthi S et.al**, 2003).

Helminths are parasitic animals living in locations such as the intestinal lumen, blood stream, or muscles of the host. The infection spreads through the contact with contaminated soil, food or water (**Weinstock J V**, 2004). The infection, in most cases, is asymptomatic; this perhaps is the most important reason why helminth infections have not been given adequate importance and are referred under the neglected Tropical diseases. Global burden of diseases caused by the major intestinal nematodes is an estimated 22.1 millions disability-adjusted life

years (DALYs) lost for hookworms, 10.5 million for *Ascaris* and 32.6 million for the two infections combined (**Stephenson LS**, 2000). These figures illustrate why there is a greater need to control the spread of these diseases through proper studies. Despite its importance the research in the field of Parasitology is fairly less in developing countries as compared to developed countries. Falagas M.E et al, 2006, studied the research productivity of different world regions in the field of Parasitology and have stressed the need for more research in this field in developing regions of the world (**Falagas ME et al.**, 2006).

Helminths are multicellular, bilaterally symmetrical triploblastic animals. Helminths of human importance are divided into two main groups: Phylum PLATYHELMINTHES which includes Classes **Cestoidea Trematoda** and Phylum NEMATODA which includes two main classes, **Adenophorea** and **Secernentea**.

Platyhelminths, commonly called as flatworms have flattened leaf

like or tape like segmented body. They lack a body cavity and are monoecious, having either an incomplete alimentary canal or entirely lack it.

Nematodes, on the other hand, are commonly called as roundworms, eelworms or threadworms. They have cylindrical, elongated,unsegmented bodies, lacking a true body cavity and appendages. They represent evolutionary beginning of complex animal body systems.

General Characters: Phylum NEMATODA

1. Nematodes have cylindrical, elongated, Unsegmented bodies covered with a tough cuticle and lack appendages. Both the ends of the body are often pointed.

2. Body size shows wide variations ranging from 4mm in *Trichuris spiralis* to 1 metre in *Dracanculus medinensis.*

3. They possess a body cavity in which the various organs float.

CLASS	SUB CLASS	ORDER
Adenophorea	Enoplia	Enoplida Isolaimida Mononchida Dorylaimida Stichosomida
	Chromadoria	Chromadrorida Monhysterida Araeolaimida Desmodorida Desmoscolecida
Secernentea	Rhabditia	Rhabditida Strongylida **Ascaridida** Drilonematida Camallanida
	Spiruria	Spiruida Filaroidea
	Diplogasteria	Diplogasterida Tylenchida Aphelenchida

Table 1.1: Broad classification of nematodes (Maggenti, 1983)

4. Nervous system and Excretory system are rudimentary.

5. Alimentary canal is complete. Mouth cavity when present is beset with teeth or cutting plates. Anus is subterminal.

6. Nematodes parasitizing man are dioecious with males generally smaller than females with their posterior end curved or coiled ventrally.

Ascaris lumbricoides

Ascaris lumbricoides, one of the largest helminth parasites is also one of the commonest helminth infections to inflict humans. More than one billion people are estimated to be infested with *Ascaris* worldwide (**Gonzlez AH et al.**, 2001).

The adult worms live in the intestine (jejunum) without any significant symptoms. However, migration to the ectopic sites in unusual circumstances such as irritation due to drugs, fever, anaesthesia and

bowel manipulation during surgery may result in life threatening complications (**Santra A et al.**, 2001). Other clinical problems associated with worm infections include fever, urticaria, malaise, intestinal colic, nausea, vomiting, diarrhoea, shortness of breath, skin rashes, biliary tract obstruction, perforation in the gut and even nervous system disorders (**Viqar Z and Loh Ah Keong**, 1989).

The adult worm which is cylindrical with tapering ends is creamy white, pinkish to light brown in colour. Sexes are separate and the female which is longer than the male measures 25 to 40 cm and 3 to 5 mm wide.The male measures 15 to 25 cm and 3 to 4 mm in width. Mouth of the worm possesses three finely toothed lips, one dorsal and two ventral. The tail end of the male is curved ventrally in the form of a hook having a conical tip. The genital pore and the anus opens into the cloaca from which two copulatory spicules protrude. In females the posterior extremity is neither curved nor pointed but is conical and straight. The anus is subterminal and opens by a transverse slit. The

vulva opens at the junction of the anterior and the middle thirds of the body. This section of the worm which is narrower is called the vulvar waist. The body cavity in which the digestive and the reproductive organs float is filled with fluid containing an irritant ascoron or ascarase. The allergic manifestations seen in infected individuals is due to this substance (**Chatterjee KD**, 1976).

The mature *Ascaris* female has an enormous egg laying capacity of 200,000 to 250,000 eggs daily (**Viqar Z and Loh Ah Keong**, 1989). The eggs liberated by the females pass out of the human host with faeces. The liberated eggs may be fertilized or unfertilized. The fertilized eggs are usually oval in shape, about 50 to 70 μm in length and 40 to 50μm in breadth (**Peng W et al.**, 2003) and are always brownish to golden brown in colour. The eggs are typically surrounded by thick smooth translucent shell with an outer albuminous coat thrown into rugosities or mammillations, which may be lost sometimes, to be referred as decorticated egg. The fertilized eggs contain a very large

conspicuous, unsegmented ovum with a clear crescentic area at each pole.

The unfertilized eggs on the other hand are longer, about 80 μm in length and 55 μm in breadth, more elliptical, brownish in colour, have a thinner shell with irregular coating of albumin and contain atrophied ovum with a mass of highly refractile granules. The fertilized eggs float in saturated solution of common salt whereas the unfertilized eggs do not (**Chatterjee KD**, 1976).

Ascaris lumbridoides has a direct life cycle and has only one definitive host-Man. Fertilized eggs containing unsegmented ovum pass through the faeces. When freshly passed they are not infective. A rhabditiform larva develops within the egg shell in 10 to 40 days time (**Chatterjee KD**, 1976) under the influence of adequate moisture, oxygen and shade (**Peng W et al.**, 2003). The ripe egg containing the coiled up embryo is infective to man. Infection is contracted *via* the faecal-oral route

when eggs are ingested with food, drink or raw vegetables (figure 1.1). The embryonated eggs pass through the duodenum and get broken under the influence of digestive juices, and the rhabditiform larvae get liberated. The larvae burrow themselves into the mucous membrane of the small intestine and are carried to the liver *via* the portal circulation. They stay in the liver for 3-4 days and then via the heart enter the pulmonary circulation. In the lungs they moult twice growing to a length of almost 2 millimeters. Breaking through the capillary walls they reach the lung alveoli. This migration takes 10 to 15 days. From the lung alveoli they crawl up the bronchi and trachea from where they are propelled to larynx and then to the pharynx and are once again swallowed into the stomach. They localize themselves in the upper part of the small intestine where one more moulting may take place. In the small intestine the larvae grow into sexually mature adults in 6 to 10 weeks. In all, four moultings take place, one outside while within the egg shell, two in the lungs and one in the intestine. The mature

female starts discharging eggs in the stool in about two months after the infection.

1.1.2 Nutritional Status

Along with helminth infection, poor nutritional status is also a cause of morbidity and mortality and thus a major health burden in children in developing countries (**WHO**, 2005). The relationship between helminth infection and nutritional status has been investigated by many (**Assis AMO**, 2004; **Kloetzel K et al.**, 1982; **Zhou H et al.**, 2005). Malnutrition is the most important risk factor for illness and deaths throughout the globe with pregnant women and children affected the most (**Muller O and Krawinkel M**, 2005). Prevalence of parasitic diseases contributes to malnutrition (**Dickson et al.**, 2000). Foundations for a better health in adults is laid during the childhood. Further, It is a well accepted fact that the future of any country lies in the hands of children. Hence, it becomes imperative to study the nutritional well

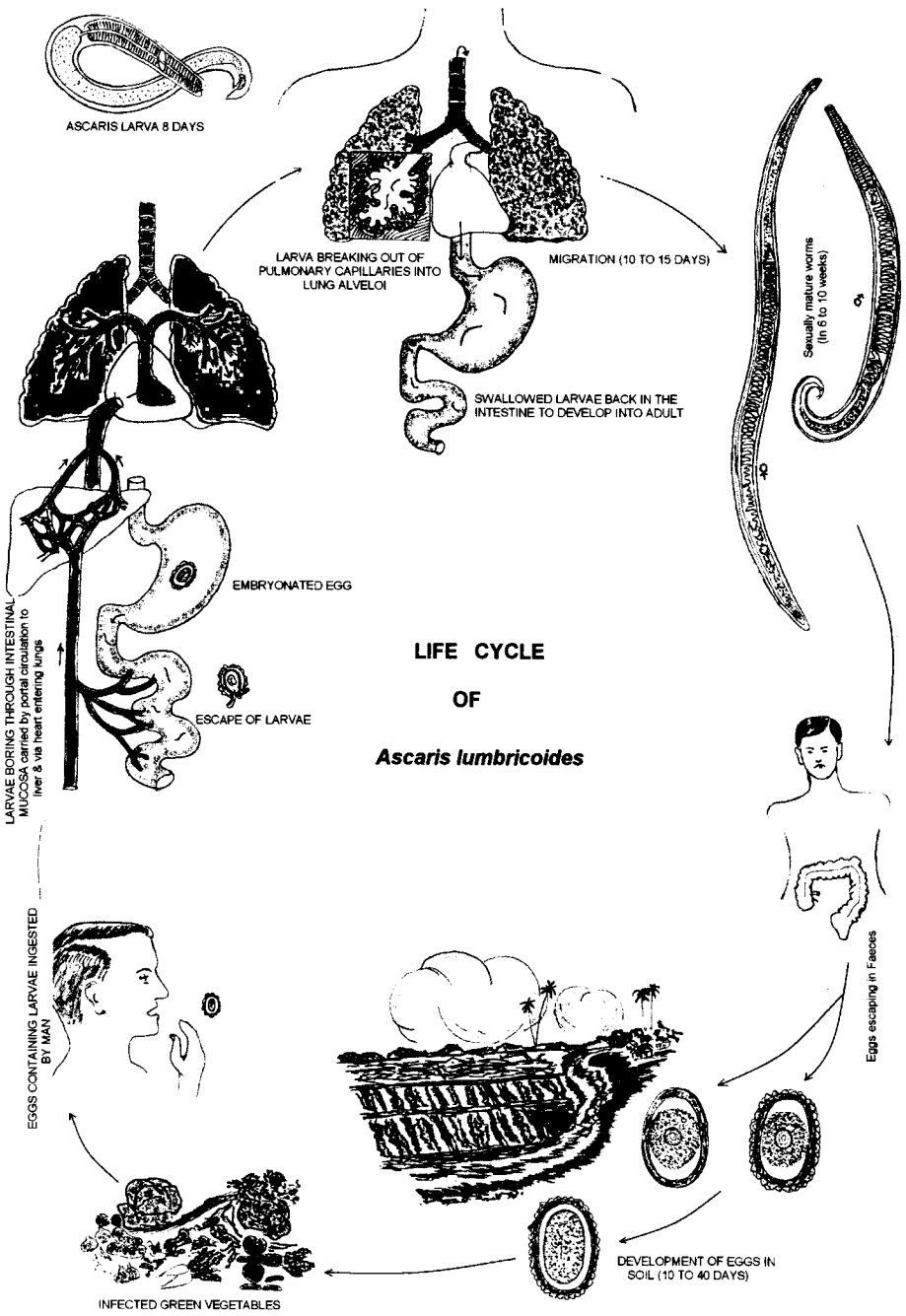


Figure 1.1: Life cycle of *Ascaris lumbricoides*

being of the children of our society. Better nutrition means stronger immune system, less illness and better health. In general nutrition and health of school children and adolescents have attracted less attention in developing countries (**Friedman JF et al.**, 2005). According to WHO 2000, better nutrition is an important criterion for ending poverty and a milestone for achieving better quality of life. Nutrition and ill health goes in cycle: Poor nutrition leads to ill health which further deteriorates the nutritional status (**WHO**, 2003). Gomez in 1955 defined malnutrition as a "**pathological condition of varying degrees of severity, and diverse clinical manifestations, resulting from the deficient assimilation of the components of the nutrient complex**" (**Gomez F et al.**, 1956); but perhaps, a more comprehensive definition defines malnutrition as a "**pathological state resulting from inadequate nutrition including undernutrition (protein energy malnutrition) due to insufficient intake of energy and other nutrients; overnutrition (overweight and**

obesity) due to excessive consumption of energy and other nutrients;deficiency diseases due to insufficient intake of one or more specific nutrients like vitamins and minerals" (Ge KY and Chang SY, 2001).

Gomez, in 1956, has gone on to add that the causes of malnutrition can be primary-insufficient food supply that is under-nutrition and secondary or conditioned-poor absorption or increased excretion. Though the overall mortality rates among children in developing countries have reduced significantly (**Onis de M.**, 2000), the, same cannot be said about their infections and poor nutritional states. Early childhood infections are exacerbated by poor nutritional status.

Inadequacies in nutritional intake eventually affect functional capacity and get manifested into abnormal physical symptoms like cessation of linear growth, that is, stunting, oedema, skin and hair changes, wasting, biochemical abnormalities like low hemoglobin, low albumin levels

in blood etc, which depend on the severity of the problem (malnutrition). Protein-energy malnutrition and micronutrient deficiencies are the two constituents of malnutrition. WHO has defined protein-energy malnutrition as measurements that fall below 2 standard deviations under the normal weight for age (underweight), height for age (stunting) and weight for age (wasting). Wasting is related to recent weight loss, whereas stunting is the result of long term effects (**Muller O and Krawinkel M**, 2005). Anthropometry, thus, has overall advantage over other methods like the biochemical, which are good at assessing the extreme cases (**Onis de M.**, 2000). Alterations in growth parameters from standard values reflect the overall health status and well being of an individual or population. Thus, assessment of growth serves as a simple, inexpensive and reliable means of evaluating the normal growth and detecting the gross abnormalities of a child even when no other clinical signs of illness are manifested, besides helping in predicting the performance and overall social and economic well being of populations

(**ICMR**, 1984; **Cogill et al.**, 2003).

The gap between the demand and supply of body's energy and nutrient requirements is reflected as malnutrition. Prolonged undernourishment in children leads to stunting, and the condition is known as "Chronic malnutrition". Whereas, rapid loss of weight-wasting over a short period of time leads to a condition referred to as "Acute malnutrition" (**Merlet O**, 2006).

Marasmus and Kwashiorkor are the two most common clinical syndromes of acute malnutrition. Poor growth/malnutrition can be attributed to a range of factors from poverty to social, cultural, direct physical environment, infections, illiteracy and poor hygiene, etc.

1.2 Literature review

It is widely believed that helminthology as a scientific discipline was established during the Renaissance period when reemergence of science

took place. Till almost 18th century the explanation and interpretation of natural phenomena including that of epidemic diseases were not proper as it was based on ancient ideas. In 16th century itself, the idea of contagious factors (pathogens) was developed but the hypothesis was not well accepted because of well-established normative concepts of those times. Parasitology got a fillip in the second half of 18th century owing to intensified urbanisation, rapid increase in population and wars. The third phase of growth in parasitology was seen from mid 19th century to the end of World War I. The concept of contagium was reestablished at this time. The efforts were supported by governments to make their colonies,settlers free from diseases. Parasitology emerged as a separate discipline during this period. Post World War II the fourth phase in the development of parasitology occurred. The main focus in this period was to study the local environment and parasitic diversity (**Plonka-Syroka B**, 2005).

Helminths, owing to their large size, must have been known to

our earliest ancestors. This fact is confirmed by some of the historians who have identified references pertaining to helminth diseases in Bible (**Cox FEG**, 2002); but perhaps the oldest mention of worms is found in Ayurveda. Charaka and Shrushut, about 3000 BC (**Sastri K and Chaturvedi G**, 1998) have described worms as 'krumi'(**Joshi Y**, 1998). Vivid description about various kinds of worms is given by these ancient Indian physicians. Further, the discovery of calcified helminth eggs in mummies as old as 1200 BC coupled with their mention in Egyptian literature also suggests the knowledge of these parasites from quite early times. Roman physicians in the early part of the millennium (AD 129 to 690) were familiar with *Ascaris lumbricoides* and have even given good clinical description of its infection (**Cox FEG**, 2002).

From the works of various workers it is clear that our ancestors were indeed infected with helminths(**Ferreira LF et al.**, 1983; **Reinhard KJ**, 1987; **Sianto L et al.**, 2005; **Sullivan Richard**, 1995). Ferreira LF et.al (1984) found Coprolites of human origin dated 4100

to 1950 B.C.containing helminth eggs identified as *Diphyllobothrium pacificum*(**Ferreira LF et al.**, 1984). In fact, the oldest record of *Diphyllobothrium pacificum* was found in northern Chile mummies which were 4000-5000 B.C (**Reinhard K**, 2003). Ferreira LF et.al in their paper mentions the presence of this tapeworm *(Diphyllobothrium pacificum)*in American Sea Lion, pointing towards the diet of people in those times and a possible explanation to the spread of the parasites from animal sources.

Ascaris lumbricoides- one of the six worms described and named by Linnaeus has retained its name ever since. Its eggs have been found in human coprolites in Peru dating 2227 B.C. (**Patrucco R et al.**, 1983).These worms have also been reported from Brazilian and Egyptian mummies as well (**Ferreira LF et al.**, 1983). According to Cox 2002, the beginning of the subdivision of helminthology is marked by the classical papers of Tyson in 1683, who gave the detailed anatomy of *Ascaris* and published a paper on tape worms of humans, and Redi

in 1684 who wrote one of the first books on parasitology, *Introno Agli Animali Viventi che si Trovano Negli Animali Veventi.* Cox has also mentioned French medical scientist Casimir Joseph Davaine as the first person in 1862 to demonstrate the transmission of *Ascaris* infection by ingesting eggs.

Many studies have examined the deleterious effects of worm infection on the nutritional status, child growth and development (**Assis AMO**, 2004; **Boivin MJ and Giordani B**, 1993; **Dickson et al.**, 2000; **Gulani et al.**, 2007; **Kloetzel K et al.**, 1982; **Northrop Clewes CA et al.**, 2001; **Oberhelman RA et al.**, 1998; **Oguntibeju OO**, 2003; **Stoltzfus RJ et al.**, 1997; **Ulukanigill M and Seyrek A**, 2000; **Watkins WE and Pollitt E**, 1996; **Willett WC et al.**, 1979; **Yahamoto R**, 2000). There is an intimate relationship between malnutrition and infections (**Hughes S et al.**, 2006). **Watkins WE and Pollitt E** (1996) reported that successful removal of *Ascaris* in school aged

children had modest effect on weight gain. **Walter et al**, 1979 randomly assigned 341 pre-school children with Levamisole (Ascaricide) or placebo treatment and found that the children treated with levamisole had 21% greater rate of weight gain as compared to children receiving placebo. However, **Kloetzel et al.** 1982, on the other hand, found that mass deworming of 165 children of rural Brazil using mebendazole and comparing them with another group of 172 untreated ones did not reveal any difference in the nutritional status; further, the fluctuations in the egg counts of *Ascaris* did not maintain any significant relation to the changes in the nutritional status at the end of 10 months. Somewhat similar observations were made by **Willett WC et al.** (1979). In 1996, **Wattkins W.E** and **Pollitt Ernesto** found that albendazole treatment as compared to the placebo administration for six months resulted in marked weight gain in children whose average age was 9.7 years. **Stoltzfus RJ et al.** (1997) concluded from their study that deworming improved weight gain in school children especially those who

were younger. **Oberhelman et al.**, 1998, found *Ascaris* and *Trichuris* were more prevalent in malnourished children. Similar results have also been reported by **Dickson et al.** (2000). Closer home, **Awasthi S** and **Pande V.K** in 2001, reported an improvement in weight in pre-school children administered Albendezol suspension over 1.5 years. More recently **Alderman et al.** (2006), in their study involving 30,000 children, have concluded that routine administration of anti-helminthic treatment contributes to the linear growth in young people up to age 16 years. Many workers have studied the effects of helminth infection on hematological indices as well (**Gulani et al.**, 2007; **Oguntibeju OO**, 2003; **Ulukanigill M and Seyrek A**, 2000). **Gulani et al.** (2007) concluded from their study involving the medline and extended medline searches (1966 to 31 July 2006) that deworming indeed improves the hemoglobin levels in children. Their study reported that administration of routine intestinal anti-helminthic agents can translate into reduction of 5 to 10% of anemia cases in a population with high prevalence of

helminthiasis.

Federico Gomez and his colleagues were the pioneers in the field of malnutrition research. In 1956, in their classical paper, they gave the classification for varying degrees of malnutrition which was based on the simple anthropometric indicator-weight (**Onis de M**, 2000). **Mercedes de Onis,** in 2000 has commented in his 'Public Health Classics' series of WHO that the classification developed by Gomez et al. was based on three prior selections: an anthropometric indicator, a reference population, and cut off points. Subsequently, in late 1970s, the US National Center for Health Statistics (NCHS) and WHO came out with international reference growth values for facilitating malnutrition research. Owing to the technical drawbacks of the NCHS/WHO, 1970, growth standards new growth standards have been worked out by WHO which are based on the multi-country study (WHO, 2007).

According to **WHO**, 2005, malnutrition is responsible for 54% of

deaths per year in children and contributes to every second death associated with infectious diseases in children below five years in developing countries (**WHO**, 2005). World Health Organisation's 2003 publication on the strategic directions for improving the health and development of children and adolescents also raised its concerns about the improvement of nutritional status among children, especially in developing countries. The poor nutritional status of children in developing countries has been reported by many workers (**Kikafunda JK**, 1998; **Olivares S et al.**, 2004; **Dang S et al.**, 2004). More than 2 million children die in India every year due to causes related to undernutrition. About 78% of the worlds 55 million wasted children live in India, Pakistan, and Bangladesh; nearly two thirds are in India (**Gross R et al.**, 2006). According to **Schaible et. al.** 2007, protein energy malnutrition (PEM) is a critical, yet underestimated factor in susceptibility to infection.

1.3 Materials and Methods

1.3.1 Area of study, Selection of subjects and Methodology

Bicholim and Sattari are among the hinterland talukas of the state of Goa. A mix of rural and urban population, it has a substantial area under mining. The region has mostly humid climate throughout, which is typical of the state of Goa.

Government primary school children of the two talukas were considered for the study. Subjects were selected at random using random number table. Children of both the sexes aged between five (5) and fifteen (15) years were recruited. Oral consent of the parents and the subjects was taken and the procedure and the aim of the study was explained to them in local language. In all one thousand sixty five (1065) children (four hundred and seventy one (471) females and five hundred and ninety four (594) males) were assessed for anthropometric indices of weight for age, weight for height and height for age. Two hundred

Figure 1.2: Map of Goa

and ninety one (291) school children, one hundred and twenty six (126) females and one hundred and sixty five boys were assessed for *Ascaris* infection as well as for the anthropometric indices.

Children were made to stand erect against a wall and their standing length was recorded to the nearest 0.5 centimeters. The weight of the subjects was recorded to the nearest 100 grams. Dates of birth of the subjects were recorded from school records. All the children were told the importance of proper hygiene and proper diet, along with the advantages of general cleanliness. A fresh stool sample in air tight non-sterile vial was collected from each of the 291 children.

Stool samples were analysed by direct smear method for assessing the extent of *Ascaris* infection within 1 hour of collection. For stool analysis, 2 milligrams of faeces was mixed in a small drop of saline on a slide. The material was evenly suspended and occurrence of air bubbles was avoided. Count of *Ascaris* eggs were recorded per smear of faeces.

Eggs per gram were calculated using the formula: N/2 X 1000, where N = number of eggs in a 2 milligrams smear (**Viqar Z and Loh Ah Keong**, 1989).

The intensity of *Ascaris* infection was quantified as described by WHO depending on the eggs per gram (epg) of feces into low (1-4999 epg) , moderate (5000-49,999 epg) and heavy (> 50,000 epg) (**Friedman JF et al.**, 2005).

Blood sample of two hundred and eight (208) children, ninety four (94) girls and hundred and fourteen (114) boys were analysed for eosinophils, hemoglobin and total protein. About three (3) milliliter of blood was drawn from the brachial vein and collected in a sterile blood collecting vial from each of the 208 children. Blood collection was made between 11 a.m to 12 p.m to maintain uniformity of diurnal variation, if any, in the blood parameters.

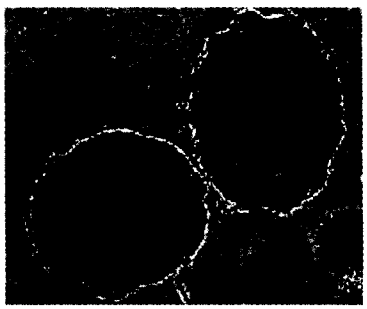


Figure 1.3: *Ascaris* eggs

Hemoglobin content was analysed by Sahli's (Acid Haematin method) method.

Absolute count of Eosinophil was also done with Improved Neubauer Haemocytometer using W.B.C pipette. Phloxine Methylene blue solution was used as the diluting fluid.

Total blood protein was estimated by Biuret method using kit supplied by Crest Biologicals, Verna, Goa.

Children were classified as mildly, moderately and severely underweight, stunted and wasted according to Gomez's and Waterlow's classifications (**Ghai OP, Gupta P and Paul VK**, 2001).

Waterlow's classification of malnutrition in children was calculated as:

% weight for height= [(weight of respondent)/ (weight of normal child of same height)] × 100

% height for age= [(height of respondent)/ (height of normal child of same age)] × 100

Gomez's classificaton of malnutrition was calculated as:

% reference weight for age = [(weight of the subject)/ (weight of normal child of same age)] × 100

Besides this, stunting, malnutrition and wasting in the subjects was calculated as Z scores less than 2 standard deviation below the median reference values.

	% Reference weight for height (wasting)	% Reference height for age (stunting)
Severe	<70	<85
Moderate	70-80	85-90
Mild	80-90	90-95
Normal	>90	>95

Table 1.2: Waterlow's classification of malnutrition in children

% Reference weight for age	Interpretation
<60	Grade III: severe malnutrition
60-74	Grade II: moderate malnutrition
74-89	Grade I : mild malnutrition
90-110	Normal

Table 1.3: Gomez's classification of malnutrition in children

1.3.2 Data entry and analysis

The entire data about the date of visit for the survey, sex, date of birth, height and weight of the children was first entered in the 'database and statistics software for public health professionals', EpiInfo version 3.4.3 released in November 2007 by Center for Disease Control (CDC), United States. The software uses CDC 2000 and WHO 1978 growth standards.

As mentioned previously, WHO, since late 1970s, has been recommending the use of Z scores for assessing the nutritional status of children (**WHO**, 1995). Z scores for the various indices namely weight for age, height for age, BMI for age and weight for height was calculated using EiInfo software and Microsoft Excel .

For 1978 WHO/CDC references, Z-scores for Weight for height are calculated for males up to 138 months (11.5 years) of age and less than 145 cm (57 inches) in height and for females up to 120 months (10 years) of age and less than 137 cm (53 inches) in height; Z-scores for Weight for Height could not be calculated for children less than 49 cm (19.3 inches) in height owing to the limitations of EpInfo software.

Similarly for CDC 2000 growth references, Z-scores for weight for height of subjects more than 121.49 cm in height could not be calculated due to the limitations of the software.

No reference values for weight for height have been released by WHO under WHO, 2007 growth standards for the concerned age group of 5 to 15 years. Also the weight for age values are not available for the subjects more than 10 years of age.

The entire study group was divided into three (03) groups, group I 5 to 8 years (High school students), group II 8 to 10 years (Pre-adolescent students), group III 10 to 15 years (Adolescents).

A z score reflects how many standard deviations above or below the population mean a raw score is. If $\overline{X}$ is the mean of the population with standard deviation σ then the Z score for the observation X would be:

$$\frac{X-\overline{X}}{\sigma}$$

For the present study, along with WHO 1978 and CDC 2000 growth

standards, WHO 2007 growth standards for school children and adolescents have been referred. The 2007 WHO growth standards have been developed by WHO by applying the state-of-the-art statistical method that is, the Box-Cox power exponential method (**Onis de M et al.**, 2007). Figure 1.4 gives the WHO 2007 growth curve for girls aged 5 to 19 years, figure 1.5 and figure 1.6 give the curves for weight for age in boys for 5 to 10 years and BMI for age for boys from 5 to 19 years as detailed by WHO 2007 growth standards. This method can take several kinds of distributions from normal to skewed or kurtotic, finally; the selected models have been simplified to LMS model. The LMS method (Cole 1988) is a way of summarizing growth standards which monitors the changing skewness of the distribution during childhood. It does so by calculating the Box-Cox power needed to transform the data to normality at each age, and displaying the results as a smooth curve of power plotted against age (**Colley DG**, 2000). The 2007 WHO growth standards for school aged students and adolescents is an extension of

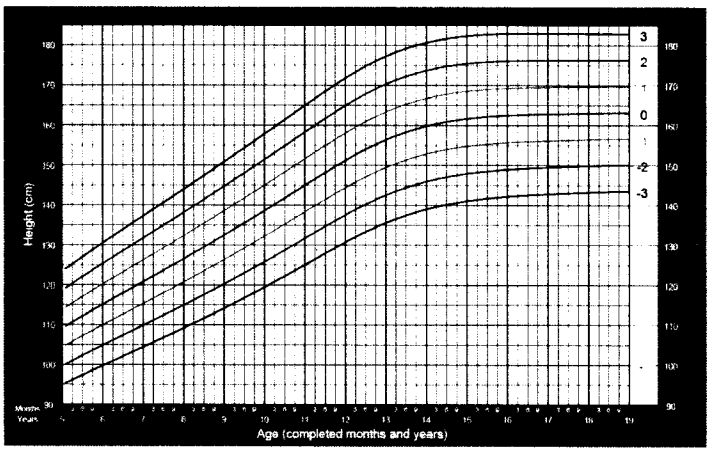


Figure 1.4: Z scores values of height for age in 5 to 19 years females as per WHO, 2007 growth standards

the standards released by WHO for children upto the age of five. For these growth references growth data from diverse ethnic backgrounds and cultural settings including India was taken (**WHO**, 2007).

The Z scores for height-for-age, weight for age and BMI for age were calculated in the following ways:

Individual Z-score for height-for-age for a measurement y at age t was computed as:-

$$\frac{y-M(t)}{StDev(t)}$$

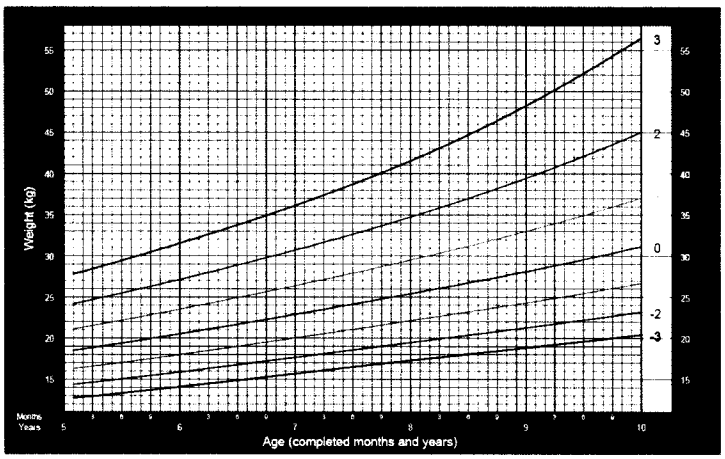


Figure 1.5: Z scores values of weight for age in 5 to 10 years males as per WHO, 2007 growth standards

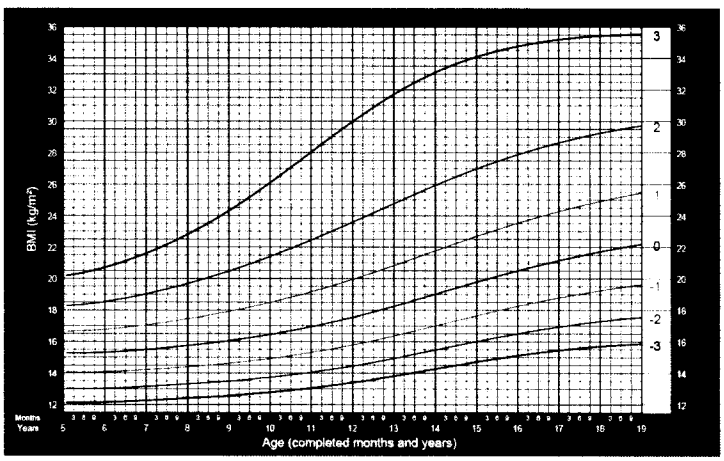


Figure 1.6: Z scores values of BMI for age in 5 to 19 years females as per WHO, 2007 growth standards

where *M(t)* is the median value of the reference population at age *t*.

The following procedure recommended by WHO multicentre Growth Reference Study Group, 2007 to calculate Z-score for Weight- for-age and BMI-for-age was used for the present study.

1. $z_{ind} = \frac{[y/M(t)]^{L(t)}-1}{S(t)L(t)}$

2. Final Z-score (z^*_{ind}) of the child was computed as:

$z^*_{ind} = z_{ind}$ *if* $|z_{ind}| \leq 3$

$z^*_{ind} = 3+(\frac{y-SD3pos}{SD23pos})$ *if* $z_{ind} > 3$

$z^*_{ind} = -3 + (\frac{y-SD3neg}{SD23neg})$ *if* $z_{ind} < -3$

where

$SD3pos$ is the cut-off 3 SD calculated at t by the LMS method:

$SD3pos = M(t)[1+L(t)*S(t)*(3)]^{1/L(t)}$;

$SD3neg$ is the cut-off -3 SD calculated at t by the LMS method:

$SD3neg = M(t)[1+L(t)*S(t)*(-3)]^{1/L(t)}$;

$SD23pos$ is the difference between the cut-offs 3 SD and 2 SD calculated at t by the LMS method:

$SD23pos = M(t)[1+L(t)*S(t)*(3)]^{1/L(t)} - M(t)[1+L(t)*S(t)*(2)]^{1/L(t)};$

$SD23neg$ is the difference between the cut-offs -2 SD and -3SD calculated at t by the LMS method:

$SD23neg =$

$\mathrm{M}(t)[1+L(t)*S(t)*(\text{-}2)]^{1/L(t)} - M(t)[1 + L(t) * S(t) * (-3)]^{1/L(t)}$

1.4 Results

The results obtained have been arranged in two sections, first section concerning anthropometry for showing the nutritional status and second to show the influence of *Ascaris* infection on the nutritional status.

1.4.1 Nutritional status

Tables 1.5 to 1.8 show the results obtained from processing the anthropometric data and its comparison with the three growth references of WHO 1978, CDC 2000 and WHO 2007. The mean age of all the subjects was 8.28 years, 8.23 (standard deviation 1.60) years for females

and 8.32 (standard deviation 1.62) years for males (Table 1.4).

The mean value of Z scores for height for age (HAZ), weight for age (WAZ), weight for height (WHZ) and body mass Index (BMIZ) for both the sexes according to the three standards is shown in table 1.5. In general, the Z-scores of the subjects are least when compared to CDC 2000 growth standards; moreover, the values for males is less than the females.

Extent of stunting (height for age) according to 1978 growth references was 38.7%, 41.9% according to CDC 2000 growth references and 39.7% according to WHO 2007 reference values.

Extent of malnutrition prevalence was 43.1% with reference to WHO 1978 growth standards, 54.9% according to CDC 2000 standards and 46% in relation to WHO 2007 growth standards.

Similarly the extent of wasting was 21.6% for WHO 1978 and 33.5% for CDC 2000 growth standards. WHO 2007 standards were not available for weight for height indices and hence extent of wasting could not be ascertained using these standards (Table 1.7).

According to WHO 1978 standards, the mean HAZ and WAZ values were least, -2.72 and -2.17 respectively, in the age group from 10 to 15 years; the mean of these indices was highest, -1.44 and -1.58 respectively, in the age group, 5 to 8 years along with -0.97 for WHZ . Mean WHZ was least (-1.12) in the age group 8 to 10 years (refer table 1.6).

According to CDC 2000 growth standards also the mean HAZ and WAZ values were least, -2.66 and -3.06 respectively for the age group 10 to 15 years. Further, the highest values of these two indices, -1.52 and -2.01 respectively, were for the age group 5 to 8 years. The WHZ value of -3.61 was also least for the age group 10 to 15 years and -1.32 which was highest belonged to the age group 8 to 10 years (refer table

1.6).

Finally, with the WHO 2007 growth standards, HAZ was least (-2.78) for 10 to 15 years age group and highest (-1.43) for the age group 5 to 8 years. The WAZ value of -2.09 was least for the age group 8 to 10 years and highest (-1.72) again for 5 to 8 years. As mentioned previously in the 'Data entry and analysis' sub-section, WHO 2007 growth standards have no reference values for weight for height; also the weight for age reference values are not mentioned for children more than 10 years old. Owing to this the Z values for weight for height and the Z-scores of weight for age for children more than 10 years of age could not be calculated. All the above mentioned results have been compiled in table 1.6

In relation to the WHO 2007 standards, independent samples T-test revealed that there was a significant difference in the mean value of Z-score for weight for age among males (-1.98) and females (-1.67) with

p-value 0.00 but there was no significant difference between the mean Z-score values of height for age among males (-1.68) and females (-1.59) with p-value 0.238. However, significant difference between the mean Z-score values for BMI of males (-1.45) and females (-1.19) with p value of less than 0.05 was obtained (refer table 1.8).

Further, according to Gomez's classification 43.0% of children were suffering from mild malnutrition, 34.3% suffered moderate malnutrition and 5.7% suffered severe malnutrition (table 1.8).

According to Waterlow's classification, 33.3% of the subjects were mildly stunted, 25.0% were moderately stunted and 8.7% were severely stunted (table 1.8).

Analysis of haemoglobin levels revealed that 27.4% of children were anemic (hemoglobin levels less than 12 gms%. The mean hemoglobin level was 13.78 gms% (std. deviation 2.65) with T-test revealing no

	Mean Age (In years)	N*	Std. deviation
F	8.23	471	1.60
M	8.32	594	1.62
Total	8.28	1065	1.61

Table 1.4: **Age characteristics of the study group**

	CDC/ WHO 1978 Standards			CDC 2000 Standards			WHO 2007 Standards		
	Mean	N*	$\sigma^{\dagger}$	Mean	N*	$\sigma^{\dagger}$	Mean	N*	$\sigma^{\dagger}$
HAZ									
F	-1.52	471	1.35	-1.70	471	1.35	-1.59	471	1.34
M	-1.72	594	1.30	-1.75	594	1.28	-1.68	594	1.27
WAZ									
F	-1.53	471	1.02	-2.06	471	1.42	-1.67	411	1.20
M	-1.83	594	1.01	-2.35	594	1.48	-1.98	508	1.24
WHZ									
F	-0.90	406	1.31	-1.34	301	1.84			
M	-1.09	571	1.38	-1.70	377	2.26			
BMIZ									
F				-1.50	471	1.65	-1.19	471	1.57
M				-1.85	594	1.88	-1.45	594	1.73

* Number of subjects, † Standard deviation of Z score

Table 1.5: **Comparisons of mean Z score values for height for age, weight for age, weight for height and Body Mass Index according to the three standards**

	WHO 1978 Standards			CDC 2000 Standards			WHO 2007 Standards		
	Mean	N♭	$\sigma^{\#}$	Mean	N♭	$\sigma^{\#}$	Mean	N♭	$\sigma^{\#}$
Age Group I*									
HAZ	-1.44	622	1.29	-1.52	622	1.27	-1.43	622	1.23
WAZ	-1.58	622	1.13	-2.01	622	1.47	-1.72	622	1.26
WHZ	-0.97	620	1.43	-1.65	513	2.14			
Age Group II⋆									
HAZ	-1.69	351	1.21	-1.85	351	1.22	-1.73	351	1.20
WAZ	-1.79	351	0.83	-2.39	351	1.32	-2.09	297	1.13
WHZ	-1.12	332	1.21	-1.32	141	1.83			
Age Group III†									
HAZ	-2.72	92	1.48	-2.66	92	1.48	-2.78	92	1.55
WAZ	-2.17	92	0.77	-3.06	92	1.58			
WHZ	-0.65	25	1.13	-3.61	24	2.04			

∗ age group 5 to 8 years; ⋆ age group 8 to 10 years; † age group 10 to 15 years; ♭ Number of subjects; # Standard deviation,

Table 1.6: **Comparisons of mean Z score values for height for age, weight for age and weight for height according to the three standards in different age groups**

	WHO 1978 Standards	CDC 2000 Standards	WHO 2007 Standards
Stunted*	38.7	41.9	39.7
Normal	61.3	58.1	60.3
Malnutrition*	43.1	54.9	46.0
Normal	56.9	45.1	54.0
Wasted*	21.6	33.5	
Normal	78.4	66.5	

* -2 standard deviation less than the normal reference values.

Table 1.7: **Percentage of stunting, malnutrition and wasting as per the three growth references**

difference in the mean levels between males and females. In all 30% of females and 25.4% of males were anemic. With reference to serum protein 11.5% of the subjects had low serum protein (less than 6.5gms%). 9.6% of the females and 13.2% of the males had low serum protein. The mean serum protein was 7.27 gms% (std. deviation 0.57)

1.4.2 *Ascaris* infection and nutritional status

There was no significant difference in the mean *Ascaris* eggs in the feces of male and female children. The mean epg of feces in females was

Variable	Males	Females	t-value	p-value
Age (years)				
Mean	8.32	8.23	0.94	0.345
SD^*	1.62	1.60		
$n^\dagger$	594	471		
Height. for age (Z score)				
Mean	-1.68	-1.59	1.18	0.238
SD^*	1.27	1.34		
Waterlow's classificaton				
% normal	31.6	34.6		
% mildly stunted	34.2	32.3		
% moderately stunted	26.3	23.3		
% severely stunted	7.9	9.8		
BMI (z score)				
Mean	-1.45	-1.19	2.53	0.01
$S.D^*$	1.73	1.57		
Gomez's classification				
Weight for age (Z score)				
Mean	-1.98	-1.67	3.76	.00
SD^*	1.24	1.20		
$n^\dagger$	508	411		
% normal	17.1	18.7		
% mild malnutrition	43.0	41.1		
% moderate malnutrition	34.3	34.8		
% severe malnutrition	5.7	5.4		

$*$ standard deviation; † number of children.

Table 1.8: **Characteristics of children by sex (WHO, 2007 Growth reference)**

37526 (standard deviation 42269) and in the males was 37338 (standard deviation 38639); the p value for the t-test to find the difference in the mean epg of feces in both the sexes was 0.969.

Table 1.9 gives the details of the mean *Ascaris* eggs per gram of feces in mild, moderate and heavy infection among the three age groups.

There was significant difference in the mean Z-scores of height for age, weight for age, weight for height and body mass index between *Ascaris* infected and non-infected subjects when CDC/WHO 1978 growth references as well as CDC 2000 standards were referred (Table 1.11, 1.12). Same results were found when the indices were compared with 2007 standards (table1.13). The Z-scores for all the indices were significantly lower in *Ascaris* infected children than in the non-infected (refer table 1.11). Table 1.10 gives the mean Z-scores of height for age, weight for age and weight for height in males and females suffering from mild, moderate, and heavy *Ascaris* infection when the Z-scores for the

various indices were compared to CDC/WHO 1978 growth references. Table 1.14 and 1.15 gives the same details for CDC 2000 and WHO 2007 growth references respectively.

The percentage of malnutrition (61.9% for WHO 1978, 49.7% for CDC 2000 and 49% for WHO 2007 standards), stunting (61.5% for WHO 1978, 59.3% for CDC 2000 and 60.2% for WHO 2007 standards), wasting (52.9% for WHO 1978, 57.4% for CDC 2000) and low BMI (49.6% for CDC 2000 and 52.5% for WHO 2007 standards) was higher in subjects who were heavily infected with *Ascaris* infection. The Z-score values of the various indices of all the infected (*Ascaris*) subjects yielded similar kind of results when compared with CDC/WHO 1978 as well as the CDC 2000 standards (table 1.16, 1.17, 1.18).

The pattern of malnutrition and stunting, according to Gomez and Waterlow's classification, in children with various degrees of *Ascaris* infection was very interesting. 100% of Grade III severely malnutritioned

Sex	*Ascaris* infection status	Age status	*N**	*Mean*†	Std. Deviation
F	No infection	5-15 years	37	00	00
	Mild infection	5-8 years	3	2666.66	1154.70
		8-10 years	8	3500.00	1119.94
		10-15 years	1	3000.00	
		Total	12	3250.00	1088.36
	Moderate infection	5-8 years	8	24125.00	10986.19
		8-10 years	20	19915.00	11244.28
		10-15 years	5	34200.00	9121.40
		Total	33	23100.00	11735.70
	Heavy infection	5-8 years	5	107600.00	19819.182
		8-10 years	24	84250.00	20939.35
		10-15 years	15	91133.33	31786.93
		Total	44	89250.00	25580.81
M	No infection	5-15 years	42	00	00
	Mild infection	5-8 years	2	4250.00	353.55
		8-10 years	4	4325.00	394.75
		10-15 years	3	4333.33	288.65
		Total	9	4311.00	310.01
	Moderate infection	5-8 years	10	26200.00	14412.95
		8-10 years	30	22050.00	12490.92
		10-15 years	14	20535.71	12674.11
		Total	54	22425.93	12796.81
	Heavy infection	5-8 years	18	88833.33	25277.86
		8-10 years	28	71946.43	23025.08
		10-15 years	14	92678.57	22320.74
		Total	60	81850.00	25014.96

∗ Number of children, † Mean of eggs per gram of feces,

Table 1.9: **Eggs per gram of feces in males and females of different age groups**

Ascaris infection status	sex		WAZ^{*}	$HAZ^{\star}$	$WHZ^{\dagger}$
No Infection	F	Mean	-0.87	-0.69	-0.28
		N	37	37	37
		SD	1.09	1.15	2.07
	M	Mean	-1.41	-1.14	-0.91
		N	42	42	42
		SD	0.86	1.16	1.28
	Total	Mean	-1.16	-0.93	-0.61
		N	79	79	79
		SD	1.00	1.17	1.71
Mild infection	F	Mean	-1.23	-0.66	-1.07
		N	12	12	12
		SD	0.66	1.02	0.89
	M	Mean	-1.48	-0.52	-1.74
		N	9	9	9
		SD	0.66	0.89	0.76
	Total	Mean	-1.34	-0.60	-1.36
		N	21	21	21
		SD	0.66	0.94	0.89
Moderate infection	F	Mean	-1.55	-1.12	-1.03
		N	33	33	33
		SD	0.54	0.74	1.02
	M	Mean	-1.74	-0.96	-1.55
		N	54	54	54
		SD	0.69	0.99	0.88
	Total	Mean	-1.67	-1.02	-1.35
		N	87	87	87
		SD	0.64	0.90	-0.97
Heavy infection	F	Mean	-2.10	-1.68	-0.96
		N	44	44	44
		SD	0.64	1.76	1.09
	M	Mean	-2.33	-1.93	-1.78
		N	60	60	60
		SD	0.68	1.17	0.83
	Total	Mean	-2.23	-1.83	-1.43
		N	104	104	104
		SD	0.67	1.44	1.03

$*$ Z score for weight for age, $\star$ Z score for height for age, † weight for height

Table 1.10: **Characteristics of mild, moderate, heavy *Ascaris* infected and non-infected children** (CDC/WHO 1978 growth standards)

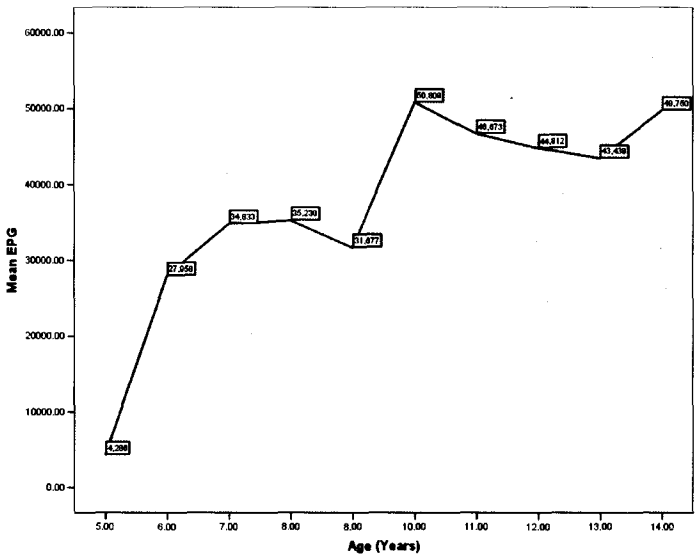


Figure 1.7: Graph showing mean eggs per gram of feces as per age

	No *Ascaris* infection	*Ascaris* infection	t-value	p-value
HAZ				
Mean	-0.93	-1.37	2.66	.00
N	79	212		
Std. dev.	1.17	1.28		
WAZ				
Mean	-1.16	-1.91	6.05	.00
N	79	212		
Std. dev.	1.009	0.73		
WHZ				
Mean	-0.618	-1.39	3.80	.00
N	79	212		
Std. dev.	1.71	0.99		

Table 1.11: **Mean HAZ, WAZ, WHZ values in *Ascaris* infected and non-infected subjects (CDC/WHO 1978 standards)**

	No *Ascaris* infection	*Ascaris* infection	t-value	p-value
HAZ				
Mean	-1.04	-1.48	2.66	.00
N	79	212		
Std. dev.	1.14	1.30		
WAZ				
Mean	-1.48	-2.46	5.93	.00
N	79	212		
Std. dev.	1.16	1.28		
WHZ				
Mean	-1.32	-2.21	2.63	.01
N	33	87		
Std. dev.	1.35	1.76		
BMIZ				
Mean	-1.33	-2.31	5.65	.00
N	79	212		
Std. dev.	1.28	1.33		

Table 1.12: **Mean HAZ, WAZ, WHZ, BMIZ values in *Ascaris* infected and non-infected subjects (CDC 2000 standards)**

	No *Ascaris* infection	*Ascaris* infection	t-value	p-value
HAZ				
Mean	-0.98	-1.39	2.40	0.01
N	79	212		
Std. dev.	1.15	1.33		
WAZ				
Mean	-1.18	-2.09	5.60	0.00
N	64	161		
Std. dev.	1.10	1.10		
BMIZ				
Mean	-1.10	-2.05	6.47	0.00
N	79	212		
Std. dev.	1.24	1.05		

Table 1.13: **Mean HAZ, WAZ, BMIZ values in *Ascaris* infected and non-infected subjects (WHO 2007 standards)**

Ascaris infection status	sex		WAZ^{*}	$HAZ^{\star}$	$WHZ^{\dagger}$	$BMIZ^{\flat}$
No Infection	F	Mean	-1.26	-0.85	-1.30	-1.16
		N	37	37	16	37
		SD	1.21	1.15	1.01	1.25
	M	Mean	-1.68	-1.20	-1.33	-1.49
		N	42	42	17	42
		SD	1.09	1.12	1.64	1.29
	Total	Mean	-1.48	-1.04	-1.32	-1.33
		N	79	79	33	79
		SD	1.16	1.14	1.35	1.28
Mild infection	F	Mean	-1.55	-0.78	-1.40	-1.74
		N	12	12	05	12
		SD	0.71	1.25	1.31	0.92
	M	Mean	-1.72	-0.60	-3.57	-2.41
		N	9	9	2	9
		SD	0.81	0.81	3.5	1.51
	Total	Mean	-1.62	-0.70	-2.02	-2.02
		N	21	21	07	21
		SD	0.74	1.06	2.07	1.22
Moderate infection	F	Mean	-1.9	-1.3	-1.2	-1.7
		N	33	33	15	33
		SD	0.89	0.87	1.32	1.08
	M	Mean	-2.04	-1.04	-1.87	-2.27
		N	54	54	17	54
		SD	0.96	0.94	1.16	1.32
	Total	Mean	-2.00	-1.16	-1.58	-2.05
		N	87	87	32	87
		SD	0.93	0.92	-1.25	1.26
Heavy infection	F	Mean	-2.73	-1.83	-1.86	-2.26
		N	44	44	16	44
		SD	1.33	1.74	1.90	1.30
	M	Mean	-3.24	-1.96	-3.07	-2.84
		N	60	60	32	60
		SD	1.38	1.24	1.90	1.36
	Total	Mean	-3.02	-1.91	-2.67	-2.59
		N	104	104	48	104
		SD	1.38	1.47	1.90	1.36

$*$ Z score for weight for age, $\star$ Z score for height for age, † Z score for weight for height, $\flat$ Z score for BMI

Table 1.14: **Characteristics of mild, moderate, heavy *Ascaris* infected and non-infected children** (CDC 2000 growth standards)

Ascaris infection status	sex		*WAZ**	*HAZ**	*BMIZ*†
No Infection	F	Mean	-0.93	-0.82	-0.91
		N	30	37	37
		SD	1.20	1.16	1.23
	M	Mean	-1.40	-1.13	-1.26
		N	34	42	42
		SD	0.97	1.13	1.24
	Total	Mean	-1.18	-0.98	-1.10
		N	64	79	79
		SD	1.10	1.15	1.24
Mild infection	F	Mean	-1.39	-0.64	-1.32
		N	11	12	12
		SD	0.69	1.18	0.86
	M	Mean	-1.45	-0.53	-2.05
		N	11	12	12
		SD	0.86	0.85	1.00
	Total	Mean	-1.41	-0.59	-2.05
		N	17	21	21
		SD	0.73	1.03	0.97
Moderate infection	F	Mean	-1.72	-1.26	-1.46
		N	28	33	33
		SD	0.74	0.88	0.89
	M	Mean	-1.87	-0.93	-2.08
		N	41	54	54
		SD	0.87	0.96	1.02
	Total	Mean	-1.18	-1.06	-1.85
		N	69	87	87
		SD	0.82	0.94	1.01
Heavy infection	F	Mean	-2.17	-1.06	-2.09
		N	29	44	44
		SD	1.31	1.78	1.06
	M	Mean	-2.72	-1.86	-2.45
		N	46	60	60
		SD	1.14	1.27	1.01
	Total	Mean	-2.51	-1.83	-2.30
		N	75	104	104
		SD	1.23	1.50	1.05

∗ Z score for weight for age, ⋆ Z score for height for age, † Z score for BMI

Table 1.15: **Characteristics of mild, moderate, heavy *Ascaris* infected and non-infected children** (WHO 2007 growth standards)

	Ascaris infection			
	No infection	Mild infection	Moderate infection	Heavy infection
Malnutrition*	12.7	0.8	24.6	61.9
Normal wt. for age	37.0	11.6	33.5	17.9
Stunting*	19.2	3.8	15.4	61.5
Normal height for age	30.0	8.5	35.2	26.3
Wasting*	8.6	7.1	31.4	52.9
Normal weight for height	33.0	7.2	29.4	30.3

* Z score less than -2 standard deviation from the mean reference values

Table 1.16: **Percentage of malnutrition, wasting and stunting in various grades of *Ascaris* infection** (CDC/WHO 1978 standards)

children had heavy *Ascaris* infection whereas 51.2% of the normal children had no *Ascaris* infection (Table 1.19). 86.7% of the severely stunted children had heavy *Ascaris* infection and 36.9% normal children (normal height for age percentage) had no *Ascaris* infection (table 1.20).

Running One-way ANOVA revealed that the mean *Ascaris* eggs per gram (epg) of faeces was significantly different (p value less than 0.05) in various grades of stunting (Waterlow's classification) i.e in severely

	Ascaris infection			
	No infection	Mild infection	Moderate infection	Heavy infection
Malnutrition*	19.0	4.9	26.4	49.7
Normal wt. for age	37.5	10.2	34.4	18
Stunting*	17.6	3.3	19.8	59.3
Normal height for age	31.5	9.0	34.5	25
Wasting*	24.1	3.7	14.8	57.4
Normal weight for height	30.3	7.6	36.4	25.8
Low BMI*	18.0	5.8	26.6	49.6
Normal BMI	35.5	8.6	32.9	23.0

* Z score less than -2 standard deviation from the mean reference values

Table 1.17: **Percentage of malnutrition, wasting, stunting and low BMI in various grades of *Ascaris* infection** (CDC 2000 standards)

	Ascaris infection			
	No infection	Mild infection	Moderate infection	Heavy infection
Malnutrition*	16.7	4.9	29.4	49.0
Normal wt. for age	38.2	9.8	31.7	20.3
Stunting*	18.2	3.4	18.2	60.2
Normal height for age	31.0	8.9	35.0	25.1
Low BMI*	13.9	5.7	27.9	52.5
Normal BMI	36.7	8.3	31.4	23.7

* Z score less than -2 standard deviation from the mean reference values

Table 1.18: **Percentage of malnutrition, stunting and low BMI in various grades of *Ascaris* infection** (WHO 2007 standards)

Gomez's classification	*Ascaris* infection			
	No infection	Mild infection	Moderate infection	Heavy infection
Grade III Severe Malnutrition				100
Grade II Moderate Malnutrition	16.3	4.7	32.6	46.5
Grade I Mild Malnutrition	33.3	9.2	36.8	20.7
Normal	51.2	12.2	22.0	14.6

Table 1.19: **Percentage of malnutrition (Gomez's classification) in various grades of *Ascaris* infection** (WHO 2007 standards)

Waterlow's classification	*Ascaris* infection			
	No infection	Mild infection	Moderate infection	Heavy infection
Severe Stunting	6.7		6.7	86.7
Moderate Stunting	19.6	3.9	15.7	60.8
Mild Stunting	22.3	5.8	36.9	35.0
Normal	36.9	10.7	32.8	19.7

Table 1.20: **Percentage of stunting (Waterlow's classification) in various grades of *Ascaris* infection** (WHO 2007 standards)

stunted (mean epg: 91200, std. dev: 35781), moderately stunted (mean epg: 55388, std. dev: 44226), mildly stunted (mean epg: 37400, std. dev: 38700) and normal (mean epg: 23312, std. dev: 30906) individuals.

Running One-way ANOVA for malnutrition grades (Gomez's classification) and epg revealed similar kind of result, except that the difference in the mean epg between normal and mild malnutrition was not significant where the p-value was above 0.05. For severe malnutrition

the mean epg was 100272 (std. dev: 20504) for moderate malnutrition, 45224 (std. dev:39056), for mild malnutrition 23189 (std. dev: 29037) and for normal individuals for weight for age the mean epg was 19129 (std. dev: 32883).

Correlation between epg of faeces and Z-score for weight for height (WAZ), Z-score for height for age (HAZ) and Z-score for BMI (BMIZ) was negative. For epg and WAZ the correlation coefficient was -0.43, for epg and HAZ -0.33 and epg versus BMIZ it was -0.35. Similar negative correlations were obtained with epg and percentage of weight for age and percentage of height for age. A positive correlation between epg and age with correlation coefficient of 0.163 was obtained. **(kindly see page 38)**.

Linear regression model was constructed to provide confounded estimates of the relationships of interest. The variables were chosen based on the results obtained from correlation between epg and WAZ, HAZ,

and age. *Ascaris* infection (in terms of epg) was taken as the dependent variable, age, sex, WAZ and HAZ were taken as the independent variables. The model was significant with F=13.54 and P 0.000. The regression model indicated WAZ (regression coefficient (β)= -0.491; SD=1.17, P= 0.00) was a significant predictor of infection (epg).

Chapter 2

IgE and gene profiles in Ascariasis and Asthmatics.

2.1 Introduction

Fighting the 'non-self' entities is one of the fundamental mechanisms to prevent 'homing' of the various pathogens in a host. All such mechanisms which an organism has either developed or acquired for the defence against the disease causing agents are part of his immune system. Derived from the Latin word 'immunis' which means 'exempt from charges such as taxes and expenses' the word immunity is used

in general parlance for exemption from any natural liability or something which is a special privilege. Immune system is indeed a privilege for any organism against the various deadly, misery causing, sickening pathogens. Although the origin of the field of immunology can be traced back to the days of Edward Jenner (1798), an English country physician who experimented with cowpox and small pox infections, the real progress in immunology started probably with the work of the great Louis Pasteur (1822-1895) who, for the first time, showed that inoculation of chicken with avirulent chicken cholera bacteria (*Pasteurella aviseptica*) failed to develop illness after re-inoculation with fresh virulent stock. Infact, Pasteur developed three kinds of vaccines-living, heat-killed and attenuated.

Modern immunology has come a long way from these earlier works done by the pioneers in the field. The original concepts of 'Cellular Immunity' given by the Russian zoologist Elie Metchnikoff (1845-1916), and 'Humoral Immunity' first suggested by the works of Fodor

(1886) and Von Behring and Kitasato (1890) have undergone refinement over the years. According to the prevailing established nomenclature, immunity is classified into two types-Innate (Natural) and Adaptive (Acquired). Innate immunity works as the 'first-line of defence'. Included in this line of defence are the constitutional factors, skin-the physical barrier, normal bodily secretions-sweat, saliva, tears, gastric secretions etc. The cellular components of innate immunity include the polymorphonuclear cells, natural killer cells, mast cells, dendritic cells, eosinophils etc (**Lydyard PM et al.**, 2003). Complement system is also a part of innate immunity. Adaptive immune system is the 'second line of defence'. Apart from being non-specific in nature, innate-immunity is present right from birth, whereas acquired immunity is highly specific in its response and is acquired through out the life of an individual.

Adaptive immunological response is either cellular (cell-mediated)

or humoral. Cell-mediated immunity involves the mediation of T-cells whereas humoral immunity involves B-cells which produce immunoglobulins.

2.1.1 The Biology of Immunoglobulins

Originally termed as gamma-globulins after Tiselius electrophoretically separated the serum proteins and later along with Kabat in 1939 found that it possessed the antibody properties (**Kanan I**, 2007), **Immunoglobulins (Igs)** are glycoproteins of animal origin. Infact, immunoglobulins are unique to vertebrates, having combining sites for antigens and are produced by B-lymphocytes mainly in response to antigenic stimulation (**Chakravarty AK**, 1996) though some may also act as receptors **on B cells or mast cells**[1] They are gamma globulin type of serum proteins consisting of basic four chain polypeptide chains

[1]Mast cells are formed in the bone marrow and are present in all vascularised peripheral tissues, connective tissues, mucous membrane surfaces of respiratory and gastrointestinal tracts, skin etc. Human mast cells are divided into two major subtypes- MC_T or MC_{TC} on the basis of the presence of tryptase, chymase or both. Mast cells have numerous membrane bound granules distributed throughout the cytoplasm (Prussin and Metcalfe, 2006).

connected by disulphide bonds. Although similar in structural organization Immunoglobulins belong to highly heterogenous family that can be arranged into various classes, subclasses and types on the basis of antigenic properties and amino acid sequences. Thus, immunoglobulins serve two main functions. First, Antigen binding - Immunoglobulins bind specifically to one or a few closely related antigens that is to say they act as receptors. Secondly, immunoglobulins have an effector function wherein they act as antibodies and bring about such effects as complement fixation and binding to the various cell types like lymphocytes, platelets, mast cells basophils etc.

In the beginning Immunoglobulins were divided into two classes (1937) based on the molecular weight, 900,000 Dalton immunoglobulin macro (IgM) and a lower molecular weight 150,000 Dalton immunoglobulin gamma (IgG); later, one more immunoglobulin was added after the introduction of immunoelectrophoretic technique, the immunoglobulin

alpha (IgA). Subsequently in the late 1960s two minor immunoglobulins IgD and IgE were identified as myeloma proteins from patients suffering from multiple myeloma. Thus, in humans five major classes of immunoglobulins are distinguished- Immunoglobulin G (IgG), IgM, IgA, IgD and IgE. Apart from being glycoprotein in nature, having unique antigen specificity, produced by B-cells and gamma globulin type of protein, all immunoglobulin molecules consist of a basic unit of four polypeptide chains connected by disulphide bonds (**Chakravarty AK**, 2006).

Immunoglobulin or antibody molecules have a common structure of four peptide chains. The structure consists of two identical **light (L) chains,** polypeptides of about 25,000 molecular weight, and two identical **heavy (H) chains**, larger polypeptides of molecular weight 50,000 or more. Each light chain is bound to the heavy chain with a disulfide bond and by noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds to form a heterodimer (H-L). Similar

noncovalent interactions and disulfide bridges exist between the two heavy and light chain combinations to form the basic four-chain $(H\text{-}L)_2$ antibody structure, a dimer of dimers. All the differences in the specificity displayed by the various antibodies can be traced to the first 110 or so amino acids of the amino terminal region of a light or heavy chain which varies greatly among them. These segments of highly variable sequence are called the "V" regions: V_L in light chain and V_H in heavy chain. The most of the differences in specificity fall under the area of the 'V' regions termed as 'Complementary-determining regions (CDRs). These CDRs on both the light and heavy chains constitute the antigen binding sites on an antibody molecule. The rest of the immunoglobulin molecule show relatively constant structural feature. The regions of relatively constant sequence beyond the variable regions are called C regions, C_L on the light chain and C_H on the heavy chain. The attachment of the carbohydrate moiety is restricted to the constant region (C_{H2}). The C_L region extends to about 110 amino acids

whereas, the C_H region extends to about 330-440 amino acids. Three dimensional images have revealed that the immunoglobulin molecule is not straight but has globular domains. Both the chains, heavy as well as light, contain several homologous units of about 110 amino acid residues. Within each unit, termed domain an intra-chain, disulfide bond forms a loop of about 60 amino acids. The constant region has 3 to 4 domains (C_L, C_{H1}, C_{H2}, C_{H3}, C_{H4}), whereas the variable region has only one domain (V_L, V_H) (figure 2.1). Besides all this electron microscopy has indicated that there is a flexible 'hinge region' at about the middle of the H chain where the two chains are connected by disulfide linkage. Studies have indicated that hinge region is rich in amino acid proline.

Immunoglobulin fragments produced by proteolytic digestion have helped to elucidate the structure and function of the immunoglobulin molecule in more detail (**Goldsby RA et al.**, 2003).

Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain disulfide bond. This results in the formation of two identical fragments that contain the light chain and the V_H and C_{H1} domains of the heavy chain. These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody. The combining site of the antibody is created by both V_H and V_L. An antibody is able to bind a particular antigenic determinant because it has a particular combination of V_H and V_L. Different combinations of V_H and V_L result in antibodies that can bind a different antigenic determinants. Figure 2.2 gives the general structure of an immunoglobulin molecule.

Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a C_{H2} and C_{H3} domain. This fragment was called Fc because it was easily crystallized.

Treatment of immunoglobulins with pepsin results in cleavage of the

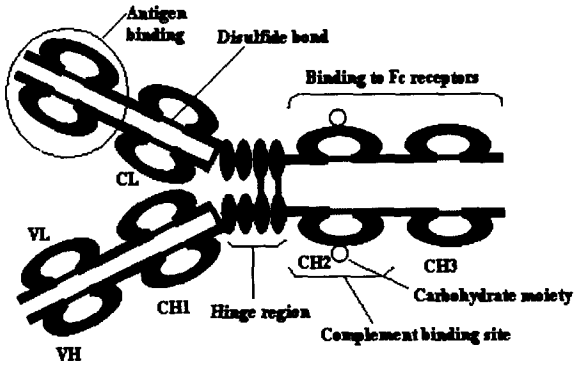


Figure 2.1: General structure of Immunoglobulin molecule

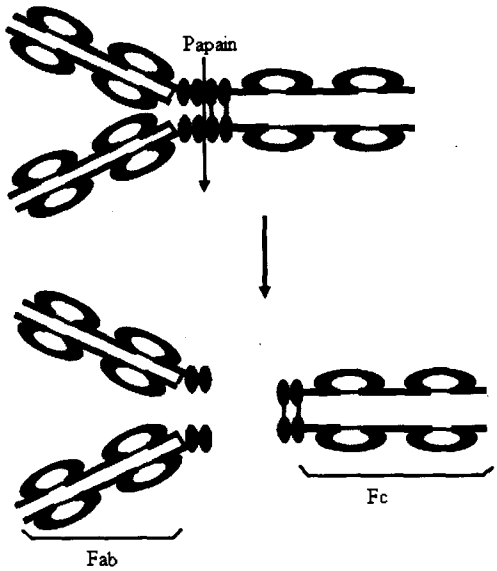


Figure 2.2: Action of enzyme papain on Immunoglobulin molecule

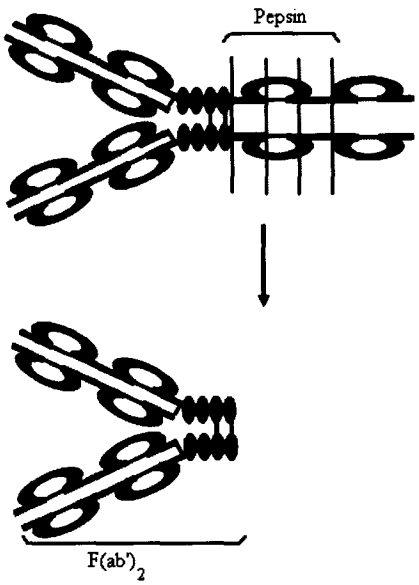


Figure 2.3: Action of pepsin on Immunoglobulin molecule

heavy chain behind the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites. This fragment is called F(ab')2 because it is divalent. The Fc region of the molecule is digested into small peptides by pepsin. Flexibility at the hinge region allows the Fab domains to swing and thus the shape of the molecule can change from Y to T (Figure 2.3).

The five different classes of immunoglobulins are identified based

on the antigenic differences between the C regions of the heavy (H) chains. Antigenically different H chains are designated as γ, μ, α, δ and ϵ corrosponding to the five immunoglobulin classes IgG, IgM, IgA, IgD and IgE respectively. Some of these classes are divided into subclasses like IgG1, IgG2, IgG3, IgG4 characterized by γ_1, γ_2, γ_3, γ_4 respectively. Subclasses also exist for IgM and IgA classes, IgM1, IgM2 bearing μ_1 and μ_2 and IgA1, IgA2 bearing α_1 and α_2. The light chains on the other hand are of two types either Kappa (κ) or lambda (λ) type in an immunoglobulin molecule but never both.

2.1.2 Immunoglobulin IgE

One of the 5 immunoglobulins of the body, IgE, is produced from the B cells and plasma cells like the other immunoglobulins. In comparison to all other immunoglobulins serum IgE concentration is lowest. Only a few plasma cells produce this immunoglobulin. Another reason for the low circulating IgE is the high affinity of mast cells for IgE via

their ϵ-heavy chain Fc receptors (FcϵR). IgE levels at birth is about 0.22 IU/ml. The adult level is reached by about 17 years. The IgE levels decline after the age of 70. Raised IgE levels have been reported in Wiskott - Aldrich syndrome, alcoholism, HIV and severe burns cases etc. Healthy, non allergic adults have an expected IgE concentration of up to 120 IU/ml^3 (**Chowdary V.S**, 2003). It has the shortest serum half life in comparison to the other Igs and is unable to activate either the classical or alternate complement pathway. Figure 2.4 gives the general structure of immunoglobulin E with its four constant region heavy chain domains. It is well established that IgE plays an important role in the interaction between humoral and cellular immunity in allergic hypersensitivity reactions in allergy (**Shakib F**, 1990).

Identification of IgE was accomplished by K. and T.Ishizaka in 1966 (**Infuhr D et al.**, 2005). However, the presence of serum component responsible for allergic reactions was first demonstrated in 1921 by K. Preusnitz and H. Kustner, who injected serum from an allergic

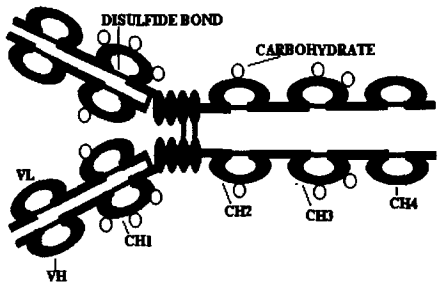


Figure 2.4: General structure of Immunoglobulin E molecule

person to a non-allergic individual intra-dermally. Injection of an appropriate antigen in such an individual at the same site caused wheal and flare reaction which was called as P-K reaction (named after its originators) (**Goldsby RA et al.**, 2003). Due to its relatively low levels in the human serum, physio-chemical studies on IgE were difficult, till in 1967 S.G.O Johansson and H.Bennich discovered an IgE myeloma, after which extensive chemical analysis of IgE could be undertaken. IgE is composed of two heavy ϵ and two light chains (κ or λ) with a combined molecular weight of 190,000. There is an additional constant region (CH4) in IgE molecule (also in IgM), the binding site

of IgE for the high-affinity receptor FcεRI is located in this domain (**Hamelmann et al.**, 2002). IgE can be detected in two forms, a secreted and membrane-bound form. Membrane bound IgE works like a classical antigen receptor on B lymphocytes (**Infuhr D et al.**, 2005).

In contrast to other immunoglobulins which bind to immunoglobulin Fc receptors after they bind antigen, IgE can bind to FcεR in the absence of any antibody. Binding of IgE to the Fc receptors on mast cells sensitizes the mast cells and makes them to degranulate when a multivalent antigen cross-links the FcεR-bound IgE (**Winter WE**, 2000). Figure 2.5 gives the allergic immune response mediated by IgE.

Elevated IgE levels are seen in allergic diseases and are also induced by parasites like helminths (**Cooper PJ**, 2004; **Vercelli**, 2005). IgE is species specific and cross reacts with related species but more importantly they can cause anaphylactic shock (**Yman L**, 2000). The most potent IgE responses in nature however are seen in helminth infection.

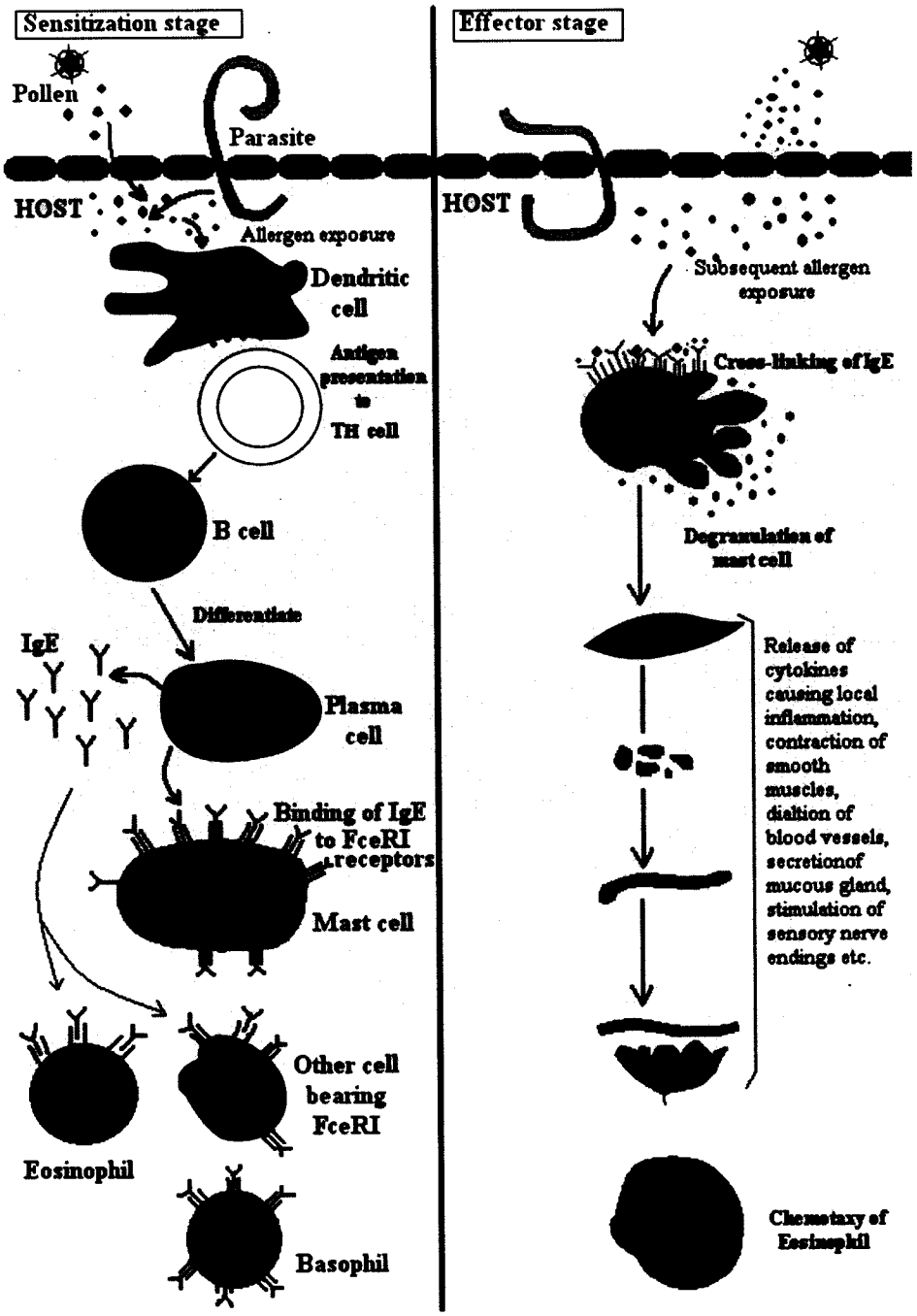


Figure 2.5: IgE mediated allergic immune response

Helminths are known to induce polyclonal stimulation of IgE (**Lynch NR et al.**, 1998). In helminth infection IgE production is brought about by the cytokines (IL-4, IL-5, IL-10) secreted by T_{H2} subset of T-helper cells which ultimately enhances worm survival, whereas the cytokines (interferon-γ, tumor necrosis factor-α, IL-2) activate pathways that are deleterious to the worms (**Lee TDG and Xie CY**, 1994).

That the IgE molecule has a bent shape, was originally suggested by Zheng Y and co-workers based on the fluorescence energy transfer experiments (**Zheng Y etal.**, 1991).

2.1.3 IgE receptors

There are two main receptors for IgE. The low-affinity IgE receptor (**FcϵRII;CD23**) present on B cells and the high-affinity IgE receptor (**FcϵRI**) on mast cells, basophils and antigen presenting cells. The high affinity FcϵRI on mast cells and basophils is a tetramer containing an

α chain, a β chain and two γ chains. The α subunit contains the IgE-binding site, the β chain and two disulfide-linked γ chains ($\alpha\beta\gamma_2$)which exert mainly cytoplasmic functions, helps in cell signaling. The same receptor is expressed in a trimeric form ($\alpha\gamma_2$) on antigen-presenting cells (APCs) (**Prussin and Metcalfe**, 2006; **Taylor et al.**, 1995). The β chain spans the membrane four times and is thought to link the α chain to the γ dimer (Figure 2.6). The γ chains extend considerable distance inside the cytoplasm and contain a conserved sequence in their cytosolic domain known as immunoreceptor tyrosine-based activation motif (ITAM). β chain is known to enhances the expression of FcϵRI on the cell surface by association with α chain. It also plays a key role in the amplification of intracellular signaling from ITAM (**Akizawa Y et al.**, 2003).

The high-affinity IgE receptor is abundant (about 200,000 molecules per cell) in the membrane of mast cell and basophils. They are also exhibited at much lower levels in Langerhans cell, monocyte, platelet,

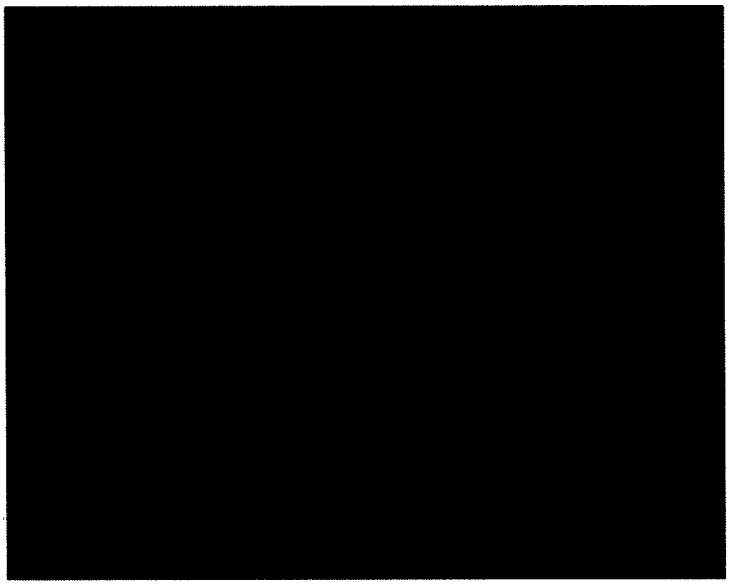


Figure 2.6: Schematic diagram of the high affinity FcεRI receptor

and eosinophil membranes. The IgE-FcεRI complex has a very high association constant (K_a) of 10^{10} M^{-1} and has a half life in serum of 3 days (**Gould HJ et al.**, 2003). Some studies have found a positive correlation between density of FcεRI on basophils and IgE levels in blood (**Macglashan DW et al.**, 1997; **Saini SS et al.**, 2000).

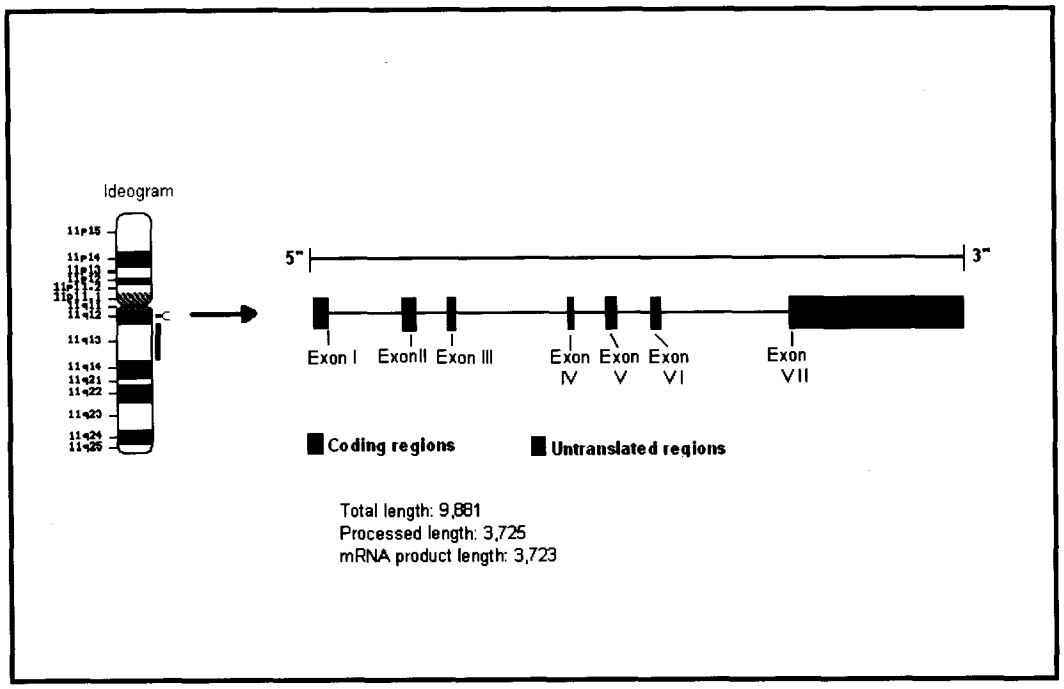


Figure 2.7: Human FcϵRIβ gene

2.1.4 FcεRIβ gene

Present on the chromosome 11, this gene is mapped to the 11q13 region. The gene is 9881 base-pairs long with a processed length of 3725 base pairs and m-RNA product length of 3723 (NCBI website). The gene encodes for the β subunit of the high affinity IgE receptor (FcεRI). This gene contains seven (7) exons and there is a single transcription initiation site which is preceded by TATA box. Out of the seven exons, the first exon codes for the 5'-untranslated region and a portion of the N-terminal cytoplasmic tail of the β chain (Refer figure 2.6 for the structure of the receptor); the transmembrane 1 portion of the chain is encoded by exon 2 and 3, transmembrane 2 portion is encoded by exon 3 and 4, transmembrane 3 by exon 5 and transmembrane 4 by exon 6. **The seventh exon encodes the C-terminal cytoplasmic tail of the polypeptide and the 3'-untranslated sequence.** Figure 2.7 shows the structure of the FcεRIβ gene in humans. The human β protein shows 69% identical amino-acid residues with rodents mouse and

rat (**Kuster H et al.**, 1992). The full name of this gene as described on NCBI website is:

Official symbol: MS4A2.

Official full name: membrane-spanning 4-domains, subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide.

The gene is also known as APY; IGEL; IGER; ATOPY; FCERI; IGHER; MS4A1; FCER1B.

2.1.5 Asthma

Asthma is a complex syndrome characterised by inflammation of the respiratory passage with intermittent, reversible airway obstruction and bronchial hyperresponsiveness (BHR) (**Busse WW and Lemanske RF**, 2001; **Wiesch DG et al.**, 1999). Genetic as well as environmental factors contribute to its inception and evolution. Even low birth weight has been suggested as a potential reason for its cause (**Steinke JW et al.**, 2003). Earliest clinical description of asthma was seen probably in

Chinese textbook of internal medicine around 2600 BC (**Walter MJ and Holtzman MJ**, 2005). Association of asthma with allergies has long been recognised (**Sanford AJ and Par PD**, 2000). Epidemiological studies have linked the severity of Asthma to IgE (**Burrows B et al.**, 1989). The characteristics of asthma can be initiated by even small amounts of allergen. The early response to the allergen are mediated by histamine, leukotrienes and prostaglandin D_2 (PGD_2). These mediators cause bronchoconstriction, vasodilation, and secretion of mucus. The late phase response which occurs after few hours is mediated by IL-4, IL-5, IL-6, tumor necrosis factor (TNF), eosinophil chemotactic factor (ECF) and platelet activating factor (PAF). These mediators cause the recruitment of inflammatory cells like eosinophils, T_{H2} cells, neutrophils etc in the bronchial tissue (**Chakravarty AK**, 2006). Such is the importance of asthma research that about 6000 articles are published every year concerning asthma (**Tattersfield AE et al.**, 2002).

2.2 Literature review

Walter MJ and Holtzman MJ (2005) has described the discovery and characterisation of IgE as a seminal event in the definition of allergy. Link of IgE with allergic diseases like asthma has been reported and acknowledged by many (**Hill MR and Cookson WOCM**, 1996; **Lynch NR et al.**, 1998; **Yahamoto R**, 2000; **Hopkin JM**, 2002; **Qiao HL et al.**, 2004; **Infuhr D et al.**, 2005). **Buckley RH and Fiscus SA in 1971** measured the concentration of IgE along with IgA in the sera of patients suffering with immunodeficiency and found that IgE levels were higher in patients with selective IgA deficiency. They also reported elevated IgE levels in atopic and in those suffering with Wiskott-Aldrich syndrome (**Buckley RB and Fiscus SA**, 1975). Qiao HL et al in 2004 in their work on patients with penicillin allergy reported that such patients have positive specific IgE not only to major antigenic determinants but also to minor antigenic determinants. Potacezek DP et al. in 2006 have reported that allergic subjects

having asthma or urticaria had serum IgE levels significantly higher than normal ones (**Potaczek DP et al.**, 2006).

The parallels between the inflammation caused by environmental allergen and parasite antigens have not only made the epidemiologists interested in finding out the relationship between helminth infection and allergy but also have intrigued immunologists. Since IgE is one of the common features of allergy as well as helminth infection, many studies have been undertaken to see the IgE levels in intestinal parasitic infection and allergy (**Lynch NR et al.**, 1998; **Scrivener S et al.**, 2001; **Selassie FG et al.**, 2000; **King EM et al.**, 2005). Lee TDG et al. have shown in their study with mice model that the body fluids of nematodes (*Ascaris*) contain B-cell mitogen which causes polyclonal stimulation of B-cell and the other nematode factors via IL-4 cause class switch to IgE thereby elevating their levels in blood (**Lee TDG and Xie CY**, 1994). Lynch NR et al. in their study to evaluate the influence of atopy on the anti-parasite response measured the total

IgE levels and specific anti-*Ascaris* IgE levels in endemically helminth infected and allergic subjects and found that the intensity of parasitic infection was considerably higher in the non-atopic children than the atopic ones. Chowdary VS et al. from their study in India found that the average IgE levels of 180 IU/ml in the normal non-allergic subjects were higher than their western counterparts with around 120 IU/ml (**Witting HJ et al.**, 1980). They also reported that IgE levels were substantially elevated in 90% of the patients with allergic rhinitis (**Chowdary V.S**, 2003).

Studies involving IgE levels among helminth infected and allergic subjects are difficult to interpret (**Cooper PJ**, 2004). Many studies have shown a dissociation between allergen-specific IgE and allergen skin test results in individuals who are helminth infected (**Van den Bigelaar AHJ et al.**, 2000; **Cooper PJ et al.**, 2002). Also, some studies have revealed a high concentration of allergen-specific IgE in the absence of skin reactivity among helminth infected subjects

(**Perzanowski MS et al.**, 2002).

Lynch NR et al. (1999) have mentioned that the most potent IgE responses in nature are seen due to the allergens of helminth parasites and since parasitic infections are endemic in most of the population, the relationship between helminth infection and IgE response is highly relevant in understanding allergic diseases (**Lynch NR et al.**, 1999). Lynch et al. have gone further to add that though most of the research in genetics of asthma is concentrated on IgE responses, the chances of finding DNA sequence variations affecting specific IgE responses are much more greater in parasitized population than in allergic/asthmatic population as most intense IgE responses in nature is seen in parasitized condition (**Lynch NR et al.**, 1998). Such views have been expressed by others also (**Lesouef PN et al.**, 1999). LeSouef PN et al. have said that "Screening the genome for variations in genes related to IgE production would be worth undertaking in parasitized populations, as such investigations should allow the identification of genes that would

not be identified in other populations". Thus, it seems more advantageous to study IgE responses in parastized population than in allergic population as parasitic infections (like helminths) are definitely more older than allergies (**Lynch NR et al.**, 1999).

Bazaral M et al. (1971) were perhaps the first to conduct study to find the genetic inheritance of IgE. They postulated that serum IgE levels are controlled by two alleles at a single locus. These findings were further confirmed by subsequent workers (**Gerrard JW**, 1978). Search for genetic variants throughout the genome modulating IgE responses is based on candidate gene approaches, linkage studies and positional cloning (**Vercelli**, 2005).

Several loci on different chromosomes have been linked to the IgE responses, like chromosome 1q 31-32 (**Hobbs K et al.**, 1998), 5q (**Marsh DG et al.**, 1994; **Xu J et al.**, 2000), 11q13 (**Shirikawa T et al.**, 1994; **Cookson WO et al.**, 1989; **Hizawa N et al**, 2001), 14q (**Mansur**

AH, 1999).

Gene FcεRIβ has been suggested to be a candidate for the linkage of chromosome 11q13 and serum IgE levels (**Sandford AJ et al.**, 1993). Some workers have reported that mutations in the FcεRIβ gene influence the underlying asthma, even in the absence of atopy (**van Herwerden L et al.**, 1995). **Trabetti E et al.** (1998) have reported this gene to be associated with bronchial hyper-responsiveness (BHR) and not with allergic asthma. Polymorphism in exon 7 of the same gene has also been reported to be associated with bronchial hyper-reactivity in 5% of Australian population by **Hill MR and Cookson WOCM** (1996). **Shirakawa T et al.** (1996) have found polymorphism in exon 7 with atopic asthma in Japenese population. **Green et al.** (1998) have shown significant difference in the prevalence of coding polymorphism in exon 7 (E237G) in blacks than in whites (blacks tend to have more severe asthma than whites). However, **Dickson et al.** (1999) reported that linkage of asthma and BHR to 11q13 region cannot be explained

by mutations in in the FcϵRIβ gene (**Dickson et al.**, 1999). Further, FcϵRIβ gene has been studied in parasitized subjects (**Palmer LJ et al.**, 1997), in allergic/atopic subjects (**Hage-Hamsten MV et al.**, 2002; **Hill MR and Cookson WOCM**, 1996), and even in people suffering from penicillins allergy (**Qiao HL et al.**, 2004), this gene has not been studied for polymorphism in parasitized as well as asthmatic subjects of the same population. Hence, there is scope for a such a study involving FcϵRIβ gene.

2.3 Materials and Methods

Blood samples of two hundred and seventeen (217) children, hundred (100) girls and hundred and seventeen (117) boys were analysed for IgE levels. Out of the two hundred and seventeen children, thirty seven (37) were asthmatic (Twenty girls (20) and seventeen boys (17)). All the thirty seven asthmatic children had no *Ascaris* infection and were diagnosed as asthmatics by local doctors. Forty one children (41) had

no *Ascaris* infection with no asthma either (seventeen (17) girls and twenty four (24) boys). Remaining 139 children, sixty three (63) girls and seventy six (76) boys had one or the other intensity levels of *Ascaris* infection.

The blood was analysed for total Eosinophils as described in chapter 1. About 1 ml of the whole blood from each subject was made to clot to recover serum. Serum was used for quantifying the IgE level as well as total protein.

IgE level in the blood serum was estimated by Enzyme-Immunoassay (EIA) method employing the kit provided by Omega diagnostics (Pathozyme Immunoglobulin E (Ref OD417)), UK, using 12 x 8 micro titration plate. The kit included standard ready to use set containing 0, 10, 50, 100, 400 and 800 IU/ml IgE Liquid, Anti-IgE HRP Conjugate, Zero Buffer, Substrate Solution (TMB), Stop Solution. Data Sheet was prepared to identify the individual wells for each sample and the standards

and all the additions were made as prescribed in the kit. Optical density of the wells was measured using microplate reader with a 450nm filter.

DNA extraction from the frozen blood samples was performed with the help of DNA extraction kit supplied by Bangalore Genei (KT23A). Detection of polymorphism in the UTR of exon 7 was performed by using following primers (**Palmer LJ et al.**, 1997):

a) 5'- TCA CTG TGT ATC ATG CTA AGC-3'

b) 5'- TGA TAC AAT ACT GCA TCG TGG-3'

The primers were custom synthesised from Bangalore Genei, Bangalore.

PCR amplifications of DNA samples were performed on Hybaid thermal cycler. The additions for PCR amplification were made as under:

Genomic DNA Template 15.0 μl, Forward Primer (100 ng/μl) 2.0 μl, Reverse Primer (100 ng/μl) 2.0 μl, dNTPs (10mM) 2.0 μl, Taq DNA assay buffer (Genei) 5.0 μl, Taq DNA Polymerase (Genei) 1.0 μl,

Distilled water 23.0 μl. Total reaction volume was 50.0 μl. Following PCR conditions were followed:

94^0	94^0	53^0	72^0	72^0
5 mins	30 sec	30 sec	45 sec	15 min
	35 cycles			

Expected size fragments (500 bp) were obtained. PCR products were restricted with RsaI endonuclease and ran in 2.0% agarose gel. Restriction Dilutions was done in 20μl volume with template 8μl, RsaI buffer 2μl, RsaI enzyme 0.5μl and double distilled water 9.5μl.

2.4 Results

Gender ratio was balanced; 117 males (53.9%) and 100 females were studied. The mean age of the subjects was 9.1 years (SD 1.91). thirty seven subjects (17.1% of the total subjects) had physician-diagnosed

asthma, one hundred and thirty nine subjects (64% of the total subjects) had *Ascaris* infection and forty one subjects (18.9% of the total subjects) had neither asthma nor *Ascaris* infection.

Total serum IgE titers exhibited a skewed distribution with a long right-hand tail and were log_e transformed prior to analysis. Figure 2.8 gives the distribution of Ln total serum IgE levels in the study population. Similarly, the absolute eosinophil count was also log_e transformed as it also exhibited skewed distribution. Figure 2.9 shows the distribution of Ln eosinophil levels in the study population.

The mean IgE levels (unadjusted) in the study population was 589.79 IU/ml of serum (SD 281.78). The mean eosinophil count was 590.34 per cu mm of blood (SD 367.65), hemoglobin 13.78 gms% (SD 2.65), serum protein 7.27 gms% (SD 0.57) and the mean serum albumin was 4.52 gms% (SD 0.80).

Variable	Males	Females	t-value	p-value
$n^{\dagger}$	117	100		
Age (years)				
Mean	9.09	9.17	0.28	0.778
SD^{*}	1.76	2.09		
Total serum IgE (IU)				
Mean	598.44	579.67	0.48	0.626
SD^{*}	252.34	313.75		
Absolute Eosinophil count				
Mean	584.64	597.01	0.27	0.806
SD^{*}	357.82	380.53		
Hemoglobin (grams%)				
Mean	13.92	13.62	0.81	0.418
$S.D^{*}$	2.78	2.49		
Total serum protein (grams%)				
Mean	7.22	7.32	1.19	0.232
$S.D^{*}$	0.57	0.57		
serum Albumin (grams%)				
Mean	4.51	4.53	0.12	0.899
$S.D^{*}$	0.84	0.76		

$*$ standard deviation; † number of children.

Table 2.1: **Characteristics of children by sex for mean IgE, eosinophils, hemoglobin, serum protein and serum albumin.**

The characteristics of the subjects by sex is presented in table 2.1. Independent-samples T test revealed no significant difference in mean serum IgE (unadjusted) levels in males (mean 598.44, SD 252.34) and females (mean 579.67, SD 313.75) with P 0.626. The mean eosinophil count in males (mean 584.64 per cu mm of blood, SD 357.82) and females (mean 591.56 per cu mm of blood, SD 385.08) was also not significantly different (P 0.891). The hemoglobin level in males (mean 13.92 gms%, SD 2.78) and females (mean 13.62 gms%, SD 2.49), the serum protein level in males (mean 7.22 gms%, SD 0.57) and females (mean 7.32 gms%, SD 0.57) and also the serum albumin in males (mean 4.51 gms%, SD 0.84) and females (mean 4.53 gms%, SD 0.76) was not significantly different with $P>$ 0.05.

ANOVA was performed to find out difference in the mean IgE levels and mean eosinophils among *Ascaris* infected, asthmatic and normal (Non-*Ascais* infected, non-asthmatic subjects) the F value of the model was 8.99 and P 0.000. The mean (unadjusted) serum IgE levels for

	Ascaris infection			
	No infection	Mild infection	Moderate infection	Heavy infection
Total serum IgE				
Mean	486.83	531.53	587.50	682.56
SD*	198.14	168.64	255.31	254.12

* Standard deviation

Table 2.2: **Mean IgE levels in various grades of *Ascaris* infection**

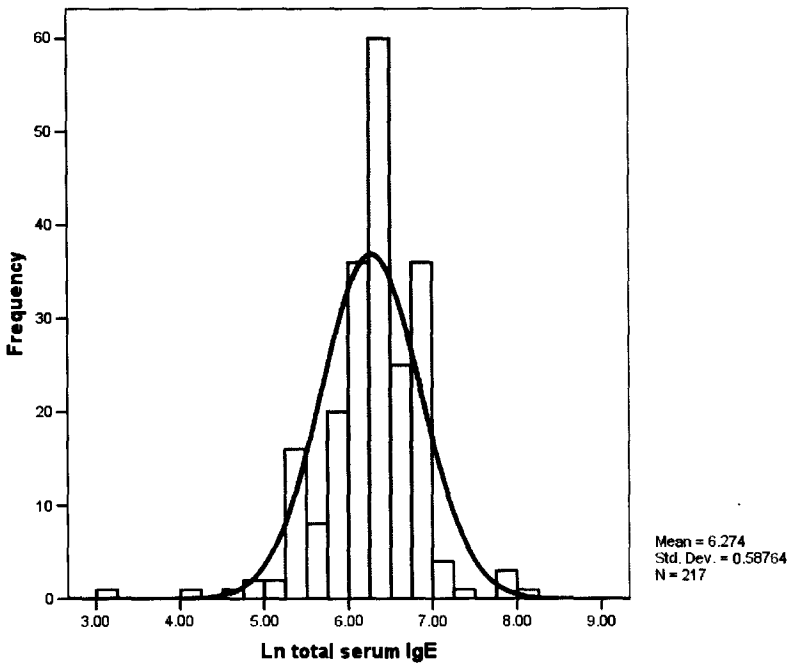


Figure 2.8: Distribution of Ln total serum IgE levels in the study population

	Ascaris infection			
	No infection	Mild infection	Moderate infection	Heavy infection
Absolute Eosinophil count				
Mean	511.68	615.83	629.32	768.48
SD*	256.13	339.89	323.68	1042.89

* Standard deviation

Table 2.3: **Mean eosinophil count in various grades of *Ascaris* infection**

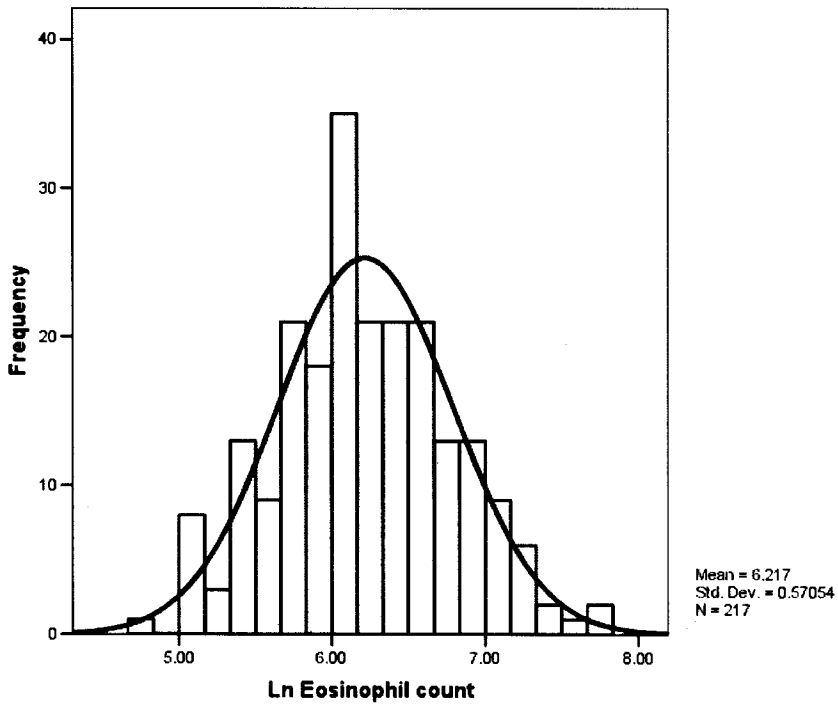


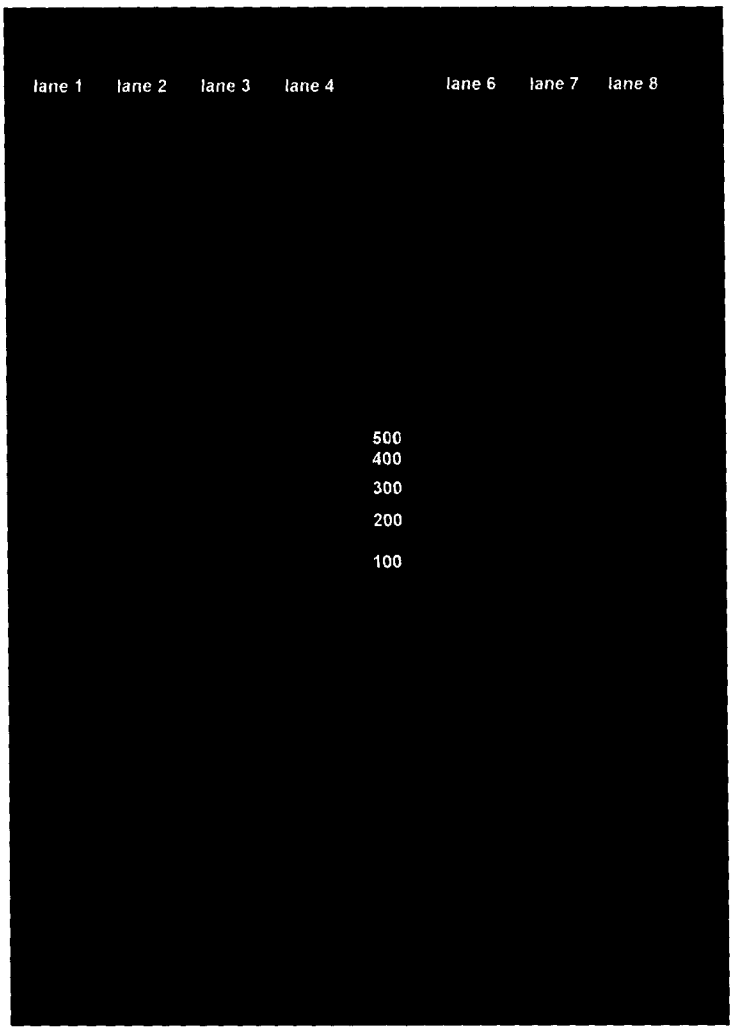
Figure 2.9: Distribution of Ln eosinophil levels in the study population

asthmatic subjects was 512.29, for *Ascaris* infected subjects, 647.44 and for the normal subjects was 464.27. The mean IgE levels were significantly different in *Ascaris* infected and Asthmatic subjects and also between *Ascaris* infected and non-infected subjects with P values 0.023 and 0.001 respectively. The mean IgE levels in normal subjects though lowest, was not significantly different from those of asthmatics (P 1.00).

ANOVA model for ascertaining the difference in mean $\log_e$ eosinophils among the three groups had a F value of 4.490 and P value 0.012. The mean eosinophil was 6.15 in asthmatics, 6.29 in *Ascaris* infected and 6.00 in non-infected children. The mean difference was not statistically significant at 95% confidence level in asthmatics and non-infected and also between asthmatics and *Ascaris* infected subjects with P values greater than 0.05. Significant difference in the mean eosinophil was found between *Ascaris* infected and non-infected individuals with P 0.013.

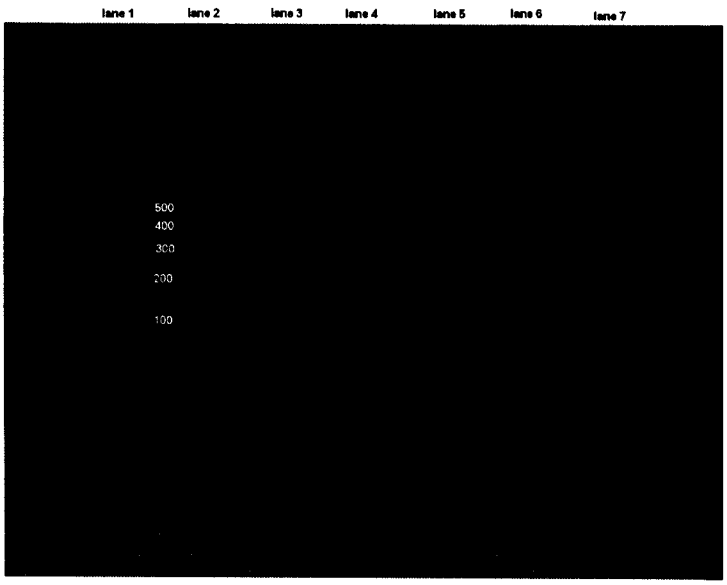
2.4.1 FcεRI-β_RsaI_ex7 polymorphism

PCR amplification with the above mentioned primers (page 100) yielded 500 base pairs of DNA segments. Figure 2.10 shows gel electrophoretic profile of the 500 base pair amplified gene segments. The amplified segments were digested with restriction enzyme RsaI and the digested products were then separated on 2.0% agarose gel. Figure 2.11 shows the electrophoretic profiles of RsaI digested products. Three sets of bands were obtained from digestion of PCR products: two 500-base pairs bands (lane 5, 6, 7 in figure 2.11), a 500, a 300 and a 200 base pairs band (lane 3 and 4 in figure 2.11) or a 300 and 200 base pairs band (lane 1 figure 2.11). The bands were genotyped as AA (two 500 base pairs bands), AB (a 500, a 300 and a 200 base pairs band) and BB (a 300 and 200 base pairs band) as mentioned previously by others (**Palmer LJ et al.**, 1997; **Hage-Hamsten MV et al.**, 2002).



Figures adjacent to lane 4 represents base pair sizes of the DNA ladder. Lane 1, 2, 3, 6 and 8 amplified DNA segments, lane 4 100 base pairs DNA ladder.

Figure 2.10: **PCR amplification of FcεRI-β gene**



Figures adjacent to lane 2 represents base pair sizes of the DNA ladder. Lane 1, 3, 4, 5, 6 and 7 RsaI digested DNA segments, lane 2 100 base pairs DNA ladder. lane 1 (BB), lane 3 (AB), lane 4 (AB), lane 5 (AA), lane 6 (AA), lane 7 (AA).

Figure 2.11: **RsaI digested PCR segments of FcεRI-β gene**

Genotype	Genotype specific Mean (SD)*	Mean difference†
AA	6.72 (0.60)	Reference
AB	6.02 (0.48)	0.696
BB	5.23 (1.06)	1.487

* Unadjusted, from ANOVA, † Unadjusted from ANOVA; data are relative to AA genotype

Table 2.4: **Genotype-specific mean values and mean differences for log_e total serum IgE levels for FcεRI-β RsaI_ex7**

Total serum IgE levels were stratified by the genotypes of the FcεRI-β_RsaI(ex7) polymorphism as shown in table 2.4. The biallelic FcεRI-β_RsaI(ex7) polymorphism were coded into three classes (AA = 1; AB = 2; BB = 3). Analysis of variance (ANOVA) was used to evaluate differences in the genotype-specific means for total serum IgE levels. ANOVA revealed that the FcεRI-β_RsaI_ex7 genotype was significantly associated with log_e total serum IgE level with F = 13.696; $P = 0.000$. The mean total serum IgE levels were highest for genotype AA, lower for AB genotype and lowest for BB genotype.

Linear regression was run to identify significant predictors of Log_e total serum IgE levels. The independent variables used were coded as sex (male = 1, female = 0), *Ascaris* infected/non infected (*Ascaris* infected = 1, *Ascaris* non-infected and asthmatics = 0), asthmatics/non-asthmatics (Asthmatics = 1, non-asthmatics = 0) and diseases/non-diseased (*Ascaris* infected/asthmatics = 1, non-*Ascaris* infected/non-asthmatic = 0) Age was analysed as a continuous variable. The linear

regression indicated that diseases or non-diseased condition i.e having *Ascaris* infection or asthma (regression coefficient $\beta = 0.226$; $P = 0.001$) was significant predictor for total IgE levels. Sex ($\beta = 0.085$; $P = 0.206$), age ($\beta = 0.048$; $P = 0.474$); *Ascaris* non-*Ascaris* infection ($\beta = 0.081$; $P = 0.352$) and asthmatic non-asthmatic ($\beta = -0.064$; $P = 0.352$) were not the significant predictors of total serum IgE levels.

Chapter 3

Interpretation and Discussion

3.1 Nutritional status and *Ascaris* infection

Poor physical growth and mental development in children have their roots in the unsatisfactory feeding practices and infections. Hence, monitoring proper growth is one of the best global indicators of children's well-being. Goa has always been a fascinating place to visit for many people around the world. It is one of the most popular tourist destinations. Boasting one of the highest literacy rates and per capita income in the country, it is indeed surprising that such a study has

never been conducted in the state (there are no published reports of the same). Nutritional status in children can be assessed under three broad heads; clinical, biochemical and anthropometric. Cumbersome assay procedures and biochemical analysis are indeed not practical tools for large scale surveys in developing countries like India. Anthropometry thus, is the most preferred and useful parameter for assessing the nutritional status of children. The present study provides a first of its kind baseline data about the health of the children of our state with reference to the standards for anthropometric indices set up by the world's premier health agency the WHO. As such there is very little information available about the anthropometric status of older children in India and elsewhere.

The study clearly shows the poor health status of the surveyed children. Such poor health if ignored leads to chronic illness among children leading to further problems like affecting their cognitive development

and ultimately affecting their future prospects (**Boivin MJ and Giordani B**, 1993). The results of the study in chapter 1 indicate high incidence of malnutrition among the surveyed children. At an average 40% stunting and 48% low weight for age prevalence with reference to the three growth standards, 27.5% wasting in comparison to the two standards (WHO/CDC 1978 and CDC 2000) looks quite alarming. Further, males exhibited poorer nutritional state than females. Prevalence of malnutrition was more in males than in females, the mean values of all the three indices were lower in males than in females. The results thus do not corroborate the assumption that malnutrition is higher among girl children in India (ICMR , 1984). Extent of severe malnutrition (5.7% of the subjects) i.e, low weight for age according to Gomez's classification and severe stunting (8.7% of the subjects) according to Waterlow's classification in the present population was lower than below 5 years old children of Madhya pradesh state as reported by **Rao DH et al.** (1994), where severe malnutrition and stunting was as

high as 26% and 18.6% respectively in Jhabua district. The children in the present study were deficient not only with regard to one of the criteria (wasting, stunting or underweight), but many were underweight as well as stunted. According to the Waterlow's and Gomez's criteria, 30.6% of the severely stunted children were also severely underweight and 55.1% of the severely stunted children were also moderately underweight. This seems obvious as those who do not reach a particular height by certain age also can't make it in terms of proper weight for that age. With reference to WHO 1978 growth standards 40.1% of the underweight children (less than -2.0 SD than the mean reference values for that age) were also wasted (less than -2.0 SD than the mean reference values for that height) with a odds ratio (OR) of 7.07 i.e to say that malnutritioned (less weight for their age) children have a far bigger chances of becoming wasted as well. Similarly the odds ratio (OR) for malnutrition and stunting was 7.23 indicating again that malnutritioned children have a high risk of becoming stunted as well. 27.0% of

the children were anemic which is again quite high.

There seems to be a pattern of developmental delay in the surveyed population at a later age (around 10 years) as the difference from the mean standard values was more pronounced in older age group of 10 to 15 years. From a different standpoint it is also possible that the malnutrition exhibited during early years become more pronounced as the age progresses. A study conducted by "Mineral foundation", a non-government organisation in the state, on college going girls in the same area revealed more than 80% of them being underweight (unpublished). Thus, it is very much likely that the poor nutritional state in early years, if neglected become more severe during later years. It is also worthwhile to note that the high prevalence of helminth infection may also have some bearing on the growth and development. The combined effect of high infection and poor nutritional state on developmental delays is potential future topic for research worth exploring. One of the limitations of the study was not quantifying the nutritional intake

of the children in question. Proper nutritive diet during the early years is essential for the normal growth and development. Whatever may be the reasons, the poor health of the surveyed children is indeed a matter to be worried about and proper intervention measures need to be taken to address this issue.

One of the important features of this study is the use of the three growth references for comparison with the study population. Thus the study gives an insight into the appropriateness and usefulness of these standards for Indian children. Although many studies in past have revealed that if fed properly the developmental potential of Indian children is similar to the US children, yet the appropriateness of NCHS and WHO standards for Indians have always been questioned and debated. **Bhandari N et al.** (2002) reported that the affluent children in Delhi showed similar growth pattern like their western counterparts and advocated the appropriateness of using the data for 'WHO Multicentre Growth Reference' study as part of developing new international growth

standards for WHO. The children of the present study showed closest resemblance with the WHO/CDC 1978 growth references followed by the new WHO 2007 growth references. The results suggest that the CDC 2000 growth standards are little stringent for Indian children.

In this study a prominent association between *Ascaris* infection and nutritional status in children has been found. *Ascaris* infection was a strong predictor for weight for age in the surveyed children. As in the case of any association study involving finding potential risk factors for acquiring a diseased state, mere association definitely does not indicate a causal link, more so for the poor nutritional state in children. However, from public health point of view such associations become important. The controlling parameters for growth and nutritional well being are multifactorial. In developing country like India these factors become all the more complex with poverty, hygiene, infections, socioeconomic variables, access to health care system etc playing major roles.

Helminth parasites are the major etiological factor for much of the morbidities among children. Studies have also shown their effect on the cognitive performance in children. The parasites not only rob the host of his precious food nutrients but can have secondary effects through the various Excretory-Secretory products. Therefore, childhood infections seem to have a definite deleterious bearing on children health. Studies have found association between helminth infections and low nutritional status. The present study shows *Ascaris* infection as being almost endemic in the study area. Though the study had its limitations in not quantifying other types of helminth infections, *Ascaris* was indeed the major helminth found in the stool samples of the surveyed children. Some of the children did have *Trichuris trichiura* and *Ancylostoma duodenale* eggs in their stool.

Extremely high prevalence of *Ascaris* infection reflects the unprotected water supply and also the defaecation practices of the subjects in the study areas. The study revealed highest prevalence of *Ascaris*

in the age group 10 to 15 years which could be due to the cumulative exposure of the infection. The study supports the view that *Ascaris* infection reaches maximum intensity around 10-14 years (**Awasthi S et.al**, 2003). Males were marginally more infected (in terms of epg) with *Ascaris* than females, probably due to more outdoor activities which makes them more susceptible to encounter the infection (*Ascaris* eggs) than females.

Nutritional status of children in general depends upon the interplay of proper diet on one hand and the incidences of chronic infection during the early part of their lives. Encountering infections depends upon the environmental factors, included in them are the hygiene practices, medical facilities available along with the existence of pathogens and education level of the concerned community/society. On the other hand a proper nutritive diet depends on the availability of food which in turn depends on the economic status and educational background. Figure 3.1 illustrates the relationship between these various factors. Studies

in the past have found a negative effect of *Ascaris* infection on the nutritional status of children (**Ulukanigill M and Seyrek A**, 2000; **Walter MJ and Holtzman MJ**, 2005; **Watkins WE and Pollitt E**, 1996). We found a negative correlation between the number of *Ascaris* eggs per gram of feces and the Z scores for weight for age, height for age and BMI. A significant association between malnutrition and infection depends upon the intensity and type of parasitic infection. The better Z scores for weight for age, height for age and body mass index in non-*Ascaris* infected children as compared to the infected children probably is a testimony to this fact. The infected children not only had low Z scores for weight for age, height for age and BMI, the Z scores for weight for age and height for age showed decreasing trend with the increase in intensity of *Ascaris* infection (table 1.10 chapter 1) suggesting further that intensity of infection does have a significant effect on the nutritional status. No significant difference in the mean WAZ and HAZ between non-*Ascaris* infected and mildly *Ascaris* infected children

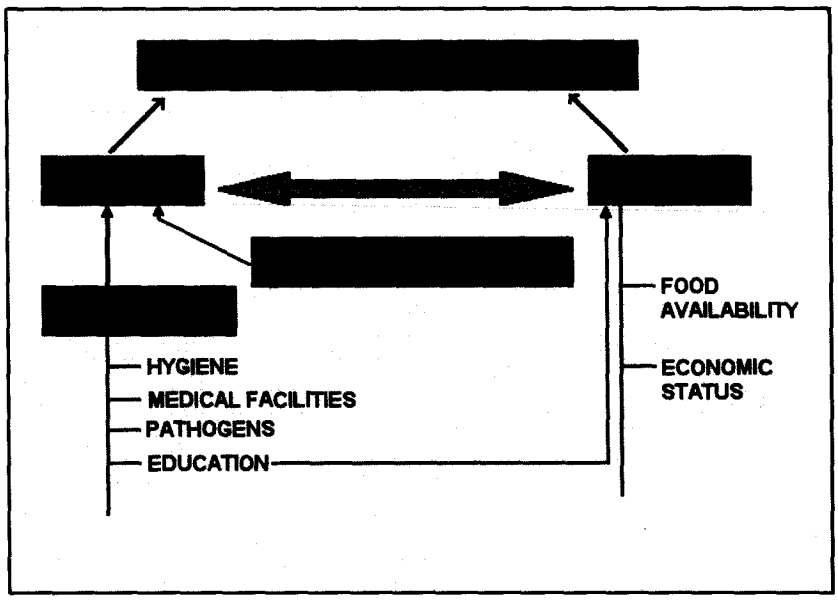


Figure 3.1: Interplay of various factors for the proper nutritional status in children

suggests that probably mild infection has no much effects on growth.

As mentioned earlier, though the present study had its limitation in not considering other factors like socio-economic status and other parasitic infections, the role of the extent of *Ascaris* infection on the anthropometric indices nevertheless cannot be underestimated. The negative effects of *Ascaris* infection on the absorption of nutrients in children cannot be ruled out as *Ascaris* is one of the largest helminth

parasites and consequently will have higher metabolic needs and thus, more dependency on the host for its nutritional requirements. Workers in the past have suggested the possible cause and effect relationship between *Ascaris* infection and intestinal lesions in children (**Tripathy K et al.**, 1972). Coupled with low protein intake high loads of *Ascaris* infection can lead to marked nutritional impairment (**Tripathy K et al.**, 1971). Though our findings do not provide conclusive evidence that *Ascaris* infection is the cause for the observed undernutrition in the study population, the results suggest that it is one of the important factors associated with low nutritional status in children. There is evidence which indicates that poor nutritional status impairs the elements of innate and the adaptive immunity which may affect the body's ability to defend parasitic infections, thus, making it more vulnerable to infections (**Hughes S et al.**, 2006). The present study, thus, highlights the importance of monitoring the nutritional status of children, especially those infected with *Ascaris* infection and provides baseline

information about the nutritional status and *Ascaris* infection in the state of Goa.

Eosinophils have been implicated with having a role in helminth expulsion (**Gleich GJ** (2000)). In the present study there was a significant difference in the mean eosinophil levels among *Ascaris* infected and non infected children. However, the difference was not statistically significant among asthmatics and *Ascaris* infected subjects. The results suggest that the eosinophils are indeed high in helminth infected subjects in comparison to the non-infected ones. Eosinophils have been found to be consistently high in allergy and asthma. Some workers have reported their ability to inflict tissue damage in bronchial asthma (Gleich GJ et al. 1998).

3.2 FcεRI-β_RsaI_ex7 polymorphism and IgE levels

IgE levels in blood are controlled by many factors including helminth infection. It is suggested and believed that the mast cell hyperplasia, eosinophilia and markedly elevated IgE in nematode infection such as *Ascaris* is not part of the immune effector mechanism for the expulsion of *Ascaris* but are part of immune evasion mechanism (**Miller HRP**, 1984). The inflammatory cytokines (interferon-γ, TNF-α, IL-2) produced by the T_{H1} subset of T helper cells activate pathways which are deleterious to helminth survival, on the other hand, the cytokines (IL-4, IL-5, IL-10) secreted by T_{H2} subset of T helper cells activate pathways which cause polyclonal synthesis of IgE which are irrelevant to or even enhance the survival of the worms. Though the mechanism of this polyclonal synthesis by helminth worms is not understood fully, the role of IL-4 has been suggested by many (**Lee TDG and Xie CY**, 1994). Many cells including mast cells are engaged in the production

of IL-4.

In the present study population there was a significant high levels of IgE in both normal (Non-*Ascais* infected, non-asthmatic subjects) as well as *Ascaris* infected and asthmatic children. **Nagraj S et al.** (2004) have also reported such high IgE levels from their study in Bangalore, India involving adult males living in slums. Many other studies have revealed that high levels of IgE in well developed communities are associated with atopic disease (**Green et al.**, 1998), whereas much higher levels of IgE levels in underdeveloped communities are not and indicate the presence of helminth and other parasitic infections, which are endemic in such areas (underdeveloped). In recent times the incidences of allergic diseases have increased many fold in the developing countries (**Hopkin JM**, 2002). The most plausible theory for such an increase in allergic diseases explains that the allergic response evolved from immune mechanisms involved with protection against parasites

and the high levels of hygiene and public health measures being followed in developed communities have practically eradicated the exposure of people in such communities to parasites. Thus, the immune responses which were against the parasites manifest as allergic diseases in the absence of parasitic infections. The present study in a way supports the view that IgE levels are indeed very high in populations where helminth infections are endemic. Studies have revealed that atopic individuals have lower total IgE levels but substantially high levels of specific anti-parasite IgE concentrations and less intense helminth infections than their non-atopic counterparts (**Lynch NR et al.**, 1998). There is a possibility of the normal individuals in the present study population belonging to such a atopic group though the prevalence of atopy was not checked in the present population. In our study the mean IgE levels were not significantly different between asthmatic and normal individuals, although the difference was significant between *Ascaris* infected and normal ones.

Asthma and allergy are considered as complex genetic diseases and do not conform to simple Mendelian inheritance (**Sanford AJ and Par PD**, 2000). There is evidence to indicate that the major genes controlling IgE levels act independently of specific responses to allergens. The switching of immunoglobulin production from immunoglobulin M to immunoglobulin E is dependent on cytokines IL-4 and IL-13 and is a key feature in helminth infection and atopic diseases (**Holt PG**, 2000). Studies have shown the role of IL-4 in the regulation of IgE levels. Ravetch J (1994) has opined that FcϵRI-β genetic variations have direct influence on the IgE levels via IL-4 production. **Hill MR and Cookson WOCM** (1996) have reported that mutation in the FcϵRI-β gene may alter the function of the receptor and may predispose to asthma through atopy. Since cross-linking of FcϵRI receptors on mast cells is essential for secreting T_{H2} cytokines (like IL-4), polymorphism in the gene for this receptor can influence IgE production via IL-4. The

present study reveals that the gene FcϵRI-β shows polymorphic profile in both *Ascaris* infected as well as asthmatic children of Goa and also demonstrates that there are significant effects of polymorphisms in FcϵRI-β_RsaI_ex7 gene on serum IgE levels.

In conclusion the study provides baseline information about the nutritional status as revealed by the anthropometric indices, and the extent of *Ascaris* infection in the children of the study area . The study also reveals that the gene FcϵRI-β is polymorphic in the study population and plays a role in the regulation of serum IgE levels.

Bibliography

Akizawa Y, Nishiyama C, Hasegawa M, Maeda K, Nakahata T, Okumura K, Ra C, and Ogawa H, 2003, 'Regulation of human FceRIβ chain gene expression by Oct-1', *International Immunology*, vol. 15, No. 5, pp. 549-556.

Alderman H, Konde-Lule J, Sebuliba I, Bundy D and Hall A, 2006, 'Effect on weight gain of routinely giving albendazole to preschool children during child health days in Uganda: cluster randomised controlled trial', *BMJ*, doi:10.1136/bmj.38877.393530.7C.

Appoh LY, Krekling S, 2005, 'Maternal nutritional knowledge and child nutritional status in the Volta Region of Ghana', *Maternal and Child Nutrition*, vol. 1, pp. 100-110.

Assis AMO, Prado MS, Barreto ML, Reis MG, Pinheiro SMC, Parraga IM, Blanton RE, 2004, 'Childhood stunting in Northeast Brazil: the role of *Schistoma mansoni* infection and adequate dietary intake', *European journal of Clinical Nutrition*, vol. 58, pp. 1022-1029.

Awasthi Shally, Bundy D A P, Savioll Lorenzo, 2003, 'Helminth infections', *BMJ*, vol. 327, pp. 431-433.

Awasthi S, Pande VK, 2001, 'Six-monthly de-worming in infants to study effects on growth', *The Indian journal of Pediatrics*, vol. 68, issue 9, pp. 823-27.

Bazaral M, Orgel HA, and Hamburger RN 1971, 'IgE Levels in Normal Infants and Mothers and an Inheritance Hypothesis', *J. Immunol*, vol. 107, pp. 794-801.

Bell RG. 1996 Aug, 'IgE,allergies & helminth parasites: a new perspective on an old conundrum', *Immunol Cell biol*, vol. 74(4), pp. 337-45.

Bhandari N, Bahl R, Taneja S, Onis de M, Bhan MK 2002, 'Growth performance of affluent Indian children is similar to that in developed countries', *Bulletin of World Health Organisation*, vol. 80, pp. 189-195.

Boivin Michael J, and Giordani Bruno, 1993, 'Improvements in Cognitive Performance for Schoolchildren in Zaire, Africa, Following an Iron Supplement and Treatment for Intestinal Parasites', *Journal of Pediatric Psychology*, vol. 18(2), pp. 249-264.

Bradley JE & Jackson JA, 2004, 'Immunity, immunoregulation and the ecology of trichuriasis and ascariasis' 2004, *Parasite Immunology*, vol. 26, pp. 429-441.

Brown M, Mawa PA, kaleebu P, Elliott AM, 2006, 'Helminths and HIV infection: epidemiological observations on immunological hypotheses', *Parasite Immunology*, vol. 28, pp. 613-623.

Buckley RB and Fiscus SA, 1975, 'Serum IgD and IgE concentrations

in Immunodeficiency diseases', *The journal of clinical investigation*, vol. 55, pp. 157-165.

Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG,1989, 'Association of asthma with serum IgE levels and skin-test reactivity to allergens', *N Engl J Med*, vol. 320, pp. 271-7.

Busse WW, Lemanske RF, 2001, 'Asthma', *N Engl J Med*, vol. 344, No. 5, pp. 350-62

Chakravarty AK, 1996, *Immunology*, Tata McGraw-Hill Company Limited, New Delhi, pp. 130-131.

Chakravarty AK, 2006, *Immunology and Immunotechnology*, Oxford University Press, New Delhi, pp. 398-99.

Cogill, Bruce, 2003, 'Anthropometric Indicators Measurement Guide', *Food and Nutrition Technical Assistance Project, Academy for Educational Development*, Washington, D.C., 2003.

Cole TJ, Sep-Oct 1989, 'Using the LMS method to measure skewness in

the NCHS and Dutch National height standards', *Annals of Human Biology*, vol. 16(5), pp. 407-19.

Cole TJ and Green PJ, 1992, 'Smoothing reference centile curves: the LMS method and penalized likelihood', *Statistics in medicine*, vol. 11(10), pp. 1305-19.

Cox FEG, Oct. 2002, 'History of Human Parasitology', *Clinical Microbiology Reviews*, vol. 15, No.4, pp.595-612.

Campbell T, Campbell A, 2007, 'Emerging Disease Burdens and the Poor in Cities of the Developing World', *Journal of Urban Health: Bulletin of the New York Academy of Medicine*, vol. 84, No. 1. doi:10.1007/s11524-007-9181-7, pp. i54-i64.

Charles McSharry, Yuxia, Celia V Holland, Malcom W Kennedy. Feb 1999, 'Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen & inflammation indicators in children', *Infection and Immunity*, vol. 67, No. 2, pp. 484-489.

Chatterjee KD, 1976, *Parasitology(Protozoology and Helminthology) In relation to Clinical Medicine*, eleventh edition, Chatterjee Medical Publishers, Calcutta, India.

Chopra M, 2006, 'Mass deworming in Ugandan children', *BMJ*, vol. 333, pp. 105.

Chowdary VS, Vinaykumar EC, Rao JJ, Rao R, Babu KR, Rangamani V, 2003, 'A Study on Serum IgE and Eosinophils in Respiratory Allergy Patients', *Indian J Allergy Asthma Immunol*, vol. 17(1), pp. 21-24.

Colley DG,2000, 'Parasitic diseases:opportunites and challenges in the 21st century', *Mem Inst Oswaldo Cruz*, vol. 95, suppl 1, pp. 79-87.

Cookson WO, Sharp PA, Faux JA, Hopkin JM 1989, 'Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q', *Lancet*, vol. 1, pp. 1292-1295.

Cooper PJ, Chico MA, Rodrigues LC, Ordonez M, Strachan D, Griffin

GE, Nutman TB, 2002, 'Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics', *J Allergy Clin Immunol*, vol. 111, pp. 995-1000.

Cooper PJ, 'Intestinal worms and human allergy', *parasite Immunology*, vol. 26, pp. 455-467.

Dang S, Yan H, Yamamoto S, Wang X, Zeng L, 2004, 'Poor nutritional status of younger Tibetan children living at high altitudes' *European Journal of Clinical Nutrition*, vol. 58, pp. 938-946.

De Onis M, 2000, 'Measuring nutritional status in relation to mortality', Public Health Classics, *Bulletin of the World Health Organisation*, vol. 78(10), pp. 1271-1274.

Dickson R, Awasthi S, Williamson P, Demellweek C, Garner P, 2000, 'Effects of treatment for helminth infection on growth and cognitive performance in children: systematic review of randomized trials', *BMJ*, vol. 320, pp. 1697-701.

Dickson PW, Wong ZYH, Harrap SB, Abramson MJ, Walters EH, 1999, 'Mutational analysis of the high affinity immunoglobulin E receptor β subunit gene in asthma', *Thorax*, vol. 54, pp. 409-412.

Falagas ME, Papastamataki PA and Bliziotis IA, March 2006, 'A bibliometric analysis of research productivity in Parasitology by different world regions during a 9-year period (1995-2003)', BMC *Infectious Diseases*, 6:56, doi:10.1186/1471-2334-6-56. Available at http://www.biomedcentral.com/1471-2334/6/56

Engle PL, Black MM, Behrman JR, Cabral de Mello M, Gertler PJ, Kapiriri L, Martorell R, Young ME, and the International Child Development Steering Group, 2007, 'Child development in developing countries 3. Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world', *Lancet*, vol. 369, pp. 229-42.

Ferreira LF, de Arajo AJ, Confalonieri UE, 1983, 'The finding of

helminth eggs in a Brazilian mummy', *Trans R Soc Trop Med Hyg*, vol. 77(1), pp. 65-7.

Ferreira LF, de Arajo AJ, Confalonieri UE, Nuez L, 1984 Aprl-jun, 'The finding of eggs of Diphyllobothrium in human coprolites (4,100-1,950 B.C.) from northern Chile', *Mem Inst Oswaldo Cruz*, vol. 79(2), pp. 175-80.

Friedman JF, Kanzaria HK, Acosta LP, Langdon GC, Manalo DL, Haiwei WU, Olveda RM, Mcgarvey ST, Kurtis JD, 2005, 'Relationship between *Schistosoma Japonicum* and Nutritional status among children and young adults in Leyte, The Philippines', *Am. J. Trop. Med. Hyg*, vol. 72(5), pp. 527-533

Ge, K-Y, Chang, S-Y, 2001, 'Definition and measurement of malnutrition', *Biomed-Environ-Sci*, vol. 14(4), pp. 283-91.

Gerrard JW, Rao DC, Morton NE 1978, 'A Genetic study of Immunoglobulin E', *Am J Hum Genet*, vol. 30, pp. 46-58.

Ghai OP, Gupta P, Paul VK, 2001, *Essential pediatrics*, Fifth edition, Mehta publishers. New Delhi. pp 65-71.

Gleich GJ, 2000, 'Mechanism of eosinophil-associated inflammation', *J Allergy Clin Immunol*, vol. 105, No.4, pp. 651-663.

Goldsby RA, Kindt TJ, Osborne BA, Kuby J, 2003, *Immunology*, fifth edition, W.H Freeman and company, New York. pp. 76-101.

Gomez F, Galvan RR, Frenk S, Munoz JC, Chavez R, Vazquez J, 1956 September, 'Mortality in Second and third degree malnutrition', *The journal of Tropical Pediatrics.* Published by WHO, 2000, vol. 78(10), pp. 1275-1280.

Gonzlez AH, Regalado VC and Ende JV, 'Non-invasive management of *Ascaris lumbricoides* biliary tract migration: a prospective study in 69 patients from Ecuador', 2001 Feb, *Tropical Medicine and International Health*, vol. 6, No 2, pp. 146-150.

Gould HJ, Sutton BJ, Beavil AJ, Beavil RL, McCloskey N, Coker HA,

Fear D, and Smurthwaite L, 2003, 'The Biology Of IgE and the Basis of Allergic Disease', *Annu. Rev. Immunol*, vol. 21, pp. 579-628.

Graves PE, Kabesch M, Halomen M, Holberg CT, Baldini M, Fritzsch, Weiland SK, Erickson RP, Martinez FD, 2000 March, 'A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children', *J Allergy Clin Immunol*, vol. 105(3), pp. 506-13.

Green SL, Gaillard MC, Song E, Dewar JB, Halkas A 1998, 'Polymorphisms of the Beta Chain of the High-Affinity Immunoglobulin E Receptor (FcϵRIβ) in South African black and white asthmatic and nonasthmatic Individuals', *Am J Respir Crit Care Med*, vol. 158, pp. 1487-1492.

Gross R, Webb P, 2006, 'Wasting time for wasted children: severe child undernutrition must be resolved in non-emergency settings', *Lancet*, vol. 367, pp. 1209-11.

Gulani A, Nagpal J, Osmond C, and H P S Sachdev, 2007,

'Effect of administration of intestinal anthelmintic drugs on haemoglobin: systematic review of randomised controlled trials', *BMJ* doi:10.1136/bmj.39150.510475.AE.

Growth and physical development of Indian infants and children. Technical report series No.18 1984. Indian council of Medical Research.

Habbarik, Tifnouti A, Bitton G, Mandil A, 2000 Feb, 'Intestinal parasitios and environmental pollution: 1343 pediatric cases in Beni-Mellal, Morocco', *Tunis Med*, vol. 78(2), pp. 109-14.

Hage-Hamsten MV, Johansson E, Kronqvist M, Loughry A, Cookson WOCM and Moffatt MF, 2001, 'Association of FcϵR1-β polymorphisma with immunoglobulin E antibody responses to common inhalent allergens in a rural population, *Clinical and Experimental Allergy*, vol. 32, pp 838-842.

Hamelmann E, Rolinck-Werninghaus C, Wahn U, 2002, 'From IgE to Anti-IgE: Where do we stand?', *Allergy*, vol. 57, pp. 983-994.

Herwerden L, Harrap SB, Wong ZY, Abramson MJ, Kutin JJ, Forbes AB, Raven J, Lanigan A, Walters EH 1995, 'Linkage of high-affinity IgE receptor gene with bronchial hyperreactivity, even in absence of atopy', *lancet*, vol. 346, pp. 1262-1265.

Hancock PL and Skinner BJ, associate editor David L. Dineley, 2000. *The Oxford companion to The Earth.*, 1st edition, pp. 763-65.

Hill MR and Cookson WOCM, 'A new variant of the β subunit of the high-affinity receptor for Immunoglobulin E (FcϵRI-β E237G): associations with measures of atopy and bronchial hyper-responsiveness, *Human Molecular Genetics*, vol. 5, No.7, pp. 959-962.

Hizawa N, Yamaguchi E, Jinushi E, Kawakami Y, 2000 Mar, 'A common FCER1B gene promoter polymorphism influences total serum IgE levels in a Japanese population', *Am. J Resp. Crit. Care Med*, vol. 161 (3pt 1), pp. 906-9.

Hizawa N, Yamaguchi E, Jinushi E, Konno S, Kawakami Y, Nishimuru M, 2001 Jul, 'Increased total serum IgE levels in patients with asthma

and promotet polymorphism at CTLA-4 & FCER1B', *J Allergy Clin Immunology*, vol. 108(1), pp. 74-79.

Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L 1998, 'Interleukin-10 and Transforming Growth Factor-β promoter polymorphisms in allergies and asthma, *Am J Respir Crit Care Med*, vol. 158, pp. 1958-1962.

Holt PG, 2000, 'Parasites, atopy and the hygiene hypothesis: resolution of a paradox? *Lancet*, vol. 356: pp. 1699-701.

Hopkin JM, 2002, 'The rise of atopy and links to infection', *Allergy*, vol. 57, suppl. 72, pp. 5-9.

Horton J, 2003, 'Human gastrointestinal helminth infections: are they now neglected diseases', *Trends in Parasitology*, vol. 19, No. 11, pp. 527-37.

Hughes S, Kelly P, 2006, 'Interactions of malnutrition and immune

impairment, with specific reference to immunity against parasites', *Parasite Immunology*, vol. 28(11), pp. 577-88.

Indian Council of Medical Research, 1984, 'Growth and physical development of Indian infants and children', *Technical Report Series*, 18.

Infuhr D, Crameri R, Lamers R, Achatz G, 2005, 'Molecular and cellular targets of anti-IgE antibodies', *Allergy*, vol. 60, pp. 977-985.

Jeffrey SD, 2007 Jan, 'The Neglected Tropical Diseases', 'Scientific American', pp. 23. available at www.sciam.com/ontheweb.

Joshi Y, 1998, *Kayachikitsa*, 3rd edition, Pune sahitya vitaran, Pune, India.

Jinabhai CC, Taylor M,Coutsoudis A, Coovadia HM, Tomkins AM, 2001 Mar, 'A health and nutritional profile of rural school children in Kwa Zulu-Natal (KZN), South Africa', *Ann Trop Paediatr*, vol. 21(1), pp. 50-8.

Kang G, Mathew MS, Rajan DP, Daniel JD, Mathan MM, Mathan VI and Muliyil JP, 1998, 'Prevalence of Inestinal parasites in rural Southern Indians', *Tropial Medicine and Internatonal Health*, vol. 3, No. I, pp. 70-75.

Kanan I, 2007, *Immonology*, First edition, MJP publishers. Chennai, India, pp. 55.

Kikafunda JK, Walker AF, Collett D, Tumwine JK, 1998, 'Risk Factors for Childhood Malnutrition in Uganda', *Pediatrics*, vol. 102;45-DOI: 10.1542/peds.102.4.e45. Available at http://www.pediatrics.org/cgi/content/full/102/4/e45

King EM, Kim HT, Dang NT, Michael E, Drake L, Needham C, Haque R, Bundy DAP Webster JP, 2005, 'Immuno-epidemiology of *Ascaris lumbricoides* infection in a high transmission community: antibody responses and their impact on current and future infection intensity', *Parasite Immunology*, vol. 27, pp. 89-96.

Koppelman GH, Reigmerink NE, Colin Stine O, Howard TD, Whittaker

PA, Meyers DA, Postma DS, Bleecker ER, 2001, 'Association of a promoter polymorphism of the CD14 gene & atopy' *Am J Respir. Care Med*, vol. 163(4), pp. 965-9.

Kloetzel K, Merluzzi Filho TJ, Kloetzel D, 1982, '*Ascaris* and Malnutrition in a Group of Brazilian ChildrenA Follow-up Study', *Journal of Tropical Pediatrics*, vol. 28, pp. 41-43.

Kuster H, Zhang Li, Brini AT, MacGlashan Don WJ, kinet JP 1992, 'The Gene and cDNA for the Human Affinity Immunoglobulin E Receptor β Chain and Expression of the Complete Human Receptor, *The Journal of Biological Chemistry*, vol. 267, No. 18, pp. 12782-12787.

Laitinen T, Kauppi P, Ignatius J, Ruotsalainen T, Daly MJ, Kaarianen H, Kruglyak L, Laitinen H, de la Chapelle A, Lander ES, Laitinen LA, Kere J, 1997, 'Genetic control of serum IgE levels & asthma: linkage disequilibrium studies in an isolated population', *Hum Mol Genet*, vol. 6(12), pp. 2069-76.

Lee JD, Wang JJ, Chung LY, Chang EE, Lai LC, Chen ER, Yen CM, 2000 Sep, 'A survey on the intestinal parasites of the school children in Kaohsiung county Kaohsiung', *J Med Sci*, vol. 16(9), pp. 452-58.

Lee TDG and Xie CY, 1994, 'IgE regulation by nematodes: The body fluid of *Ascaris* contains a B-cell mitogen.', *J Allergy Clin Immunol*, vol. 95, Number 6, pp. 1246-54.

Lesouef PN, Goldblatt J, Lynch NR, 1999 Dec, 'Genome Screen and candidate gene studies in parasitized populations', *Clinical Exp Allergy*, vol. 29, supp.14, pp. 31-34.

Lightowlers MW, Rickard MD, 1998, 'Excretory-Secretory products of Helmith parasites:effects on host immune responses', *Parasitiology*, vol. 96, pp. 5123-66.

Lydyard PM, Whelan A, Fanger MW, 2003, *Instant Notes in Immunology*, First edition, Viva books private limited, New Delhi, pp. 13-21.

Lynch NR, Goldblat J, Le Souef PN 1999, 'Parasite infections and the risk of asthma and atopy, *Thorax*, vol. 54, pp. 659-660.

Lynch NR, Hagel IA, Palenque ME, Di Prisco MC, Escudero JE, Corao LA, Sandia JA, Ferreira LJ, Botto C, Perez M, Le Souef PN, 1998 Feb, 'Relationship between helminthic infection and IgE response in atopic and non-atopic children in a tropical environment', *J.Allergy Clin Immunol*, vol.101, No.2, pp. 217-21.

Macglashan DW, Jr, Bochner BS, Adelman DC, Jardieu PM, Togias A, Mckenzie-White J, 1997, 'Down-regularegulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody', *J Immunol*, vol. 158, pp. 1438-1445.

Malla B A, Sherchand JBA, Ghimire PB, Kumar R C and Gauchan Pa, 2004, 'Prevalence of Intestinal Parasitic Infections and Malnutrition among Children in a Rural Community of Sarlahi, Nepal', *Journal of Nepal Health Research Council*, vol. 2 No.1.

Mansur AH, Bishop DT, Markham AF, Morton NE, Holgate ST, Morrison JF, June 1999, 'Suggestive Evidence for Genetic linkage between IgE phenotypes & chromosome 14q markers', *Am. J. Respir. Crit. Care Med*, vol. 159, Number 6, pp. 1796-1802.

Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, and Beaty TH 1994, 'Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations' *Science*, vol. 264, pp. 1152-1156.

Mathers CD, Ezzati M, Lopez AD, 2007, 'Measuring the Burden of Neglected Tropical Diseases: The Global Burden of Disease Framework', *PLoS Negl Trop Dis*, vol. 1(2): e114. doi:10.1371/journal.pntd.0000114.

McKerrow JH, 2005 August, 'Designing drugs for parasitic diseases of the developing world', *PloS Medicine*, vol. 2, issue 8, e210.

Merlet O, 2006, 'A Beginners Guide to Malnutrition', *Medecins Sans Frontieres.* Available at

www.msf.or.jp/info/pressreport/pdf/pressMalnutritionE.pdf

Meyers DA, Beaty TH, Freidoff LR, Marsh DG, 1987 July, 'Inheritance of total serum IgE (basal levels) in man', *Am J. Hum Genet*, vol. 41 (1), pp. 51-62.

Meyers DA, Beaty TH, Colyer CR, Marsh DG, 1991, 'Genetics of total serum IgE levels: regressive model approach to segregation analysis', *Genet. Epidemiol*, vol. 8(5), pp. 351-59.

Meyers DA, Postma DS, Panhuysen CI, XUJ, Amelung PJ, Levitt RC, Bleeecker ER, 1994 Sep, 'Evidence for a locus regulating total serum IgE levels mapping to chromosome 5', *Genomics*, vol. 23(2), pp. 464-70.

Miller HRP, 1984, 'The protective mucosal response against gastrointestinal nematodes in ruminants and laboratory animals', *Vet Immunol Immunopathol*, vol. 6, pp. 167-259.

Mitra M, Kumar PV, Chakrabarty S, Bharti P, 2007 April, 'Nutritional

status of Kamar tribal children in Chhattisgarh', *Indian J of Pediatr*, vol. 74(4), pp. 381-384.

Morales G, Loaiza L, Pino LA, 1999 Jul-dec, 'Risk markers in subjects with high loads of *Ascaris lumbricoides* in rural community of the Cojedes State, Venezula', *Bol Chil Parasitol*, vol. 54(3-4), pp. 88-96.

Muller O, Krawinkel M, 2005 Aug, 'Malnutrition and health in developing countries', *CMAJ*, vol. 173(3), pp. 279-286.

Murugkar DA, 2005, 'Nutritional status of Khasi schoolgirls in Meghalaya', *Nutrition*, vol. 21, pp. 425-431.

Nagraj S, Raghavan R, Macaden R, Kurpad AV, 2004, 'Intestinal parasitic infection and total serum Ige in asymptomatic adult males in an urban slum and efficacy of antiparasitic therapy', *Indian journal of Medical Microbiology*, vol. 22(1), pp. 54-56.

Northrop-Clewes CA, Rousham EK, Mascie-Taylor CGN, LunnPG, 2001, 'Anthelmintic treatment of rural Bangladeshi children: effect

on host physiology, growth, and biochemical status', *Am J Clin Nutr*, vol. 73, pp. 53-60.

Nutman TB, Withers AS, Ottesen EA, 1985 oct, 'In vitro parasite antigen-induced antibody responses in human helminth infections', *J Immunol*, vol. 135(4), pp. 2794-9.

Oberhelman RA, Guerrero ES, Fernandez ML, Silio M, Mercado D, Comiskey N, Ihenacho G, Mera R, 1998, 'Correlations between intestinal parasitosis, physical growth, and psychomotor development among infants and children from rural Nicaragua', *Am. J. Trop. Med. Hyg*, vol. 58(4), pp. 470-475.

Oguntibeju Oluwafemi O, 2003, 'Parasitic Infestation and Anaemia : The Prevalence in a Rural Hospital Setting.Journal, *Indian Academy of Clinical Medicine*, vol. 4, No. 3, pp. 210-12.

Olivares S, Kain J, Lera L, Pizarro F, Vio F, Moron C, 2004, 'Nutritional status, food consumption and physical activity among Chilean

school children: a descriptive study', *European Journal of Clinical Nutrition*, vol. 58, pp. 1278-1285.

OLorcain P, Holland CV, 2000, 'The public health importance of *Ascaris lumbricoides*', *Parsitology*, vol. 121 suppl:551-71.

Onis de M, Onyango A W, Borghi E, Siyam A, Nishidaa C, Siekmanna J, 2007, 'Development of a WHO growth reference for school-aged children and adolescents', *Bulletin of the World Health Organization*, vol. 85, pp. 660-667.

Onis de M, Frongillo EA, Blossner M, 2000, 'Is malnutrition declining? An analysis of changes in levels of child malnutrition since 1980', B*ulletin of the World health organisation*, vol. 78(10), pp. 1222-1233.

Palmer LJ, Pare PD, Faux JA, Moffatt MF, Daniels SE, LeSouefPN, Bremner PR, Mockford E, Gracey M, Spargo R, Musk AW, and Cookson WOCM, 1997, 'FceR1-β Polymorphism and total Serum IgE Levels in Endemically Parasitized Australian Aborigines', *Am. J. Hum. Genet*, vol. 61, pp. 182-188.

Patrucco R, Tello R, and Bonavia D, 1983, 'Parasitological studies of coprolites of pre-Hispanic Peruvian populations', *Curr. Anthropol*, vol. 24, pp. 393-394.

Pelletier L David, FwngUlo A Edward Jr, Habicht Jean-Pierre, 1993, 'Epidemiologic Evidence for a Potentiating Effect of Malnutrition on Child Mortality', *Am J Public Health*, vol. 83, pp. 1130-1133.

Peng W., Zhou X, Gasser RB, 2003, '*Ascaris* egg profiles in human faeces: biological and epidemiological implications', *Parasitology*, vol. 127, pp. 283-290.

Perzanowski MS, Ng'ang'a LW, Carter MC, Odhiambo J, Ngari P, Vaughan JW, Chapman MD, Kennedy MW, Platts-Mills TAE, 2002, 'Atopy, asthma, and antibodies to Ascaris among rural and urban children in Kenya', *The Journal of Pediatrics*, vol. 140, Issue. 5, pp. 582-588.

Plonka-Syroka B, 2005, 'Scientific standards in parasitology in historical perspective', *Wiad Parazytol*, vol. 51(3), pp. 197-207.

Potaczek DP, Sanak M, Mastalerz L, Setkowicz M, Kaczor M, Nizankowska E, Szczeklik, 2006, 'The α-chain of high-affinity receptor for IgE (FcϵRIα) gene polymorphisms and serum IgE levels', *Allergy*, vol. 61, pp. 1230-1233.

Prussin C and Metcalfe DD, 2006, 'IgE, mast cells, basophils, and eosinophils', *J Allergy Clin Immunol*, vol. 117, Number 2, pp. 450-55.

Qiao HL, Yang J, Zhang YW, 2004 Mar, 'Specific serum IgE levels and FcϵRIβ genetic polymorphism in patients with penicillins allergy, *Allergy*, vol. 59, pp. 1326-1332.

Rao DH, Rao KMG, Radhaiah G, Rao NP, 1993, 'Nutritional Status of tribal preschool children in three ecological zones of Madhya pradesh', *Indian Pediatrics*, vol. 31, pp 635-640.

Reinhard KJ, Hevly RH, Anderson GA, 1987, 'Helminth remains from prehistoric Indian coprolites on the Colorado Plateau', *J Parasitol*, vol. 73(3), pp. 630-39.

Reinhard K, Urban O, 2003, 'Diagnosing ancient diphyllobothriasis from Chinchorro mummies', *Mem Inst Oswaldo Cruz*, vol. 98, Suppl 1, pp. 191-93.

Rhoads ML, Fetterer RH, Hill DE, Urban JF Jr, 2000, '*Trichuris suis*:a secretory chymotryphism/elastase inhibitor with potential as an immunomodulator', *Exp Parasitol*, vol. 95(1), pp. 36-44.

Saini SS, Klion AD, Holland SM, Hamilton RG, Bochner BS, MacGlashanDW, 2000, 'The relationship between serum IgE and surface levels of FceR on human leukocytes in various diseases: Correlation of expression with FceRI on basophils but not on monocytes or eosinophils', *J Allergy Clin Immunol*, vol. 106, No. 3, pp. 514-20.

Sanford AJ and Par PD, 2000, 'Genetics of Asthma-The Important Questions', *Am J Respir Crit Care Med*, vol. 161, pp. 202-206.

Sandford AJ, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA,

Young RP, Nakamura Y, Lathrop GM, Cookson WO 1993, 'Localisation of atopy and beta subunit of high-affinity IgE receptor (Fc epsilon RI) on chromosome 11q', *Lancet*, vol. 341, pp. 332-334.

Santra A, Bhattacharya T, Chowdhury A, Ghosh A, Ghosh N, Chatterjee BP and Guha DN, Muzumder, 2001, 'Serodiagnosisof ascariasis with specific IgG4 antibody and its use in an epidemiological study', 'Transactions of the Royal Society of Tropical Medicine and Hygeine', vol. 95, pp. 1-4.

Sastri K,Chatuvedi G, 1998, '*The Charaka Samhita of Agnivesa revised by Caraka and drdhabala with introduction by vaidya samrata Sri Satya narayana Sastri*', Chaukhambha Bharati academy, Varanasi.

Schaible UE, Kufmann Stefan HE, 2007, 'Malnutrition and infection: Complex mechanisms and global impacts', *Plos Med*, 4(5): e115. doi:10.1371/ournal.pmed.0040115.

Scrivener S, Yemaneberhan H, Zebenigus M, Tilahun D, Girma S, Ali

S, McElroy P, Custovic A, Woodcock A, Pritchard D, Venn A, Britton J, 2001, 'Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study', *Lancet*, vol. 358, pp. 1493-99.

Selassie FG, Stevens RH, Cullinan P, 2000, 'Total and specific IgE (house dust mite and intestinal helminths) in asthmatics and controls from Gondar, Ethiopia', *Clin Exp Allergy*, vol. 30, pp. 356-58.

Sianto L, Reinhard KJ, Chame M, Chaves S, Mendona S, Gonalves ML, Fernandes A, Ferreira LF, Arajo A, 2005, 'The finding of Echinostoma (Trematoda: Digenea) and hookworm eggs in coprolites collected from a Brazilian mummified body dated 600-1, 200 years before present', *J Parasitol*, vol. 91(4), pp. 972-75.

Shakib, 1990, 'Is IgE-mediated hypersensitivity an autoimmune disease?', *Allergy*, vol. 45, pp.1-9.

She Sherrill DL, Stein R, Martinez FD, 1999 July, 'Total serum IgE

and its association with asthma symptoms and allergic sensitization among children', *J allergy clin immunol*, vol. 104(1), pp. 28-36.

Shirikawa T, Li A, Dubowitz M, Dekker JW, Shaw AE, Faux JA, Ra C, Cookson WO, Hopkin JM 1994, 'Association between atopy and variants of the b subunit of the high-affinity immunoglobin E receptor, *Nat Genet*, vol.7(2), pp. 125-129.

Shirakawa T, Mao XQ, Sasaki S, Enomoto T, Kawai M, Morimoto K, Hopkin J 1996, *Human Molecular Genetics*, vol. 5, No. 8, pp. 1129-1130.

Shubair ME, Yassin MM, al-Hindi AI, al-wahaidi AA, Jadallah SY, Abu Shanban N D al, 2000 Aug, 'Intestinal parasites in relation to haemoglobin level and nutritional states of school children in Gaza', *J Egypt Soc Parasitol*;, vol. 30(2), pp. 365-75.

Steinke JW, Borish L and Rosenwasser LJ, 2003, 'Genetics of hypersensitivity', *J Allergy Clin Immunol*, vol. 111, No. 2, pp. S495-S501.

Stephenson LS, Latham MC, Ottesen EA, 2000, 'Malnutrition and parasitic helminth infections', *Parasitology*, vol. 121, pp. s23-38.

Stoltzfus RJ, Albonico M, Tielsch J, Chwaya H, Savioli L, 1997, 'School-based deworming program yields small improvement in growth of Zanzibar school children after one year', *J Nutr*, vol. 127, pp. 2187-2193.

Sullivan Richard, 1995, 'A brief journey into medical care and disease in Ancient Egypt', *J R Soc Med*, Vol. 88, pp. 141-145.

Suzuki, Hizawa N, Yamaguchi E and Kawakami Y, 2000, 'Association between a C + 33T polymorphism in the IL-4 promoter region & total serum IgE levels', *Clinical and experimental allergy*, vol. 30, pp. 1746-1749.

Tattersfield AE, Knox AJ, Britton JR, Hall IP, 2002, 'Asthma', *Lancet*, vol. 360, pp. 1313-22.

Taylor JA, Karas JL, Ram MK, Green OM, and Dugan CS, 1995 Aug,

'Activation of the High-Affinity Immunoglobulin E Receptor FceRI in RBL-2H3 Cells Is Inhibited by Syk SH2 Domains', *Molecular And Cellular Biology*, vol. 15, No. 8, pp. 4149-4157.

Trabetti E, Cusin V, Malerba G, Martinati LC, Casartelli A, Boner AL, and Pignatti PF 1998, 'Association of the FcepsilonRIbeta gene with bronchial hyper-responsiveness in an Italian population', *J Med Genet*, vol. 35(8), pp. 680-681.

Tripathy K, Duque E, Bolanos O, Lotero H, Mayoral LG, 1972, 'Malabsorption syndrome in ascariasis', *The American Journal of Clinical Nutrition*, vol. 25, pp. 1276-1281.

Tripathy K, Gonzlez F, Lotero H, Bolaos O, 1971, 'Effects of Ascaris Infection on Human Nutrition', *Am. J. Trop. Med. Hyg*, vol. 20(2), pp. 212-218

Ulukanligil1 M, Seyrek A, 2000, 'Anthropometric status, anaemia and intestinal helminthic infections in shantytown and apartment

schoolchildren in the Sanliurfa province of Turkey', *European Journal of Clinical Nutrition*, vol. 58, pp. 1056-1061.

UNICEF. *Progress for children- A report card on nutrition.* Number 4, May 2006.

Van den Bigelaar AHJ, Ree RV, Rodrigues LC, Lell B, Deelder AM, Kremsner PG, Yazdanbakhsh M, 2000, 'Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10', *lancet*, vol. 356, pp. 1723-1727.

Vercelli D, 2005, 'Genetic Regulation of IgE responses; Achilles and the tortoise, *J Allergy Clin Immunol*, vol. 116, pp. 60-64.

Viqar Zaman and Loh Ah Keong, 1989, *Handbook of Medical Parasitology*, second edition, KC Ang Publishing Pte. Ltd, Singapore, pp. 153-157.

Walia RK, Bajaj HK, 2003, *Textbook on Introductory plant Nematology*,

Directorate of Information and publications of Agriculture, Indian Council of Agricultural Research. New Delhi.

Walter CW, Kilama WL, Kihamia CM, 1979, '*Ascaris* and Growth Rates: A Randomized Trial of Treatment', *AJPH*, vol. 69, No. 10. pp. 981-991.

Walter MJ and Holtzman MJ, 2005, 'A centennial History of Research o Asthma Pathogenesis, *Am J Respir Cell Mol Biol*, vol. 32, pp. 483-489.

Watkins WE, Pollitt E, 1996, 'Effect of Removing *Ascaris* on the Growth of Guatemalan Schoolchildren', *Pediatrics*, vol. 97, pp. 871-876.

Weinstock JV, Summers R and Elliot DE, 2004, 'Helminths and Harmony', *Gut*, 53, pp. 7-9.

Weiss ST, 2000 Feb, 'Parasites and asthma/allergy:what is the relationship?', *J Allergy Clin Immunol*; vol. 105(2), pp. 205-10.

Winter Winter WE, Hardt NS, Fuhrman S, 2000, 'Immonoglobulin E', *Arch Pathol Lab Med*, vol. 124, pp. 1382-1385.

Witting HJ, Belloit J, De Fillippi I, Royal G, 1980, 'Age related immunoglobulin E levels in healthy subjects and in patients with allergic diseases, *J. Allergy Clin Immunol*, vol. 66, pp. 305-313.

WHO, *WHO child growth standards: methods and development*, 2007, NLM classification: WS 103. World Health Organization 2007.

WHO, growth standards 2007. Available at http://www.who.int/growthref/en

WHO, *Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee*, 1995, World Health Organ Tech Rep Ser 1995;854.

World Health Organisation (2005) *Nutrition:Challenges.* Available at http://www.who.int/nutrition/challenges.

World Health Organization, 2003, *Strategic directions for improving the*

health and development of children and adolescents, ISBN 92 4 159106 4.

Wiesch DG, Meyers DA, and Bleecker ER, 1999, 'Genetics of Asthma. Current reviews of allergy and clinical immunology', *J Allergy Clin Immunol*, vol. 104, pp. 895-901.

Willett WC, Kilama WL, Kihamia CM, 1979, 'Ascaris and growth rates: a randomized trial of treatment' *Am J Public Health*, vol. 69, pp. 987-991.

Xu J, Postma DJ, Howard TD, Koppelman GH, Zheng SL, Stine OC, Bleecker ER, Meyers DA, 2000 Nov, 'Major genes regulating total serum immunoglobulin E levels in families with asthma', *Am J. Hum Genet*, vol. 67(5), pp. 1163-73.

Yamamoto R, Nagai N, Kawabata M, Leon WU, Ninomiya R, Koizumi N, 2000 Dec, 'Effect of intestinal helminthiasis on nutritional status of school children', *Southeast Asian J Trop Med Public Health*, vol. 31(4), pp. 755-61.

Yman L, 2000, 'Specific IgE in the diagnosis of parasite-induced allergy', *Allergy*, vol. 55, Suppl 59, pp. 14-17.

Zheng Y, Shopes B, Holowka D, Baird B, 1991, 'Conformations of IgE bound to its receptor Fc"RI and in solution', *Biochemistry*, vol. 30, pp. 9125-32.

Zhou H, Ohtsuka R, He Y, Yuan L, Yamauchi T, Sleigh AC, 2005, 'Impact of parasitic infections and dietary intake on child growth in the schistosomiasis-endemic dongting lake region, china', *Am. J. Trop. Med. Hyg*, vol. 72(5), pp. 534 - 539.

Glossary

Allergy:an abnormal reaction of the body to a previously encountered allergen introduced by inhalation, ingestion, injection, or skin contact, often manifested by itchy eyes, runny nose, wheezing, skin rash, or diarrhea.

Allergic rhinitis: Allergic rhinitis, known as hay fever, is caused by pollens of specific seasonal plants, airborne chemicals and dust particles in people who are allergic to these substances. It is characterised by sneezing, runny nose and itching eyes.

Anaphylaxis: Anaphylaxis is an acute systemic (multi-system) and severe Type I Hypersensitivity allergic reaction in humans and

other mammals. Minute amounts of allergens may cause a life-threatening anaphylactic reaction. Anaphylaxis may occur after ingestion, skin contact, injection of an allergen or, in rare cases, inhalation.

Atopy: A predisposition toward development of allergic reactions.

Coprolites: Coprolites are the fossils that result when human or animal dung is fossilized.

cytokines: Low-molecular weight proteins produced by many different cell types that mediate inflammatory and immune reactions.

DALYS: The Disability Adjusted Life Year (DALY) is a health gap measure that extends the concept of potential years of life lost due to premature death (PYLL) to include equivalent years of 'healthy' life lost by virtue of being in states of poor health or disability.

Dendritic cells: Professional antigen-presenting cells in different tissues that have long membrane processes resembling dendrites of

neurons.

Gene polymorphism: Coexistence of multiple alleles at a locus.

PCR: Polymerase chain reaction is a technique widely used in molecular biology to amplify a single or few copies of a piece of DNA across several orders of magnitude, to generate millions or more copies of the DNA piece.

Stunting: Defined as low height for age.

Urticaria: Urticaria (or hives) is a skin condition, commonly caused by an allergic reaction, that is characterized by raised red skin wheals (welts). It is also known as nettle rash or uredo.

Wasting: Defined as low weight for height.

Z score: Z score reflects how many standard deviations above or below the population mean a raw score is.