جامعة بابل
كلية الطب

دراسة حول تأثير زيت الحبة السوداء على الاستجابة المناعية للقاح شلل الأطفال

رسالة مقدمة إلى مجلس كلية الطب –جامعة بابل كجزء من متطلبات نيل

درجة الماجستير في الأحياء المجهرية

من قبل الطالبة

رشا جاسم موسى الورد

الخلاصة

تضمنت الدراسة مائة وسبعة وعشرين(127)طفلاً تتراوح أعمارهم بين يوم واحد و خمسة سنوات. باستخدام فحص التلازن الدموي المنفعل تم قياس المستوى المناعي لهؤلاء الأطفال من خلال علاقتة بالعمر وعدد الجرع المأخوذة من لقاح شلل ألاطفا ل.

تم اختيار تسعة وسبعين(79) طفلاً عشوائيا لدراسة أثر زيت الحبة السوداء في الاستجابة المناعية للقاح شلل الأطفال ضمن جرع وفترات زمنية مختلفة.

أظهرت النتائج أن هنالك علاقة بين عدد الجرع المأخوذة من اللقاح والمستوى المناعي لدى الأطفال حيث كان يتراوح بين (1 : 20) للأطفال غير الملقحين و (1 : 40) للأطفال المستلمين جرعة واحدة ليصل إلى (1 : 80) للاطفا ل المستلمين ثلاث جرع ليبقى على هذا المستوى مع اختلاف بسيط للجرع المتقدمة .

كما تبين وجود علاقة بين عمر الطفل والمستوى المناعي لديهم إذ أن اوطىء مستوى خلال فترة (الخدج) وأعلى مستوى خلال الثلاث سنوات الأولى من عمر الطفل حيث تراوح بين (1 : 40) و (1 : 320) أما بالنسبة للأعمار الأكبر فأنة يتراوح بين (1 : 40) و (1 : 160) .

ظهر الدور التحفيزي لزيت الحبة السوداء في الاستجابة المناعية للقاح واضحاًَ ومرتبطاً مع الجرعة المأخوذة و مدة العلاج وعدد الجرع المستلمة من اللقاح أيضاً.

وباستخدام طريقة التلازن الدموي المنفعل وطريقة الانتشار المناعي المنفرد لحساب المستوى المناعي لدى الأطفال

أظهرت التحليلات الإحصائية (المنوال) النتائج الآتية:

-المستوى المناعي للأطفال الملقحين بجرعة واحدة هو (1 :40) و (1 : 80) للأطفال الملقحين بجرعتين إلى خمس جرع .

- المستوى المناعي للأطفال المستلمين زيت الحبة السوداء بجرعة(0.6مل/كغم) لمدة 6 أسابيع كان (1 :80) و (1 :160) و (1 : 320) للأطفال الملقحين بجرعة واحدة وبجرعتين وثلاث جرع فأكثر من اللقاح على التوالي

-المستوى المناعي للأطفال المستلمين زيت الحبة السوداء بجرعة (0.3مل/كغم) لمدة ثلاثة أسابيع كان (1 :40) للأطفال الملقحين بجرعة واحدة و (1 :80) للأطفال الملقحين بجرعتين وثلاث جرع و (1: 160) للأطفال الملقحين بأربع جرع أو أكثر .

تم أخيراً دراسة مستوى الكلوبيولينات المناعية نوع (ج) للأطفال قبل وبعد إعطائهم زيت الحبة السوداء وكان الاختلاف واضحاً حيث أن المعدل لدى الأطفال قبل إعطائهم الزيت كان ((488.325 ملغم/لتر)) وقد تصاعد إلى((986.625ملغم/لتر)) بعد إعطائهم(0.6 مل/كغم)ولمدة ثلاث أسابيع ومن ثم أصبح ((1025.314 ملغم/لتر)) للأطفال المستلمين) بعد ست أسابيع من إعطائهم الزيت.

بسم الله الرحمن الرحيم

﴿ وَمَا ذَرَأَ لَكُمْ فِي الْأَرْضِ مُخْتَلِفاً أَلْوَانُهُ إِنَّ فِي ذَلِكَ لَآيَةً لِقَوْمٍ يَذَّكَّرُونَ ﴾

صدق الله العلي العظيم
(الآية ١٣ من سورة النحل)

We certify that this thesis was prepared under our supervision at the University of Babylon as partial requirements for degree of M.Sc. in Microbiology .

supervisor

Dr. Mohammed Aboud AlQarguli

Assistant Professor

supervisor

Dr.Abdul Razzaq AbdulLatif

Assistant Professor

According to available recommendation , I forward this thesis to discussion .

Dr. Mohammad Sabry

Chairman of Department of Microbiology
College of Medicine
University of Babylon

We the examining committee, after reading this thesis and examining the student in its contents , find it adequate as a thesis for the degree of master of Science in Microbiology.

Signature :
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Assist. prof.Jahfar K. Naama **Assist.prof.Yahia A.Shaheed**

Member **Member**

Approve for the College Committee on Graduate Studies

Dr. Hussein S. AL-Janabi
Dean
College of Medicine

Dedication

To my Parents, my sisters and brothers,
My husband and my best wishes in life :
Yousif and Sama

ACKNOWLEDGMENTS

I would like to express my sincere gratitude and deep appreciation to my supervisors **Dr.MohmmedA.Muhsin and Dr. Abdul –Razzaq A. Latif** for their great help, instructive advice, guidance and support throughout the course of my study.

I am also grateful to Dr. Jafer R. Mohammed and all staff in AL-.Imam-Ali Primary Health Care Center for their great assistance in sample collection. My thanks also go to Dr. Amal M. Hassan for her help and Mr.Hatam Abdul Latif, assistant professor of statistics, in the College of Medicine for his statistical advice.

I am greatly indebted to the children and their families for their great help and co-operation.

Sincere thanks to professor Salih Mahdi Hamid due to languagestic evaluation of the thesis.

Rasha

CONTENTES

TABLES

FIGURES

Abbreviations

Ab	Antibody
Abs	Antibodies
Ag	Antigen
APAbTs	Anti Polio Antibody Titers
EPI	Expanded Program Immunization
IPV	Inactivated Polio Vaccine
IR	Immune Response
$\mathcal{M}$	Mode of Antipolio Antibody Titers
$\mathcal{M}s$	Modes of Antipolio Antibody Titers
MOH	Ministry of Health
N.S oil	Nigella Sativa Oil
NIDs	National Immunization Days
R	Range
VAPP DPT	Vaccine Associated Paralytic Poliomyelitis Diphtheria Tetanus Pertusis.
WHO	World Health Organization

Abstract

One-hundred and twenty seven children between one day and five years of age were included in this study. The pretreated level of anti polio virus antibodies titers(APAbTs) was estimated and evaluated according to the number of oral polio vaccine (OPV) doses received by children as well as their ages. Seventy-nine children were selected to study the effect of N.S oil (which is used in different doses and in different durations) on specific immune response to polio virus. The total immunoglobulin class G (IgG) concentration was studied for the children who took ٠.٦ ml/Kg /day. A Passive heamagglutination test was found to be a sensitive method for measuring specific anti polivirus antibodies. The immunoglobulins class IgG was, however, determined by using the single radial immunodiffusion test.

The results reveal that there is a clear relationship between the number of OPV doses received by children and the level of APAbTs. This titers increases gradually from ١:٢٠ for unvaccinated children to ١:٤٠ for the children who had one OPV dose and ١:٨٠ for those who had three OPV doses and remained stable at this level with slight variability for further doses. There is some correlation between the age of children and APAbTs: the lowest level is during neonatal period, and the range of APAbTs during the first three years (apart from

neonatal period) is from ١:٤٠ to ١:٣٢٠, whereas for older age it is ١:٤٠ to ١: ١٦٠.

The role of N.S oil on specific IR to polio appears to be an inducer and exhibits an enhancing effect on APAbTs. In addition to is magnitude this enhancing effect correlates with the dose of N.S oil, duration of treatment, and the number of OPV doses received by children before treatment. The Modes to APAbTs ($\mathcal{M}$) in the pretreatment condition or the untreated children are ١:٤٠ for the children who had one OPV dose, and ١:٨٠ for those who had two or more OPV doses. For the children treated by ٠.٦ ml/kg / day of N.S oil for ٦ weeks, the $\mathcal{M}$ is ١:٨٠, ١:١٦٠, and it is ١:٣٢٠ for those who had one, two, three and four (or more) OPV doses respectively. The $\mathcal{M}$ in children treated with ٠.٣ ml/kg / day for three weeks is ١:٤٠ for those who had one OPV dose, ١:٨٠ for those who had two or three OPV doses, and ١:١٦٠ for those who had four (or more) OPV doses.

The stimulation effect of N.S oil on total IgG for children before and after treatment with N.S oil has been studied. It correlates with the duration of treatment, where the mean of total IgG is ٤٨٨.٣٢٥ mgdl before treatment, and ٩٨٦.٦٢٥ and ١٠٢٥.٣١٤ mgdl for children treated by ٠.٦ ml/kg / day for three and six weeks respectively.

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الخلاصة

تضمنت الدراسة مائة وسبعة وعشرين(١٢٧)طفلاً تتراوح أعمارهم بين يوم واحد و خمسة سنوات. باستخدام فحص التلازن الدموي المنفعل تم قياس المستوى المناعي لهؤلاء الأطفال من خلال علاقتة بالعمر وعدد الجرع المأخوذة من لقاح شلل ألاطفا ل.
تم اختيار تسعة وسبعين(٧٩) طفلاً عشوائيا لدراسة أثر زيت الحبة السوداء في الاستجابة المناعية للقاح شلل الأطفال ضمن جرع وفترات زمنية مختلفة.
أظهرت النتائج أن هنالك علاقة بين عدد الجرع المأخوذة من اللقاح والمستوى المناعي لدى الأطفال حيث كان يتراوح بين (١ : ٢٠) للأطفال غير الملقحين و (١ : ٤٠) للأطفال المستلمين جرعة واحدة ليصل إلى (١ : ٨٠) للاطفا ل المستلمين ثلاث جرع ليبقى على هذا المستوى مع اختلاف بسيط للجرع المتقدمة .
كما تبين وجود علاقة بين عمر الطفل والمستوى المناعي لديهم إذ أن اوطىء مستوى خلال فترة (الخدج) وأعلى مستوى خلال الثلاث سنوات الأولى من عمر الطفل حيث تراوح بين (١ : ٤٠) و (١ : ٣٢٠) أما بالنسبة للأعمار الأكبر فأنة يتراوح بين (١ : ٤٠) و (١ : ١٦٠) .
ظهر الدور التحفيزي لزيت الحبة السوداء في الاستجابة المناعية للقاح واضحاً ومرتبطاً مع الجرعة المأخوذة و مدة العلاج وعدد الجرع المستلمة من اللقاح أيضاً.
وباستخدام طريقة التلازن الدموي المنفعل وطريقة الانتشار المناعي المنفرد لحساب المستوى المناعي لدى الأطفال
أظهرت التحليلات الإحصائية (المنوال) النتائج الآتية:
-المستوى المناعي للأطفال الملقحين بجرعة واحدة هو (١ :٤٠) و (١ : ٨٠) للأطفال الملقحين بجرعتين إلى خمس جرع .
- المستوى المناعي للأطفال المستلمين زيت الحبة السوداء بجرعة(٠.٦مل/كغم) لمدة ٦ أسابيع كان (١ :٨٠) و (١ :١٦٠) و (١ : ٣٢٠) للأطفال الملقحين بجرعة واحدة وبجرعتين وثلاث جرع فأكثر من اللقاح على التوالي
-المستوى المناعي للأطفال المستلمين زيت الحبة السوداء بجرعة (٠.٣مل/كغم) لمدة ثلاثة أسابيع كان (١ :٤٠) للأطفال الملقحين بجرعة واحدة و (١ :٨٠) للأطفال الملقحين بجرعتين وثلاث جرع و (١: ١٦٠) للأطفال الملقحين بأربع جرع أو أكثر .
تم أخيراً دراسة مستوى الكلوبيولينات المناعية نوع (ج) للأطفال قبل وبعد إعطائهم زيت الحبة السوداء وكان الاختلاف واضحاً حيث أن المعدل لدى الأطفال قبل إعطائهم الزيت كان ((٤٨٨.٣٢٥ ملغم/لتر)) وقد تصاعد إلى((٩٨٦.٦٢٥ملغم/لتر)) بعد إعطائهم(٠.٦ مل/كغم)ولمدة ثلاث أسابيع ومن ثم أصبح ((١٠٢٥.٣١٤ ملغم/لتر)) للأطفال المستلمين) بعد ست أسابيع من إعطائهم الزيت.

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CHAPTER ONE

INTRODUCTION

CHAPTER ONE
INTRODUCTION

A great variety of infectious microbes such as virus, bacteria, fungi, protozoa and parasites are found in our environment. In normal individuals, most of these microbes are short living and of little morbidity and mortality, except in few situations, this is due to the immune system which combats infectious agents in many different forms . A wide variety of immune responses are required to deal with each type of infection (Roitt *et.al.*,٢٠٠١) .

Man is supplied with all capabilities of defense mechanisms. Innate immunity is the first line of defense followed by the specific immune response.

Following exposure to an antigen (Ag), the development of specifically altered reactivity is called the immune response (IR). It is triggered when the Ag enters the body and encounters specialized cells, called the Antigen Presenting Cells(APC). Then complexes and intricately regulated sequences of events involve several cell types. (Hyde,٢٠٠٠ ; Parslow ,٢٠٠١). The (IR) involves two phases: the Recognition Phase and the effector phase. During the Recognition Phase, Ag activates specific cells that recognize it, whereas in the Effector Phase the lymphocytes coordinate with an IR that eliminates the source of Ag, and this is achieved by clonal selection (Roitt and

Rabson ,٢٠٠٠ ; Paraslow , ٢٠٠١). The Recognition Phase involves the recognition of the Ag by particular lymphocytes. B-cells usually recognize intact Ag molecule, where Tcells recognizes Ag fragments on the surface of other cells. The recognition leads to the clonal expansion and differentiation of the effector and memory cells (Brooks *et.al.*,١٩٩٨;Roitt and Rabson,٢٠٠٠). The IR has at least four major functional properties : these are (Hyde, ٢٠٠٠) :

A-Self-nonself discrimination : It responds to foreign micro-organisms and even to cells and tissues from other hosts but not the constituents that make up the host body.

B-Specificity : It is the ability to detect subtle difference target epitopes, and to respond to each of these individually.

C-Memory: It is the capacity of the immune system to provoke more rapid and more vigorous responses in the subsequent encounters than those occurring at the inial encounter.

D-Adaptiveness : It is the ability to respond to previously unrecognized Ags.

Many different ways were used to enhance or help immune system to control and cure from infectious agents i.e.; (herbal plant, vaccines, antibiotics, immunoglobulin) .

It has been reported that Nigella sativa (N.S) is an important herbal plant to cure many of the common sicknesses. Reported the Black Seed is important to heal many illnesses except death (Al-Rawi,١٩٨٨). The reason might be due to the complexes of

the chemical structure of the N.S;which is reported to have over one hundred different chemical constituents in addition to sources of the essential fatty acids. Thus, the oil of N.S is used medically in many aspects including the enhancement of the IR. (Meral *et.al.*, ٢٠٠٠ ; Cindy, ٢٠٠٠) .

Poliomyelitis disease is caused by a dangerous virus , that has received considerable attention all over the such World Health Organization (WHO) and all countries. Oral polio vaccine (OPV) induce humeral immunity and also systemic immunity, particularly inside the intestine (Ghendon and Robertson ,١٩٩٤). So, an important program vaccination scheme has been adopted and implemented, and is still continuous to control the disease .

Aims of Study

- Estimation of anti polio antibody titers (APAbTs) and evaluate for both response to poliovirus vaccine and poliovirus vaccination programs.
- Study the effect of N.S. oil on specific humoral immunity to poliovirus in vivo.
- Estimation the best dose of N.S oil and duration of treatment for reaching a protective level of immunity.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

٢.١.History of Poliomyelitis

The disease of poliomyelitis has a long history. The first example may even have been more than ٣٠٠٠ years. Two Egyptian mummies showed limp deformities characteristic of poliomyelitis. The first one was ٣٧٠٠ B.C and the second ١٢٠٩ B.C (Melnick ,١٩٨٤;Robbins,١٩٨٦). In ١٢٠٩ B.C, Mummy Giptah was found with an equines foot, and the priest Ruma with a withered leg and equines foot, shown on a plaque and probably poliomyelitis in ١٥٨٠-١٣٥٠B.C(Grist and bell,١٩٨٤;Stanely and Walter,١٩٩٩).In ١٥٥٩ a painting by Pieter Bruegel shows a crippled beggar, not necessarily polio although it did probably occur during that period in England (Sabin ,١٩٨١). In the eighteenth century the first description of poliomyelitis was given by Underwood ; while in the nineteenth century the first epidemic of poliomyelitis occured in Island of St.Helena.(Thomas,١٩٨٤;Samuel *et.al.*, ١٩٩٨).

Hein was the first who described some scattered sporadic cases in ١٨٤٠ and noted apparently major epidemic in ٤٤ cases in Stockholm during the summer of

١٨٨٧. The first description of the pathological processes in poliomyelitis with the involvement of the anterior horn cells of the spinal cord was given by Duchene in ١٨٥٥(Samule *et.al.,* ١٩٩٨;Stanely and walter, ١٩٩٩). The first major polio epidemic reported in the United States occurred in Vermont during the summer of ١٨٩٤. It consisted of ١٣٢ total cases, including some adults (Melnick ,١٩٨٤;Robines,١٩٨٦)Not until ١٩٠٨ was the actual polio virus identified by two Austrian physicians, Karl Land Steiner and Popper by experiment producing paralytic disease in monkeys by spinal cord inoculation of brain tissue from fetal cases (Samuel *et.al.,* ١٩٩٨) (Stanely and waltar,١٩٩٩). In ١٩٠٩, the passage of the virus through a monkey was experimented by Flexner and , the year ١٩١٦ saw a large outbreak of polio in the United States. The total number of affected individuals was unknown; over ٩٠٠٠ cases were reported in New York City alone (Melnick ,١٩٨٤ ;Robines ,١٩٨٤). In the ١٩٣٠s Paul and Track recovered the virus in feces of both patients and healthy carriers repeatedly over a period of weeks and the concept of poliomyelitis as an enteric infection became established. In ١٩٤٨, Bodian established that three closely related but antigenically distinct virus strain could cause poliomyelitis. These viruses which are designated polio virus types ١, ٢, ٣ are classified as picorna viruses which belong to the enterovirus group(Melnick ,١٩٩٥). The growth of the virus on tissue cultures happened in ١٩٤٩

(Stanley and Walter,١٩٩٩;Melnick,١٩٩٠), the first large scale trial of Salk (dead vaccine) by injection, did in ١٩٥٤ and eventually the first general use of sabin (live attenuated vaccine) by mouth in ١٩٥٨ (Stanley and Walter ,١٩٩٩).

٢.١.٢.Epidemiology of Poliomyelitis

Poliomyelitis is an acute viral infection which ranges in severity from a non-specific illness to paralysis with permanent disability. Polioviruses have become established throughout most of the world's population and survival for many centuries in an endemic fashion, reaching almost all infants very early in life. Passive immunity is transferred from mother to offspring and many infants subsequently experienced their first polioviruses infection during the first few months of life while maternal antibodies still provide protection. Furthermore, because such large proportion of poliovirus infection take an inapparent or sub clinical form, the paralytic cases that occur could go unnoted in population faced with very high infant and child mortality rates (Christie, ١٩٨٧ ; Streble ,١٩٩٢ ; Melnick, ١٩٩٥). Poliomyelitis has had three epidemiologic phases: endemic, epidemic and vaccine era. The first two reflect prevaccine patterns, and the generally accepted explanation is that improved systems of hygiene and sanitation in cooler climates promote the transition from endemic to epidemic paralytic disease in those societies (Minor ,١٩٩٦ ; Jawtez,١٩٩٨;

Stanely and Walter,١٩٩٩). Most infections with poliovirus are asymptomatic and less than ١٪ result in paralysis. Paralysis rates are highest with poliovirus type ١ (the most frequent cause of epidemics and may be less than ١ case per ١٠٠٠ infections with type ٣). A syndrome identical with polio is caused by other enteroviruses notably enterovirus ٧١, and some atypical cases may be difficult to differentiate clinically from Guillian Bare syndrome (Jawtez , ١٩٩٨; Munsat, ١٩٩١).

Symptomatic cases are typically characterized by two phases. The first, a non specific febrile illness followed (in a small percentage of cases) by aseptic meningitis and/or paralytic disease. Depending on the site of paralysis, poliomyelitis can be classified as a spinal, bulbar or spin bulbar disease. Case fatality is variable. It is highest in the oldest patients and may reach ٥-١٠٪. The disease is transmitted from person to person primarily by direct fecal oral contact. However, the virus can also be transmitted by indirect contact with infectious saliva or feces, or by contaminated sewage or water (Gromeir and Wimmer, ١٩٩٨; WHO, ١٩٩٩).

Viruses can be recovered from the pharynx and intestine of patients and healthy carrier, the prevalence of infections is highest among household contacts when the first case is recognized in a family, all susceptible in the family are already infected (Jawetz , ١٩٩٨). There is no evidence of a persistent wild poliovirus carrier state or animal or insect reservoir and the

virus can survive only for finite periods of time in the environment.(Streble et.al., ١٩٩٢).

Higher non-human primates (chimpanzees and gorillas) are susceptible to infection and disease but these populations are not sufficiently large to sustain polio virus transmission in the absence of human infection. Humans are the only natural reservoir of poliovirus; therefore, once poliovirus is deprived of its human host through immunization, it will rapidly die out (Dowdle,١٩٩٧ ; WHO, ١٩٩٩).

Poliovirus in sewage reflects the prevalence of infection in the community; contamination of surface water may occur through discharge of untreated or inadequately treated sewage or run off from contaminated soil (WHO, ١٩٨٨).

٢.١. ٣.Viral Entry, Spread and Neurovirulance

Poliovirus enters the body through the mouth and implants itself in the mucosa of the throat (when large numbers are ingested) and in the intestine (Faden *et.al.,* ١٩٩٣). The virus multiplies in the intestinal epithelium or within the collection of the lymphoid tissue known as payer's patches (Onorato *et.al.,* ١٩٩١;Arie *et.al.,*٢٠٠٠).Newly synthesized virus is released into the intestinal lumen and make its way to local lymph nodes. It can be detected very soon after infective virus, then it reaches the systemic lymph nodes and the blood stream producing a primary viremia(WHO,١٩٨٨ ; Onorato *et.al.,*١٩٩١).

The appearance of the virus in the blood coincides with the period of what is called the ‘minor illness’; non specific syndrome which occurs in the majority of infection and subsides in ١-٢ days (Racaniello, ١٩٩٨). Maintenance of persisting viremia is required for the viral invasion of the central nervous system, a rare event that occurs in approximately ١-٢٪ of all infections and the ability of the poliovirus to infect certain types of virus-binding activity in these tissues such as brain spinal cord and intestine (Modlen *et.al.,*١٩٩٧ ;Munsat ,١٩٩١ ;Melnick, ١٩٩٥). In Central Nervous System (CNS) poliovirus replicates mainly in the motor neurons in the anterior horn of the spinal cord, in the brain stem and in the motor cortex. Paralysis results when viral replication destroys sufficiently large number of motor neurons in region of the central nervous system that controls specific muscles(Arie *et.al.,*٢٠٠٠). Neurovirulance in the general refers to the ability of poliovirus to replicate in, and destroy cells of, the central nervous system(Melnick ,١٩٩٠). Both naturally occurring polioviruses and variants isolated in the laboratory display a wide range of neurovirulance as determined by the inoculation of different experimental animals by different routes (Jublett *et.al.,* ١٩٨٠ ; Racaniello ١٩٨٨ ;Racaniello ,١٩٩٢ ;Modlen *et.al.,*١٩٩٧).

٢.١.٤. Physical and Chemical Characteristics of Poliovirus

Poliovirus is stable at acid pH and can survive for weeks at room temperature and for many months at zero to ٨°C(WHO,١٩٩٩). As with other enteroviruses, poliovirus is resistance to ether, ٧٠٪ alcohol and other laboratory disinfectants. Treatment with ٠.٣٪ formaldehyde, or free residual chlorine at a level of ٠.٣ to ٠.٤ parts per million rapidly inactivates poliovirus, as does exposure to a temperature of ٥٠°C or higher and to ultraviolet (Minor and Bell, ١٩٩٠).

٢.١.٥. The Nature of Immunity against Poliomyelitis

A- Response to natural infection : Following natural exposure, IgM appears in the serum about ٧-١٠ days after infection. The IgM Response is ٢-٨ fold and level peaks at greater than IgG response about two week after exposure, and disappears from the serum within ٦٠ days. Immunoglobulin G (IgG) level increases steadily starting from two weeks after infection and persisting in the body for months to years . IgA antibody appears in the serum two to six weeks after exposure, remains at low levels, and persist for years (EPI, ١٩٩٣ ;Samuel *et.al.,*١٩٩٩). Serum antibodies are type-specific. There may be low degrees of heterotypic antibody induced infection especially between type ١ and type ٢ polioviruses (Stanly and Walter ,١٩٩٩). It is believed that serum

neutralization antibodies (primary IgG) persist for life (Ogra ,١٩٨٤ ;EPI, ١٩٩٣;). Passive immunity is transferred from mother to fetus via the placenta (Onorato *et.al.,* ١٩٨٨ Ananthakrishman *et.al.,* ١٩٨٨). Poliovirus infection also induces development of secretary IgA antibody (Ogra,١٩٨٤ Ramsay *et.al.,*١٩٩٤; Fortran *et.al.,* ١٩٩٥).

B-The serum and secretary immunity after natural infection.

a.Duration of serum antibody: After natural infection with poliovirus, serum IgG antibodies persist for many years. For example, in an isolated Eskimo population, IgG persists for at least ٤٠ years after natural infection in the absence of any further exposure to poliovirus (Orenstein *et.al.,* ١٩٨٨).

b.Duration of immunological reaction in the intestine: The persistence of local intestinal IgA can be analyzed by studying intestinal resistance to infection with polioviruses. It was suggested that a dcrease in serum antibody titer could be a good indicator of reduction in intestinal local immunity. (Ghendon and Robertson ,١٩٩٤).

c.Duration of local immunity in the nasopharynx: Secretary IgA antibody has been detected in the nasopharyngeal secretion of individuals ١٠-١٥ years after natural infection. (Ogra ,١٩٨٤ ;Fortuin *et.al.,* ١٩٩٥).

C-Risk factors:

A number of factors may affect the potential for infection with poliovirus or the severity of clinical poliomyelitis.

a.Immunodeficiency

Persons with congenital and acquired B-cell immuno-deficiency have markedly increased risk of (OPV) vaccine associated with paralytic poliomyelitis (VAPP). Approximately ٢٩٪ of the ٨٩ VAPP cases reported to occur between ١٩٨٥ and ١٩٩٧ are immunodeficient. Two thirds of immunodeficint VAPP cases have occurred among infant OPV recipients and the remaining immunodeficient patients who are mostly adults have acquired VAPP via exposure to infants recently fed OPV. The risk of VAPP among newborn infants with a congenital B-cell immunodeficiency disorder is estimated to be ٢٠٠٠ higher than for an immunocompetent infant (Vernon *et.al.,*١٩٩٠;Streble *et.al.,*١٩٩٢;WHO,١٩٩٢) .

b.Injection

it has been shown that administration of multiple intramuscular injection during the incubation period of wild poliovirus increases the risk of paralytic disease more than ten folds (Streble *et.al.,*١٩٩٤). Cases of DPT injection associated paralysis are usually reported in children six months of age or older, reflecting the fact that most infants are protected from

poliomyelitis during the first few months of life by maternal antibodies. Therefore, it is desirable to complete a primary series of OPV/DPT immunization by ٤ months of age during which time the risk of post-injection poliomyelitis is extremely low (Sutter *et. al.,* ١٩٩٢).

c.Physical activity:

Early studies show that for persons who develop paralytic poliomyelitis, the intensity of physical activity in the first ٤٨ hours after the onset of paralysis correlated with the severity of paralysis. In contrast, physical activity prior to the onset of paralysis dose not relate to subsequent paralysis (Sabin,١٩٨٥).

d. Pregnancy, age and sex:

poliovirus infections occur equally in male and female although paralysis is more common in boys (Ryder *et.al.,*١٩٩٣).Among adults, women are at greater risk of infection but are not necessarily at a greater risk of paralysis. Clinical data suggest that both the incidence and the severity of poliomyelitis may increase in pregnant women (Harjulehto *et.al.,*١٩٨٩, Harjulehto *et.al.,*١٩٩٣).

e.Tonsillectomy

Persons with tonsillectomies have a more risk than those with intact tonsils. Later studies indicated that previous tonsillectomy at any time increased the risk of bulbar

poliomyelitis compared with children who had intact tonsils , seronegative childrense who had their tonsils removed had a lower level of secretory antibody response in the pharynx when immunized with OPV (Ogra and Karzon ١٩٧١;Patriarca *et.al*., ١٩٩٧).

٢.١.٦.Vaccine:

Immunization is the process of inducing immunity artificially by either vaccination (active immunization) or administration of antibodies(passive immunization).(Behrman *et.al.*,٢٠٠٠, ٢٠٠٤).

Vaccination is the administration of any vaccine or toxoid (inactivated toxin) for preventing disease. The first vaccine was named after Vaccinia, the cow pox virus. Jenner pioneered its use two hundred years ago and it was considered the first scientific attempt to prevent an infectious disease (small pox) (Behrman *et.al.*,٢٠٠٠, Roitt *et.al.*, ٢٠٠١). The work of Pasteur emerged one hundred years later when the general principle governing vaccination was known and altered preparation of microbes used to generate enhanced immunity against virulent organisms (Roitt *et.al.*, ٢٠٠١; Geison, ١٩٩٥). With Brunet's clonal selection theory (١٩٥٧)and the discovery of B and T lymphocyte, the key mechanism became clear and the antigens of a vaccine must induce clonal expansion in specific T or B cells leaving behind a population of memory cells (Bottiger,١٩٨٤;Linder,١٩٩٤;Roitt *et.al*,٢٠٠١). The principle of

vaccination is based on two key elements of adaptive immunity, namely specificity and memory. The memory cells allow the immune system to mount a much stronger response on a second encounter with antigen, and this secondary response is both faster to appear, usually within four to five days and more effective than the primary immune response(Sabin,١٩٨٥;Roitt,*et.al.*,٢٠٠١;Behrman,*et.al.*,٢٠٠٤).The response to vaccine in clinical practice is assessed by the serum concentration of specific antibodies .The presence of antibodies usually correlates with protection . (Behrman *et.al.*,٢٠٠٠, ٢٠٠٤). The art of vaccination to produce antigenic preparations from the pathogen depend on the fact that

a. It is safe to administer.

b. It induces the right sort of immunity.

c. It is affordable by the population at which they are aimed (Linder *et.al.*,١٩٩٤;Roitt *et.al.*, ٢٠٠١).

The type of antigens used in a vaccine depends on many factors. The more antigens of the microbe retained in vaccine, the better. Live vaccine can be natural or attenuated organisms. It is a part from vaccinia; no other completely natural organism has ever come into standard use. Like bovine and simian, rota virus has been tried in children, and in the Middle East and Russia, Leishmania infection from mild cases is reputed to induce immunity (Adim, ١٩٨٥; Roitt *et.al.*,٢٠٠١) but the attenuation (changes) of micro-organisms makes them less able

to grow and cause disease in their natural host. Galmette and Guerin were the first who successfully done with a bovine strain of *Mycobacterium tuberculosis*, which during ١٣ years (١٩٠٨-١٩٢١) of culture in vitro, changed into much lesser virulent form currently known as BCG (Bacilli Calmette – Gurine) that has at least protective effects against TB. The real successes with virus vaccine started with the [١٧D] strain of yellow fever virus obtained by passage in mice and chicken embryos (١٩٣٧);this was followed by similar approach with polio, measles, mumps and rubella (Fulginiti,١٩٨٥ ;Roitt *et.al.*, ٢٠٠١). Killed vaccines are intact but non living organisms like virus vaccines (polio, rabies ,influenza ,hepatitis -A-)and bacterial vaccine (pertusis ,typhoid ,cholera ,plague ,Qfever). Inactivated toxins and toxoids are the most successful of all bacterial vaccines (Linder *et.al.*,١٩٩٤).

In the ١٩٢٠s, during work on the production of animal sera for human therapy, it was discovered that certain substances (aluminum salts) added to, or emulsified with, an antigen to enhance antibody production act as adjuvant. Aluminum hydroxide is still widely used with diphtheria and tetanus toxoids (Roitt *et.al.*, ٢٠٠١). In addition, an adjuvant must be safe, stable and affordable and should target particular cells of the immune system with reasonable specificity (Funkhous *et.al.*, ١٩٨٧; Lieberman *et.al.*, ١٩٩٥).

In technological developments it is apparent that the number of general strategies for making new vaccines is rapidly expanding such that all antigens or epitopes can be presented in a highly immunogenic form in the context of a live or non live vaccine (Rechman *et.al.*,١٩٨٨). Protein antigens alternatively can be expressed through a DNA-based vaccine. Further understanding of gene function in viral and bacterial pathogens should enable live vaccines to be more stably and predictably attenuated as vaccines and as live vectors for immunization against pathogens (Williams *et.al.*,١٩٩١; Darji *et.al.*,١٩٩٧). Adjuvant technologies should advance to the point at which formulations that are more potent than aluminum salts to gain a wide spread should use killed vaccines and an oral delivery of purified proteins to become feasible for immunization(Wessels *et.al.*, ١٩٩٠;Darji *et.al* .,١٩٩٧) .

٢.١.٧. Development of the Concept of Polio Vaccine

Attenuated viruses may be isolated by the passage of the virus in different animal hosts or in various cultured cells, or in a combination of both (Sabin, ١٩٨٥).

Flexner and Amoss in the ١٩٢٠s were the first who suggested the concept of using such “weakened” polio virus to immunize against poliomyelitis (Racaniello, ١٩٨٨). The first use of the term “attenuated” in connection with polio virus vaccine is attributed to Kolmer who has believed that extensive passage

of the strain of poliovirus in monkeys [MV] produces a virus "of greatly reduced infectivity for human being" (Sutter, ١٩٩٥).

Theiler (١٩٤١) was the first who truly isolated polio virus strain after ١٥٠ passages in mice. The P٢/Lansing strain of polio virus no longer caused paralysis after intracerebral inoculation of rhesus monkeys (Morens *et.al.*,١٩٩١). The early mouse-passage P٢/Lansing virus induced paralysis in monkeys at high rate then subsequently Enders and his colleagues showed that the growth of the P١/Brunhild strain of poliovirus in cultured human non-neural tissues produced a virus with reduced neuro-virulence in monkeys (Dalakas *et.al.*,١٩٨٤). The passage of type ٢ strain of poliovirus in rodents produces a strain which when administered orally to humans induces an immune response in the absence of the disease (Murdin *et.al.*,١٩٩٢).

٢.١.٨.Poliovaccines

Two different kinds of polio vaccines are available. A live attenuated oral polio vaccine (OPV) developed by Albert Sabin in ١٩٦١ and an inactivated killed injectable polio vaccine (IPV) originally developed in ١٩٥٥ by Jones (EPI, ١٩٩٧; Stanely and walter, ١٩٩٩). The two vaccines are highly effective against all three types of poliovirus, but there are significant differences in the way each vaccine works (WHO, ١٩٩٦).

Inactivated polio vaccine (IPV) works by producing protective antibodies in the blood, thus preventing the spread

of poliovirus to the CNS (Behrman,*etal.*, ٢٠٠٠) . The inactivated polio vaccine provides only very low level of immunity to poliovirus inside the gut (EPI, ١٩٩٥); therefore , the inactivated polio vaccine provides an individual protection against polio paralysis but only marginally reduces the spread of wild poliovirus in a person immunized with IPV because the virus multiplies inside the intestine and is shed in stools(Evans, ١٩٨٤ ;WHO,١٩٩٦; Hinman ,*etal.*, ١٩٨٧). Consequently, the IPV can not be used to eradicate polio, let alone its cost which is over five times then that of OPV. The cost includes that of needles and syringes as well as the need for trained health workers to administer the vaccine using sterile injection procedures(Salk,١٩٨٤;Beale,١٩٩٠ ; Behrman,*et.al.*,٢٠٠٠).

The advantage of the IPV is that it does not carry the risk of paralysis associated with OPV (WHO, ١٩٩٧).OPV limits the multiplication of wild (natural occurring) virus inside the gut (Behrman,*et.al.*, ٢٠٠٠; Jawetz, ١٩٩٨).This vaccine works by inducing not only humeral immunity but also mucosal immunity particularly inside the intestine. This vaccine therefore, produces good barrier against circulation of virus and creates passive immunity to close contacts by means of shedding of vaccine virus in the stools of recently immunized children (Salk,١٩٨٤; Behrman *et.al.*, ٢٠٠٤).Another advantage is that it is relatively

٢.٢. Nigella Sativa

The plant is also called Black Seed, Black Cumin, Nigella Sativa, Kalonji, Schwarz Cummel, Sinonj Nutmeg flower, Black Caraway, Habba Sowda, Fennel Flower (AL- Rawi,١٩٨٨;EL-Hazmi *et.al.*,١٩٩٢) .It is indigenous to the Mediterranean region(AL-Rawi,١٩٨٨). In Iraq, it is distributed in Mosul, Erbil, Kirkuk, Sulaimaniya as well as south Jazira (AL-Rawi,١٩٨٨). It is a herbaceous plant and originates from the common fennel flower plant (Cindy,٢٠٠٠). N.S is sometimes mistakenly confused with the fennel herb plant [Foeniculum Vulgare] (EI-Feki *et.al.*, ١٩٩٧). The black seed is a plant which has been used widely as a folk treatment for about ٢٠٠٠ years(EI-Tahir *et.al.*, ١٩٩٣). In the Prophet Tradition, it is written that the N.S oil prevents and, indeed heals all illnesses except death itself (AL-Rawi,١٩٨٨).In the Easton's Bible dictionary it is clarified that the Hebrew word for black cumin, "Ketsah",undoubtealy refers to the N.S, a small annual of the order Ranunculaceae which grows widely in the Mediterranean countries and is cultivated in Egypt and Syria for its seed (EI-Kadi and Kandil ,٢٠٠٤) N.S was discovered in Tutankhamen's tomb implying that it played an important role in ancient Egyptian practices(Sanamin ٢٠٠٤), whereas the famous Greek physicians used black cumin seed to treat headaches, toothaches, nasal congestion and intestinal worms. They are also used as a diuretic to promote menstruation

and increase milk production(Shamis AI-Deen ,١٩٩٠ ; Morsi ,٢٠٠٠).

٢.٢.١.Description of Nigella Sativa:

It is an aromatic plant from the Ranceculaceae family. This family includes many kinds which vary in shapes and chemical structure. Among the list the most important kinds are:

a.The common Nigella Sativa: This kind has a fast and strong growth with an average height of ٦٠ cm. It also has heavy ramification. The leaves are divided into small parts of filamentary shape. Roses are white mixed with blue colour. The seeds have aromatic smell. (El-Hazmi *et.al.*, ١٩٩٢).

b.The Damascus Nigella Sativa: This resembles the first kind but differs in the shape of leaves, which are divided into very long and thin parts. The roses are big and blue in color (El-Hazmi *et.al.*, ١٩٩٢).

c.The Eastern Nigella Sativa: This kind is characterized by its weak growth it's length is no more than ٤٠ cms. The roses are yellow with red spots. (El-Hazmi *et.al.*, ١٩٩٢).

٢.٢.٢.Chemical Composition of Nigella Sativa :

Black Seed contains about ١٠٠ valuable nutrients(Ali,١٩٩٧). It contains about ١٢٪ protein, ٣٨٪ carbohydrates and ٣٥٪ plant fat and oils(Hammed *et.al.,* ٢٠٠٢ ;AI –Kaisey *et.al.,* ٢٠٠٢) and also contains vitamin B١, B٢, niacin and vitamin C (Shamis AI-Deen, ١٩٩٠)The active ingredients of Black Seed (Thymoquinon Nigellon) and Black Seed oil is ٣٥٪ more concentrated than the raw seed and they are rich in polyunsaturated fatty acid known as essential fatty acid (AI –Kaisey, ٢٠٠٢).

Table (١) : The Biochemical Constituents of Nigella Sativa Arranged in an alphabetical Order(Haq *et.al.*,١٩٩٥ Ali, ١٩٩٧;Ghosheh *et.al.*,١٩٩٩).

١	Alanine	٢٤	Lysine
٢	Ametole	٢٥	Magnesium
٣	Arginine	٢٦	Methionone
٤	Ascorbic acid	٢٧	Myristic acid
٥	Asparagines	٢٨	Nigellin
٦	Calcium	٢٩	Nigellon
٧	Campsterole	٣٠	Oleic acid
٨	Carvine	٣١	Palmitic acid
٩	Cymene	٣٢	Phenyl alanine
١٠	Cystine	٣٣	Phytostorols
١١	Dehydroascorbic acid	٣٤	Potassium
١٢	Eicosadienoic acid	٣٥	Beta sitosterol
١٣	Glucose	٣٦	Alpha spinasterol
١٤	Glutamic	٣٧	Sodium
١٥	Glycine	٣٨	Stearic acid
١٦	Iron	٣٩	Stigma sterol
١٧	Isoleucine	٤٠	Tann
١٨	Kaempferol	٤١	Threonine
١٩	Leucine	٤٢	Thymohydroquinine
٢٠	d-limonene	٤٣	Thymoquinone
٢١	Linoleic acid	٤٤	Tryptophan
٢٢	Linolenic acid	٤٥	Tyrosine
٢٣	Lipase	٤٦	Zinc

Table (٢) : Composition of Black Cummin Seed Oil Essential Fatty Acids (EFAs)(AI –Rawi ,١٩٨٨).

Essential Fatty Acids (EFAs)	%
Myristic acid	٠.٥٪
Palmitic acid	١٣.٧٪
Palmiteic acid	٠.١٪
Stearic acid	٢.٦٪
Oleic acid	٢٣.٧٪
Linolenic acid (omega-٦)	٥٧.٩٪
Linolenic acid (omega-٣)	٠.٢٪
Arachidonic acid	١.٣٪

٢.٢.٣.Medical Uses of N.S Oil

The Oil of N.S is used in different folk treatments: burns, acne, skin rash, falling hair, asthma, aches and pain. It also

regulates blood sugar level, health supplement and blood cholesterol (Shamis AI –Deen, ١٩٩٠; Sanamin, ٢٠٠٤) .In addition, the oil of N.S is used in the treatment of liver diseases, gall bladder problems as well as heart(EL-Tahir *et.al.*, ١٩٩٣). This oil is also used for treating immune disorders; it also has antimicrobial activities: the gram positive bacteria represented by S*taphylococcus aureus*, gram negative bacteria represented by *Pseudomonas aeruoginosa*, *Escherischia coli* and pathogenic yeast *Candida albicans* (Hanafy, ١٩٩١; Baqir *et.al.*, ٢٠٠٢). The oil is used as anti-tumor and the active ingredients are thymoquinone and dithymoquinone both of which inhibit tumor cell in vitro experiments even tumor cells resistance to anti-cancer drug (Worthen *et.al.*, ١٩٩٨). Recent studies show that when the oil of N.S is incubated with cancer cell, it cannot produce fibroblast growth factor and the protein colleagues, both necessary for blood vessel growth in the tumor (Worthen *et.al.*,١٩٩٨; Baqir *et.al.*, ٢٠٠٢); without blood supply tumor cannot grow.

٢.٢.٤.Nigella Sativa and Immune System

N.S oil has been used in many countries of the Middle and Far East as a natural treatment for more than ٢٠٠٠ years. There are many Traditions that have mentioned this seed for more than ١٤٠٠ years(EI –Kadi and Qandil ,٢٠٠٤). The role of the N.S in natural immunity was not estimated until ١٩٨٦ (Sanamin ,٢٠٠٤). N.S oil enhances both the immunity system of the body and improves its function (Cindy, ٢٠٠٠). N.S oil has been mentioned to stimulate the immune system when the T-cells treated with Nigella Seed protein produce greater amount of cytokines, specifically interleukin-١ beta and tumor necrosis factor (Haq-A *et.al.*,١٩٩٥, ١٩٩٩). It has been also mentioned that it supports increased production on immune cells, bone marrow cells and B-cells that produce antibodies and stimulated neutrophil activity (Sanamin, ٢٠٠٤). N.S oil has been found to provide children with all the energy required for the active stage of life, and the regular use of N.S oil increases the strengthening of immune system as well as acts as a antiviral effect (Salim and Hossian, ,٢٠٠٠;Burtis and Bucar,٢٠٠٠).

٢. ٢.٥.Properties of Nigella Sativa Oil :

a. calcium antagonist activity

It has been shown in vitro that the volatile oil of N.S inhibits intestinal and tracheal smooth muscle contractions. This effect is attributed to the calcium antagonist activity of Nigella oil (Aqel, ١٩٩٢). This observation is further supported by other

studies which show that thymoquinone and dithymoquinone (active ingredients of N.S) inhibit the release of histamine from rat peritoneal mast cells, through a mechanism which involves the entry of calcium ions (Chakravarty, ١٩٩٣). It seems likely that the observed bronchodilatation action or the antihypertensive effects of N.S oil reported in vivo could in part be explained by the calcium antagonist activity of N.S (El-Tahir *et.al.,* ١٩٩٣; Gilani, ٢٠٠١).

b. anti-oxidant activity

The essential oil of N.S seeds has an anti-oxidant activity (Meral *et.al.,* ٢٠٠١). Such activity is attributed to thymoquinone, carvacrol and t-anethol consistent of the oil (Burits and Bucar, ٢٠٠٠). Furthermore, thymoquinone has been shown to have free radical scavenging activity, and through this mechanism it inhibits benopyrene-induced for stomach carcinogenesis in mice (Badary *et.al.,* ١٩٩٩).

CHAPTER THREE

MATERIALS &METHODS

CHAPTER THREE

MATERIALS AND METHODS

٣.١.Subjects :

This study which covers the period from October ٢٠٠٣ to August٢٠٠٤ ,includes One hundred and twenty Seven(١٢٧) children whose ages between one day and five years. For all of them, estimations of APATs. according to number of oral polio vaccine doses received have been done, as well as according to their ages.

Seventy-nine(٧٩) children were studied to evaluate the effect of N.S oil on specific immune response to polio virus . On a random selection basis, they were divided into two major groups, forty- one (٤١)children were assigned in the first major group and thirty- eight (٣٨) children were in the second. The children within the first major group were subjected to treatment at ٠.٦ ml/kg / day in two divided doses. However, they were subdivided according to duration of treatment into :

A:- Twenty (٢٠) children for those who received the treatment for six weeks duration, then depending on number of OPV doses received by children before treatment with N.S oil they were subdivided into five subgroups these are first ,second ,third ,fourth and fifth subgroup were assigned for children who had

one ,two ,three four and five OPV doses respectively. There are three children in the first, as well as in the second subgroup, four children in the third subgroup and five children in the fourth and so as in the fifth subgroups.

B:- Twenty –one(٢١) children for those who were treated for three weeks duration , as in group A they were divided according to number of OPV doses into five subgroup. There are three children in the first as well as second subgroup and four, five and six children involved in the third ,fourth and fifth subgroup respectively .(Fig-١-)

The total IgG was also studied for this major group .

In the second major group ,thirty eight children were subjected to treatment at o.٣ ml/kg/day, as in first major group they were subdivided into .

A:-Eighteen(١٨) children for those who had received the treatment for six weeks duration ,also this group subdivided into five subgroups according to number of OPV doses there are three children for each of the first ,second and third subgroup, where as there are four in the fourth and five in the fifth subgroups

B:- Twenty(٢٠) children for those who had received the treatment for three weeks duration ,each of the first ,second and third subgroup had three children and there are five and six children in fourth and fifth subgroup respectively. (Fig-١-)

٢.٢ Data Collection Formula :

A. For all children :

a. Name

b. Age

c. Number of OPV doses (EPI & NIDS).

d. Date of the last OPV dose.

e. Date of the first blood sample collection.

f. Number of sample.

g. Titer of Anti Polio Antibody Titers

B. For children who have taken N.S oil :

a. Time of N.S oil administration:

b. Time of the second blood sample collection:

c. Number of Sample.

d. Body weight: Dose of N.S oil: Duration:

e. Titer of Anti Polio Antibody Titers

f. History of OPV during administration of N.S :

Yes: No:

٣.٣. Oil of Nigella sativa :

The oil of Nigella sativa has been obtained from the local medical supply centers in Baghdad, produced by Mousul Factory by the aid of supervisor Dr. Mohammed .A. Muhsin. As dose ٠.٦ ml /kg /day(Abdeen,*et.al.,* ٢٠٠٣).

٣.٤. Blood samples :

Blood samples were collected by the method of venipuncture. The area was first wiped with alcohol and left to dry. A disposable syringe of ٥ ml was used to aspirate about ٢-٣ ml of blood in plain tube then transferred to laboratory after labeled with the name and number of children. In the laboratory, the blood was left at room temperature for ٣٠ minutes till it clot then centrifuge at ٣٠٠٠ rpm for ١٥ minutes. The sera of whole blood were aspirated and divided into ٠.٥ ml test tubes and storageat-٢٠^{o}C till testing time. Each tube was used once to avoid repeated freezing and thawing. The sera before using were allowed to slaut at room temperature. All serum samples were heated at ٥٦C for ٣٠ minutes to inactivate complement (Garvey *et.al.,* ١٩٧٧).

٣.٥. Equipments and Tolls :

The equipments used during this work are :

The instrument	Company	Country
Autoclave	Webeco Gm bH	Germany
Automatic Pipettes	Organon Teknik	Belgium
Centrifuge	Damon IEC Division	USA
Water bath	Memmert	Germany
Disposable Syringe	Meheco	China
Disposable Tips	Nethler hinz	Germany
DisposablePlastic Tube	AFMA-Dispo	Germany
Sensitive Balance	Sartorious	U.K.
Oven	Memmert	Germany
Micro titration plate	Cookengineering Co-Alexandria	USA
Incubator	Memmert	Germany
Refrigerator		
Glass centrifuge tube	Stevilin	England
pH Meter	Philips	Holland
Filter Paper	Whatman, ٠.٢٢ Mm	Germany
Pasteur Pipette	Biomerieux	France

٣.٦. Solutions :

٣.٦.١. Normal Saline :

The solution was prepared by dissolving ٠.٨٥ g sodium chloride (NaCl; BDH Company; UK; MW=٥٨.٤٤) in ٢٠ ml distilled water, then the volume was completed to ١٠٠ ml. The final concentration was ٠.٨٥٪. The solution was then sterilized by autoclaving (١٢١^{o}C / ١٥ bar for ١٥ minutes. This solution was used for titration purposes.

٣.٦.٢. Tannic Acid Solution :

The solution was prepared by dissolving ٠.٥ g tannic acid powder (٧٦ $H_{٥٢}O_{٤٦}$; BDH company; MW=١٧٠١.٢٢) in ٢٠ ml distilled water, then the volume was completed to ١٠٠ ml with distilled water to obtain a solution with concentration of ٠.٥٪, used to tan RBCs, i.e. to mope the Ags found on sheep erythrocyte surfaces (Garvey ***et.al.***, ١٩٧٧).

٣.٦.٣.Citric Acid Solution

The solution was prepared by dissolving ١٥ citric acid Powder in ٢٠ ml distilled water, then the volume was completed to ١٠٠ ml. The final concentration was ٠.١٥%.

٣.٦.٤. Alsever's Solution :

Alsever's solution is an isotonic solution, anticoagulant blood preserving solution that permits the storage of whole blood refrigeration temperature for about ten weeks (Talib, ١٩٩٦). The solution was prepared by dissolving ٢٤.٦ g glucose (BDH company); ٩.٦ gm tri-sodium citrate (BDH company) and

٥.٠٤ g sodium chloride (BDH company)in ١٢٠٠ ml distilled water; the pH was then adjusted to ٦.١ with ١٥٪ citric acid and filtered by using a ٠.٢٢ Mm pore's diameter membrane filter using micro filtration unit.

٣.٧. Reagents :

٣.٧.١. Prepared reagents: Preparation of tanned erythrocytes.

Sheep blood (١٠ ml) was obtained from the animal in a sterile condition, and then mixed with equal volume from Alsever's solution. The mixture kept at ٤^{o}C then from this mixture, ٣ ml taken in a sterile centrifuge tube and centrifuged at ٢٥٠٠ rpm for ٥ minutes. By using sterile Pasteur's pipette, the supernatant was discarded and the deposit was resuspended in ١٠ ml normal saline and centrifuged at ٢٥٠٠ rpm for ٥ minutes. The supernatant was discarded and the deposit was resuspended in ten ml normal saline and mixed thoroughly.

From this mixture, three ml were taken in sterile centrifuge tube which contains three ml of ٠.٥٪ tannic acid solution mixed and placed in the water bath at ٣٧^{o}C for ١٠ minutes. The centrifugation was carried out at ٢٥٠٠ rpm for five minutes. The supernatant was discarded and to the precipitate three ml of normal saline were added and gently mixed.

٣.٧.٢. Ready made reagents: Single radial immunodiffusion (SRID) test kits . Kallested endplate (SRID) test kits were used

for the quantitative determination of human immunoglobulin IgG in human serum (Sanofi diagnostic pasture ,USA)

٣.٨. Labratory Investigaion :

٣.٨.١. Passive Haemagglutination Test (PHAT) :

According to the modified method of Garvey *et.al.*, (١٩٧٧), PHAT was used to determine the level of immunity against polio in vaccinated or unvaccinated children , with or without Nigella sativa oil.

Determination of antibody level in children's sera:

Tanned sheep erythrocyte (٣ ml) was mixed with ١ ml of polio vaccine and left at room temperature for about ١٥ minutes. It was then centrifuged at ٢٥٠٠ rpm for five minutes, the supernatant was discarded, and the deposit was re-suspended in ٣ ml normal saline. Then centrifugation was done at ٢٥٠٠ rpm for five minutes the supernatant was discarded and the deposit was re-suspended in ٢ml normal saline and mixed.

Amicrotitration plate was used to estimate the level of antibodies in children's sera according to Garvey *et.al.*, (١٩٧٧).

The procedure was carried out as follows:

١. Fifty µl of normal saline was added in each well of plate (١٢ wells).
٢. Ten µl of children's sera was added to ٩٠ µl of normal saline in a sterile test tube and mixed well.

٣. Fifty µl of diluted children's sera (١:١٠) was added to the first well and mixed. Then double folds dilutions were prepared by transferring fifty µl from the first well to the second well, mixed well and fifty µl out of it was transferred to the third well and so on. From the eleventh well, fifty µl was discarded. The serial dilution was thus obtained from the first well till the eleventh one; ١:٢٠, ١:٤٠, ١:٨٠, ١:١٦٠, ١:٣٢٠, ١:٦٤٠, ١:١٢٨٠, ١:٥١٢٠, ١:١٠٢٤٠, and ١:٢٠٤٨٠ accordingly.

٤. The twelfth well contains fifty µl of tanned sheep erythrocyte covered with the polio vaccine was added (the last well represented the negative control well, which was not taking the presence of children's sera.

٥. The micro titration plates were shaken gently for ٢ minutes and then covered and incubated at ٣٧^{o}C for ٢٤ hours. Then, the presence or absence of haemagglutination was recorded. PHAT titer represented the last dilution that gave a positive result.

٣.٨.٢. Single Radial ImmunoDiffusion (SRID) Test :

To estimate the (IgG) level between children who received N.S oil and children who did not ,SRID test kits (Sanofi kits)were used as in Mancini *et.al.*, ١٩٦٥ .A linear relationship exists between the Ag concentration and the

corresponding squared immune precipitin ring diameter that formed in an Ab gel system .

The test procedure was carried out as follows **:**

Five µl serum from each individual was dispensed into wells of the plate containing agarose gel mixed with amonospecific antiserum .The plate was then covered and incubated at room temperature on a level surface for ٤٨ hours for IgG estimation .The sample was diffused a radically through the gel and the Ag form aprecipiting ring with the monospecific antiserum .The immuno –precipitation ring diameter was measured by the optical reader and the relevant concentration corresponding to the precipitates ring diameter was calculated from the conversion table provided with the kit.

٣. ٩.Statistical Methods

We used the (Mode) for study and analysis of data (Anti polio Antibody titers) for sampled children who were treated or not with N.S oil , so as the Range.

The Mean had been used to compare the results of total IgG for children who were treated with N.S oil with other not treated .

CHAPTER FOUR

RESULTS AND DISCUSSION

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٤.١. Assessment of Children Immunity against Poliovirus

The relationship between the number of OPV doses received by children and the anti - polio antibodies titers (APAbTs)have been shown in Fig (٢). For unvaccinated children, seventy-five percent (٧٥٪) have ١:٢٠ APAbTs against polio. The blood samples have been aspirated immediately after birth from umbilical cord and this indicates transplacental maternal antibodies (Ananthakrishman *et.al.*١٩٨٨; Onorato *et.al.,* ١٩٨٨). The other twenty-five percent (٢٥٪) of unvaccinated children have ١:٤٠ APAbTs titer and all of them are of older ages. That may be due to contact with polio virus (vaccine-associated or wild virus) from others. For children who had one OPV dose, seventy-five(٧٥٪) percent of them have APAbTs of ١:٤٠, the other twenty five(٢٥٪) percent of children had APATs of ١:٨٠.For children who received two and three OPV doses, the APATs are similar, where seventy five percent of them have ١:٨٠ APAbTs titer and other twenty five percent of them have APAbTs titer of ١:٣٢٠.As for the children who received four and five OPV doses, the APAbTs are nearly similar where fifty four percent (٥٤%) of them have APAbTs titer of ١:٨٠ and

almost eight percent (٨٪) have ١:٣٢٠.On the other hand, for the children who received four OPV doses, twenty eight percent (٢٨٪)have ١:١٦٠ and ten percent (١٠٪) have ١:٤٠;whereas for children who received five OPV doses, sixteen percent (١٦٪) have ١:١٦٠ and twenty percent (٢٠٪) of them have a titer of ١:٤٠.

From the findings above, there is a clear relationship between the number of OPV doses and the APAbTs titer, for the unvaccinated children have the lowest APAbTs titer. The APAbTs titers increase with subsequent OPV doses to reach a steady level of ١:٨٠ after the second and third OPV doses, and remain relatively stable in the fourth and fifth OPV doses with slight variability that consist, with (Behrman *et.al.,* ٢٠٠٠,٢٠٠٤).

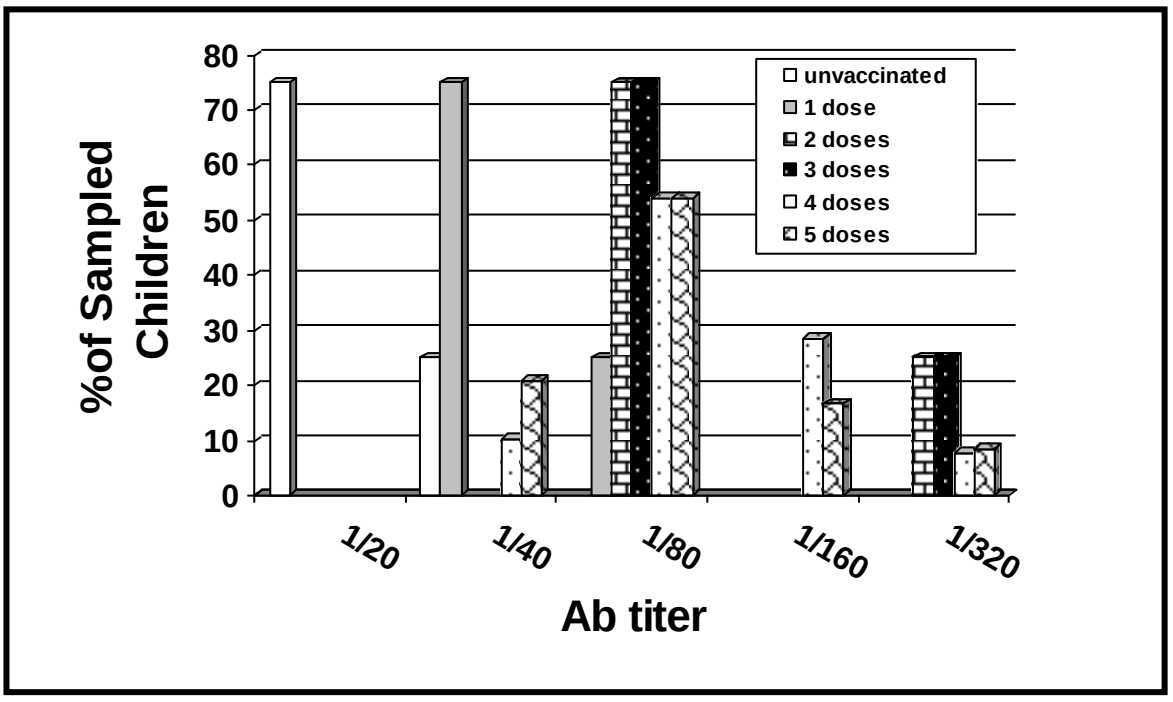

Figure (٢) :The Relation between the Immune Level against Polio virus and Number of OPV received by Children

The modes of anti-polio antibodies titers according to the number of OPV doses received by children (***Ms***)are represented in (Fig ٣) independent of age, which also account for more than fifty four percent (٥٤٪) of the APAbTs titer of the corresponding OPV dose. These titers are ١:٢٠ for unvaccinated children, ١:٤٠ for children who received only one OPV dose, and ١:٨٠ for children who did two, three, four and five OPV doses. For unvaccinated children, this ***M*** of antibody titer (١:٢٠) reflects a transplacental passage of maternal antibodies during fetal life, which in turn reflects maternal serum level of polio antibodies. This, however, consists with (Ananthakrishman, *et.al.,* ١٩٨٨; Onorato *et.al.,* ١٩٨٨). As for children who received only one OPV dose, this ***M*** of APAbTs titer represents the primary response to a vaccine antigen. it consists with (Behrman *et.al,*٢٠٠٠, ٢٠٠٤).The antibodies titer achieve the highest level after the second OPV dose and remain constant with subsequent OPV doses; whereas the heightened humoral or cell mediated responses are elicited by a second exposure to the same T-dependant antigen the secondary responses occur rapidly, usually four –five days (Behrman *et.al* ٢٠٠٠;٢٠٠٤). The persistence of a stable APAbTs titer after a second OPV dose might be attributed to the repeated stimulation of immune system (memory cells) by the same antigen (vaccine).

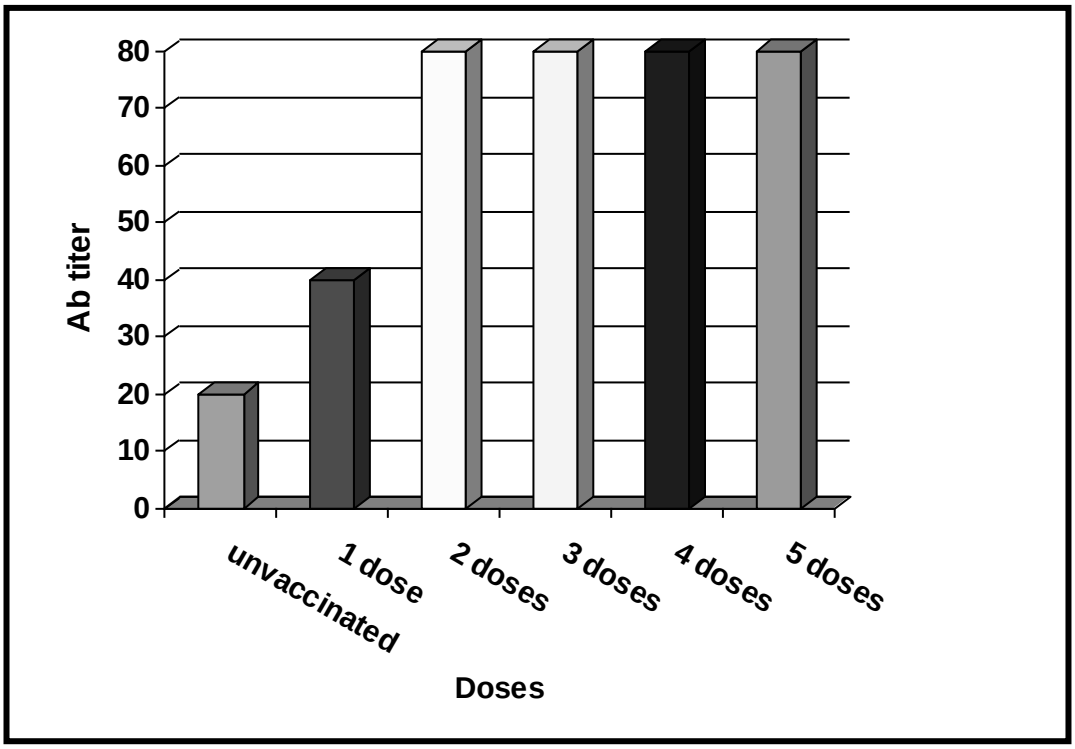

Figure (٣) The titers of APAbTs according to the number of OPV Doses .

The level of (APAbTs) according to the age of children under study and independent the number of OPV doses has been evaluated in Fig (٤). The children have been divided into four groups as follows :

١. The first group, represents the neonatal period (less than one month old children).In this group, the children under study have APAbTs of ١:٢٠ and all of them have been investigated immediately after birth, as discussed

previously. So, the lowest level of antibody titer for children less than five years old occurs during this period.

٢. The second group of children are one month to one year old of age (infants period).The Range (**R**) of APAbTs is ١:٤٠ -١:٣٢٠. Fifty two percent of the children have APAbTs equal to or less than, ١:٨٠ and, the other children have a titer of equal to, or more than, ١:١٦٠.

٣. The third group of children are one to ٣ years old (toddler age). **R** is ١:٤٠ -١:٣٢٠ ,and Fifty six percent have APAbTs equal to or less than, ١:٨٠ and the rest have a titer equal to, or more than, ١:١٦٠.

٤. The fourth group are three to five years old (preschool age), **R** is ١:٤٠ -١:١٦٠), ٢٢٪ have APAbTs of ١:٤٠, ٦٦٪ had ١:٨٠ and ١١٪ had APATs of ١:١٦٠. .

The **R** for children younger than three years old (apart from neonatal period) is ١:٤٠-١:٣٢٠, whereas the **R** for older age is ١:٤٠-١:١٦٠ . Higher percentage of children who are younger than three years old had higher antibodies titer (≥١:١٦٠) in comparison to older age . This might be due to repeated stimulation of the immune system by regular OPV doses through EPI applied in Iraq. The APAbTs titer for children older than three years old is nearly maintained at a stable titer (١:٨٠), which might be due to infrequent stimulation of the immune system by National immunization days (NIDs) administered annually at spring and fall seasons.

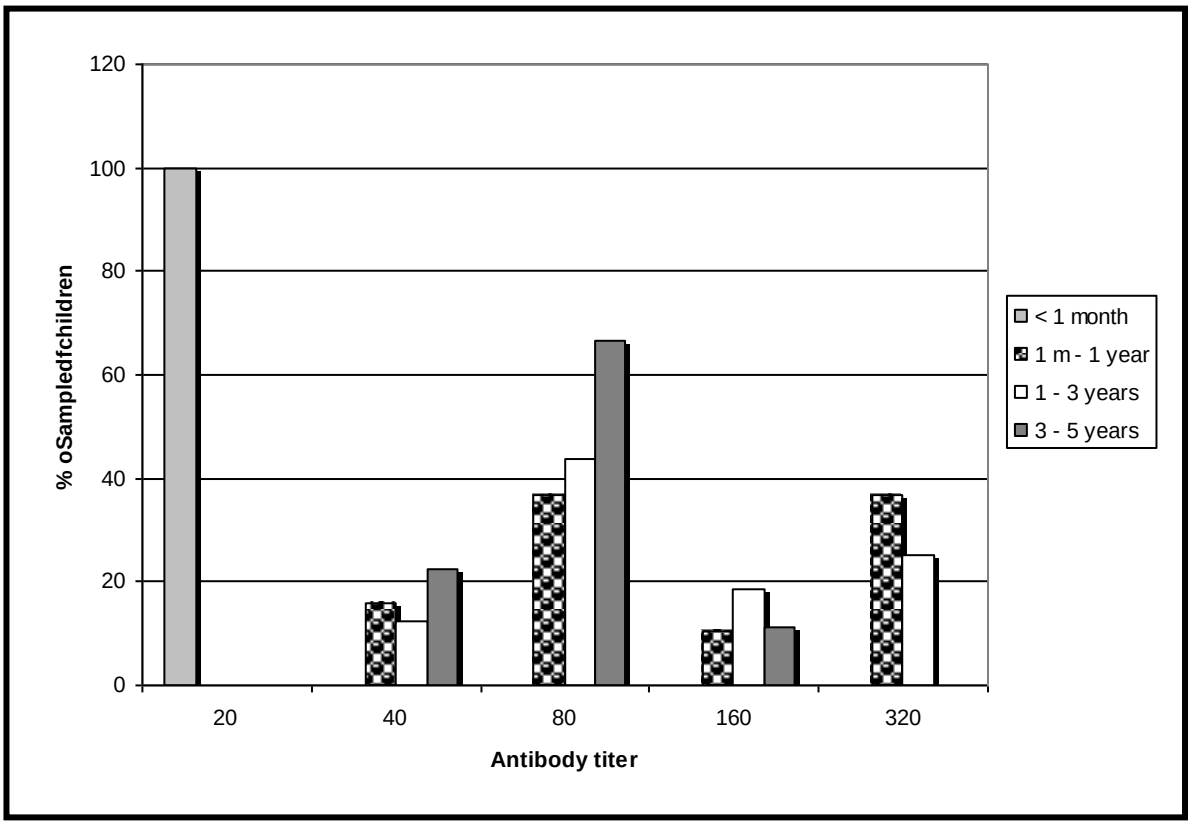

Figure (٤): Anti Polio Antibody Titers according to age independent the number of OPV Received by the child.

٤.٢. Evaluation of Nigella Sativa Oil effects on Specific Humoral Immune Response against Poliovirus in Children

The effect of N.S oil on specific humoral IR to polio has been studied , ٠.٦ ml/Kg /day in two divided doses for three and six weeks duration were used . There are two groups of children and each group is subdivided into five subgroups depending on the number of OPV doses received by children fig.(١) The *Ms* are used to make a comparison between the children taking N.S oil and the others who had not taken the oil . The *Ms* are also similarly compared between the children in different subgroups in Table (٣).

The *Ms* for children who have not taken N.S oil (Fig ٣) have been used as standard for comparison with *Ms* of matched children in the first, second and third subgroups. But the *Ms* of the same children before and after taking N.S oil have been used for comparison in the fourth and fifth subgroups.

١-The first group for children who had taken the prescribed dose (٠.٦ ml/Kg /day) for a six- week duration. In the first subgroup that represents the children who had only one OPV dose, the *M* is ١:٨٠ after taken the oil ,whereas the *M* in this subgroup for the children who had not taken N.S oil is ١:٤٠. In the second subgroup that represents children who had two OPV doses, the *M* is ١:١٦٠ after taken N.S oil , whereas *M* in this subgroup for the children who had not taken N.S oil is ١:٨٠. The

third subgroup that represents the children who had three OPV doses ,the $\mathcal{M}$ is ١:٣٢٠ after taken N.S oil ,whereas $\mathcal{M}$ in this subgroup for children who had not taken N.S oil is ١:٨٠. In the fourth and fifth subgroups that represent the children who had four and five OPV doses respectively ,the $\mathcal{M}s$, before taking N.S oil were ١:٨٠ for each subgroup, and had changed into ١:٣٢٠ after taken the oil .

٢-The second group for children who had taken the prescribed dose (٠.٦ ml/Kg /day) for a three- week duration. In the first and second subgroups that represent the children who had one and two OPV doses respectively, the $\mathcal{M}s$ after taken the oil, are ١:٤٠ for the first and ١:٨٠ for the second subgroups, which is similar to the $\mathcal{M}s$ for the children who had not taken N.S oil. In the third subgroup that represents the children who had three OPV doses ,the $\mathcal{M}$ after taken N.S oil is ١:١٦٠, whereas the $\mathcal{M}$ for the children who had not taken N.S oil and received three OPV doses is ١:٨٠. In the fourth and fifth subgroups that represent the children who had four and five OPV doses respectively, the $\mathcal{M}s$ before taking N.S oil, are ١:٨٠, and had changed to ١:٣٢٠ after taken N.S oil as prescribed in dose and duration. The total of both first and second groups is collected and restudied regardless of the duration by which the prescribed dose (٠.٦ ml/Kg /day) is administered. The results in $\mathcal{M}s$ after having taken the N.S oil are similar to those in the first group.

From the results above , the positive role of N.S oil in the enhancement of specific IR to polio appears clearly and this enhancement is effected by the duration through which the N.S oil is given. The number of OPV doses received by children before taking N.S oil have significant effects on the induced IR. When they have taken (٠.٦ ml/Kg /day) for a six- week duration, the $\mathcal{Ms}$ increase one fold for the children who had one and two OPV doses ,and increase two folds for the others who had three or more OPV doses, whereas for children who had taken ٠.٦ ml/Kg /day for a three week duration, the $\mathcal{Ms}$ have not changed for the children who had one and two OPV doses, but increased one fold for the children who had three OPV doses, and two folds for others who had four or more OPV doses. So, in the fixed dose of N.S oil, the more duration and OPV doses, the better IR against polio and vice versa. Although there is no similar study in which the N.S is used to enhance the IR specifically against polio, there are few studies by which the N.S is used to enhance general immunity in general (Cindy, ٢٠٠٠); (El-Kadi and Qandil, ٢٠٠٤;Meral *et.al.*, ٢٠٠٣ and Sanamin ,٢٠٠٤). All these studies explain the effect of N.S oil and might be related to the increase of production of antibodies, bone marrow cells and B-cells as well as the increase of the number ofmemorycells.

Table (٣): Modes of APAbTs according to no. of OPV doses before and after ٠.٦ ml/Kg /day of NS oil

N.S Oil Dose (ml/Kg/day)	Duration (wks)	Total No.	Children received one OPV doses			Children received two OPV doses			Children received three OPV doses			Children received four OPV doses			Children received five OPV doses		
			No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil
٠.٦	٦	٢٠	٣	١:٤٠	١:٨٠	٣	١:٨٠	١:١٦٠	٤	١:٨٠	١:٣٢٠	٥	١:٨٠	١:٣٢٠	٥	١:٨٠	١:٣٢٠
	٣	٢١	٣	١:٤٠	١:٤٠	٣	١:٨٠	١:٨٠	٤	١:٨٠	١:١٦٠	٥	١:٨٠	١:٣٢٠	٦	١:٨٠	١:٣٢٠
Total no. of ٠.٦ ml		٤١	٦	١:٤٠	١:٨٠	٦	١:٨٠	١:١٦٠	٨	١:٨٠	١:٣٢٠	١٠	١:٨٠	١:٣٢٠	١١	١:٨٠	١:٣٢٠

In Fig (٥) there are three curves of *Ms* :two of them are for the children taking ٠.٦ ml/Kg /day of N.S oil for three- and six-week durations, the other curve for the children who have not taken N.S oil. The lower curve that represents the *Ms* of children who have not taken N.S oil as in Fig (٣) and considered as standard to compare for other two curves, this curve starts to rise from a titer ١:٢٠ to ١:٤٠ then to ١:٨٠ for unvaccinated, who received one and two OPV doses respectively, then the curve keeps stable at a level of ١:٨٠ for further OPV doses (three or more).

In the second curve that represents the *Ms* of children who have taken the prescribed dose for a three week duration, the curve rises gradually from a titer of ١:٤٠ for the children who had only one OPV dose to ١:٨٠ for those who had two OPV doses, to ١:١٦٠ for those who had three OPV doses then reaches ١:٣٢٠ for those who had four OPV doses and persist at this level for further doses.

In the third (the upper) curve which represents *Ms* of the children who had taken the prescribed dose (٠.٦ ml/Kg /day) for a six week duration, the curve rises from ١:٨٠ for the children who had one OPV dose to ١:١٦٠ for those who had two OPV doses, then reaches ١:٣٢٠ for those who had three OPV doses and persists at this level for further OPV doses.

From the three curves above, the undoubted effects of N.S on the enhancement of IR appear clearly. The magnitude of

the immune response also correlates well with the number of OPV doses and durations. This correlation might be related to the immaturity of the immune system during the first few months of life during which the child receives ٢-٣ OPV doses(Behrman *et.al*, ٢٠٠٠,٢٠٠٤),whereas the correlation with duration might be due to prolonged stimulation of memory cells or other B-cells to achieve higher Abs titer.

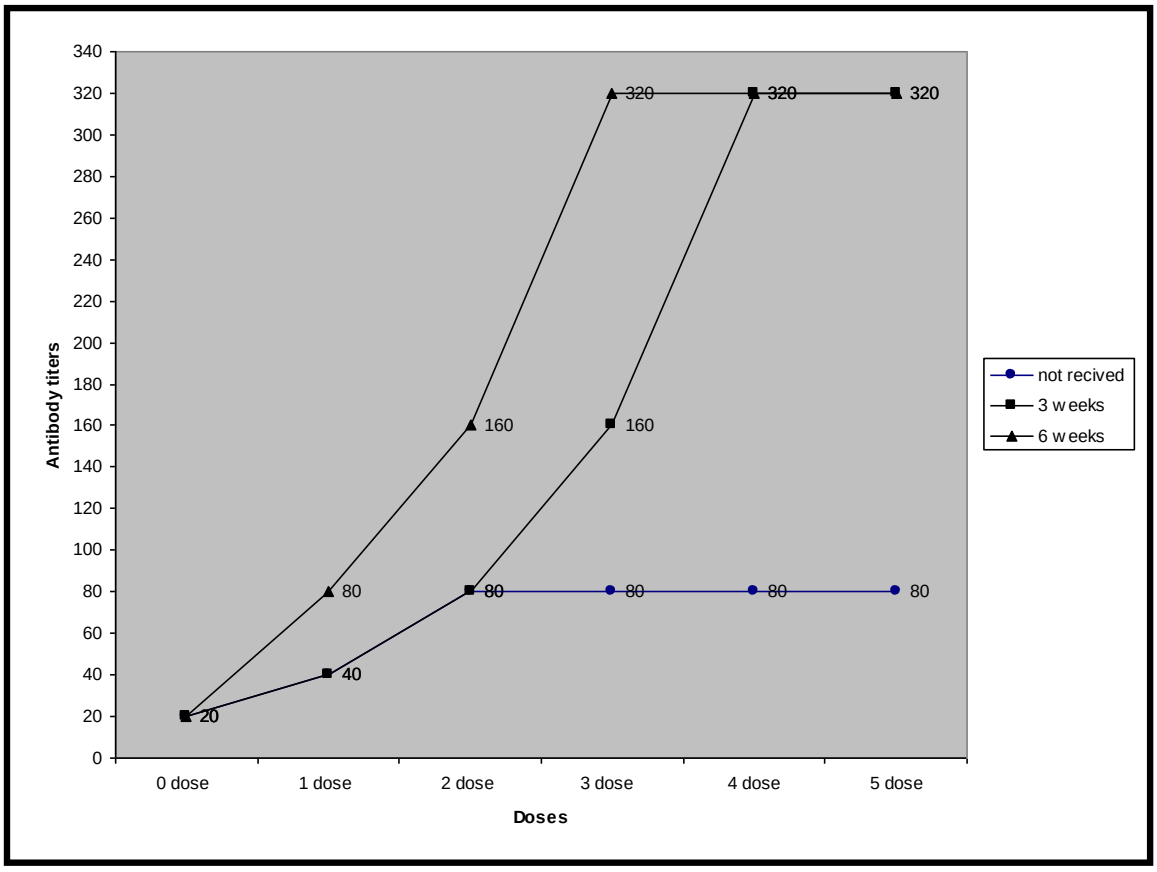

Fig (٥): Correlation between number of OPV doses and Modes of APAbTs at ٠.٦ml/kg/day of N.S oil.

The effects of N.S oil when using ٠.٣ ml/kg/ /day for three and six week durations (which is the half dose used in Table -٣-) on specific immune response to polio have been studied in Table(٤) The same statistical way applied in Table (٣)has been used :

١- The first group, for children who had taken the prescribed dose for a six- week duration. The *Ms* without N.S oil are ١:٤٠ for the children who had one OPV dose and ١:٨٠ for those who had two or more OPV doses, and increased to ١:٨٠ for the children who had one OPV dose and ١:١٦٠ for the children who had two or more OPV doses after taken N.S oil as described in dose and duration .

٢- The second group, for children who had taken the prescribed dose of N.S oil for a three- week duration. For the first, second, and third subgroups the *Ms* are ١:٤٠, ١:٨٠ and ١:٨٠ respectively after taken the prescribed N.S oil. That is similar to *Ms* of children who had not taken N.S oil and had similar OPV doses. In the fourth and fifth subgroups, the *Ms* for the children who had taken N.S.oil are ١:٨٠ for each subgroup that change to ١:١٦٠ after they were taken (٠.٣ ml/Kg /day) of N.S oil for a three- week duration.

As in Table (٣), the total of both first and second groups is collected and restudied regardless of the duration. The results in *Ms* after having taken the N.S oil are similar to those in the first group.

From the results above, although we have used half the dose of N.S oil used in Table (٣) in three- and six- week durations, there is still a positive role in the enhancement of specific humeral IR. This effect correlates with the number of OPV doses received by children before having taken N.S oil and duration through which we had administered the dose of N.S oil, whereas in the children who had taken ٠.٣ ml/Kg /day for a six week duration, the *Ms* increased in the children who had one, two, three, four and five OPV doses by one fold as compared to those who had not taken N.S oil. When taking the same dose (٠.٣ ml/Kg /day) of N.S oil for half the duration (٣ week), there are no changes in *Ms* for the children who had one, two and three OPV doses, but there is an increase in *Ms* by one fold for those who had four and five OPV doses.

In comparison with Table (٣), there is a good relationship between the dose of N.S oil received by the children and the induced APAbTs . The *Ms* after having taken a full dose (٠.٦ ml/Kg /day) regardless of the duration are ١:٨٠, ١:١٦٠, ١:٣٢٠, ١:٣٢٠ and ١:٣٢٠ for the children who had one, two, three, four and five OPV doses respectively, whereas the *Ms* after having taken half the dose (٠.٣ ml/Kg /day) regardless of the duration are ١:٨٠, ١:١٦٠, ١:١٦٠, ١:١٦٠ and ١:١٦٠ for the children who had one, two, three four and five OPV doses respectively.

Table (٤): Modes of APAbTs according to number of OPV Doses before and after ٠.٣ ml/Kg /day of NS Oil

N.S Oil Dose ml/Kg /day	Duration (wks)	Total no.	Children received one OPV dose			Children received two OPV doses			Children received three OPV dose			Children received four OPV dose			Children received five OPV dose		
			No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil	No. of children	Mode without NS oil	Mode with NS oil
٠.٣ ml /Kg /day	٦	١٨	٣	١:٤٠	١:٨٠	٣	١:٨٠	١:١٦٠	٣	١:٨٠	١:١٦٠	٤	١:٨٠	١:١٦٠	٥	١:٨٠	١:١٦٠
	٣	٢٠	٣	١:٤٠	١:٤٠	٣	١:٨٠	١:٨ ٠	٣	١:٨٠	١:٨٠	٥	١:٨٠	١:١٦٠	٦	١:٨٠	١:١٦٠
Total of ٠.٣ ml		٣٨	٦	١:٤٠	١:٨٠	٦	١:٨٠	١:١٦٠	٦	١:٨٠	١:١٦٠	٩	١:٨٠	١:١٦٠	١١	١:٨٠	١:١٦٠

In Fig (٦) there are three curves of $\mathcal{Ms}$: two of them are for the children who had taken ٠.٣ ml/Kg /day of N.S oil for six -and three- week durations; and the other curve is for the children who had not taken N.S oil. The lower curve that represents the $\mathcal{Ms}$ of children who had not taken N.S oil is discussed previously in Fig (٥).

In the second curve that represents the $\mathcal{Ms}$ for the children who had taken the N.S oil for a three week duration, the curve is similar to the first one (standard) for children who had one, two and three OPV doses, then rises to ١:١٦٠ for those who had four and five OPV doses.

The $\mathcal{Ms}$ for the children who had taken the prescribed dose for a six -week duration are represented in the third curve (upper). The curve rises from ١:٨٠ for the children had one OPV dose to ١:١٦٠ for those who had two OPV doses, then remains stable at this titer for further OPV doses.

From the results above and in comparison with Fig (٥),it appears that in a fixed duration, the more N.S dose and more OPV doses produced, the better humoral IR against polio and vice versa. We have also noted that ٠.٦ ml/Kg /day of N.S oil for a six- week duration is considered the best dose(Abdeen *et.al,*٢٠٠٣) and optimal time to produce the best enhancement of APAbTs.

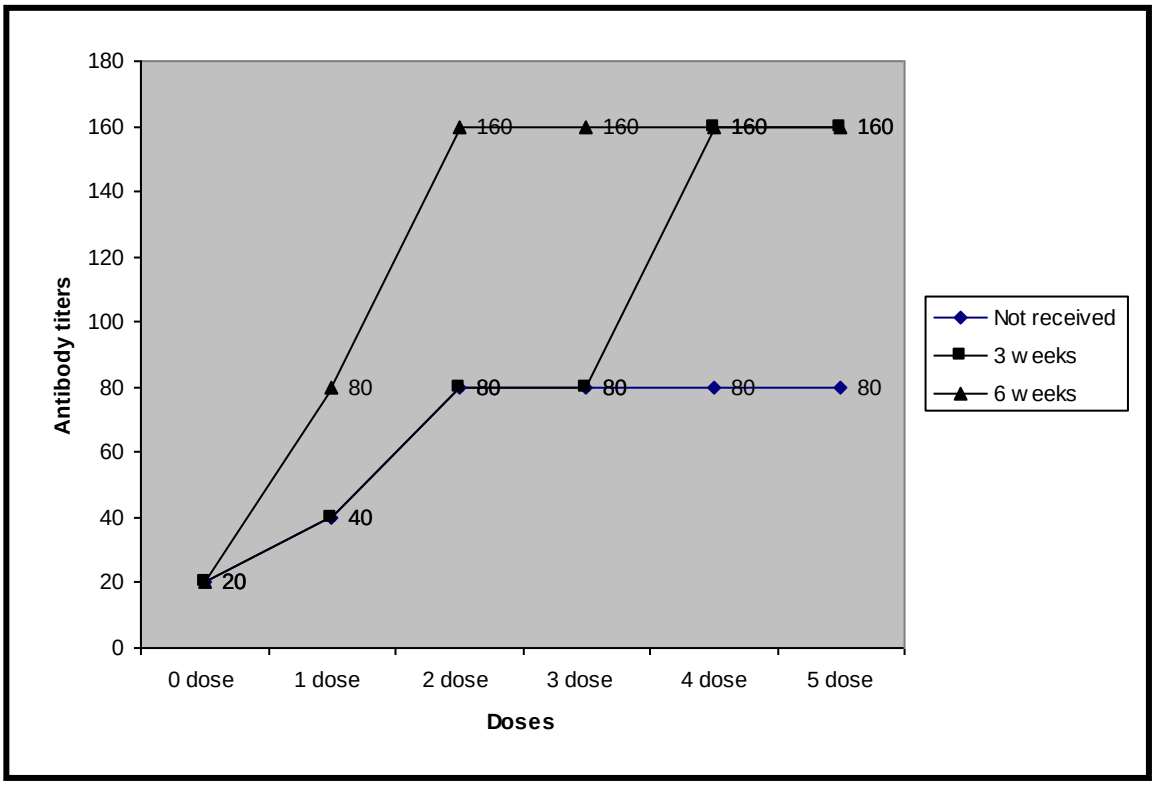

Fig (٦): Correlation between number of OPV doses and Modes of APAbTs at ٠.٣ml/kg/day of N.S oil.

The magnitude of immune response induced by using ٠.٦ ml/Kg /day was evaluated in Table(٥) and figures (٧,٨ and ٩)for the children who had four and five OPV doses(the same children were evaluated in the fourth and the fifth subgroups in Table-٣-) Characteristically, there is no recent polio vaccination for this group of children. Ten children had been chosen as a control group.

There are ten children who had taken the prescribed oil for a six week duration; eight out of them (٨٠٪)have an increase in their APAPTs ,and six of whom (٦٠٪)for two folds and two (٢٠%) for one fold .The other two children (٢٠٪) had no change in their APAbTs .

On the other hand , there are eleven children who had taken the prescribed oil for a three week duration ;eight (٧٢٪) out of them have their APAbTs increased ,five (٤٣٪)for two folds and three (٢٧٪) for one. The other three children(٢٧٪)had no change in APAPTs .When the duration of taking the prescribed oil is excluded and the total children re-evaluated ,there are sixteen (٧٦٪) whose APAbTs have increased; eleven (٥٣٪) by two folds ,and five (٢٣٪) by one .There are also five children (٢٣٪) who had no change in their APAbTs.

For the control, group the basal level of APAbTs has been assessed, after three and six week durations. Two children (٢٠٪)had one fold rise in their APAbTs after six weeks,one of them has the APAbTs increase during the first three weeks.

From the results above, the magnitude of IR appears correlated well with the duration of N.S oil ,where the longer duration (six weeks) proves better than the shorter one (three weeks). This might be due to the prolonged stimulation of memory cells or other B-cells that result in higher Abs titers.

There are no changes in APAbTs for (٢٠٪)and (٢٧٪)of children who received N.S oil for six and three weeks respectively. This might be due to ecological, genetic, and nutritional factors, sub clinical illness or other causes.

The increase in APAbTs in few children in the control group might be due to contact with polio virus from other (vaccine related virus or wild virus) during this period of follow up, although a real attempt has been made to prevent contact with newly vaccinated children .

Table (٥): The Magnitude of APAbTs after Taking ٠.٦ ml/kg /day from N.S Oil.

Dose ml/Kg /day	Duration (wks)	Total no. of children	Total no. of children who show increment	%	No. of children had one fold increment	No. of children had two fold increment	No change in titer	%
٠.٦	٦	١٠	٨	٨٠	٢	٦	٢	٢٠
	٣	١١	٨	٧٢	٣	٥	٣	٢٧.٢
Total of ٠.٦ ml		٢١	١٦	٧٦	٥	١١	٥	٢٤
Control for ٦ wks duration		١٠	٢	٢٠	٢	-	٨	٨٠
Control for ٣ wks duration		١٠	١	١٠	١	-	٩	٩٠

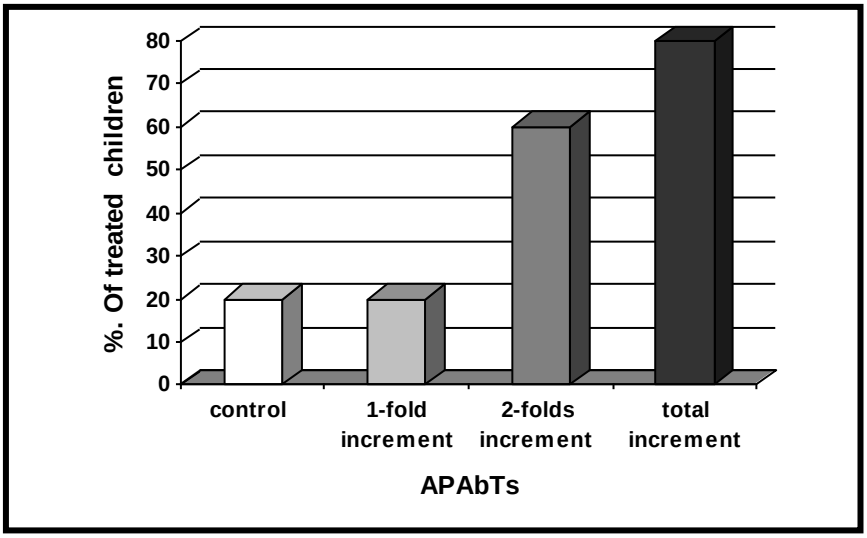

Figure (٧): The Magnitude of Immune Response with ٠.٦ ml/Kg /day for a six- week Duration of N.S Oil Treatment.

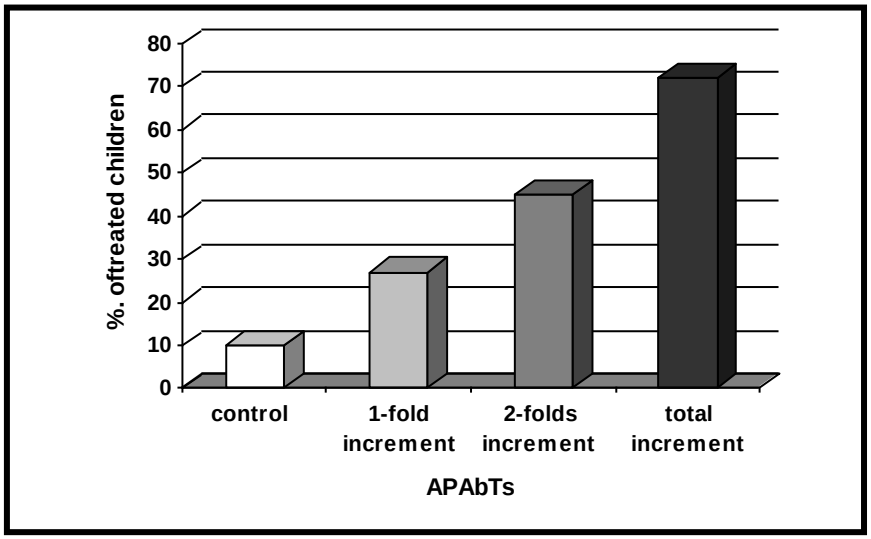

Figure (٨): The Magnitude of Immune Response with ٠.٦ ml/Kg /day for a three- week Duration of N.S Oil treatment.

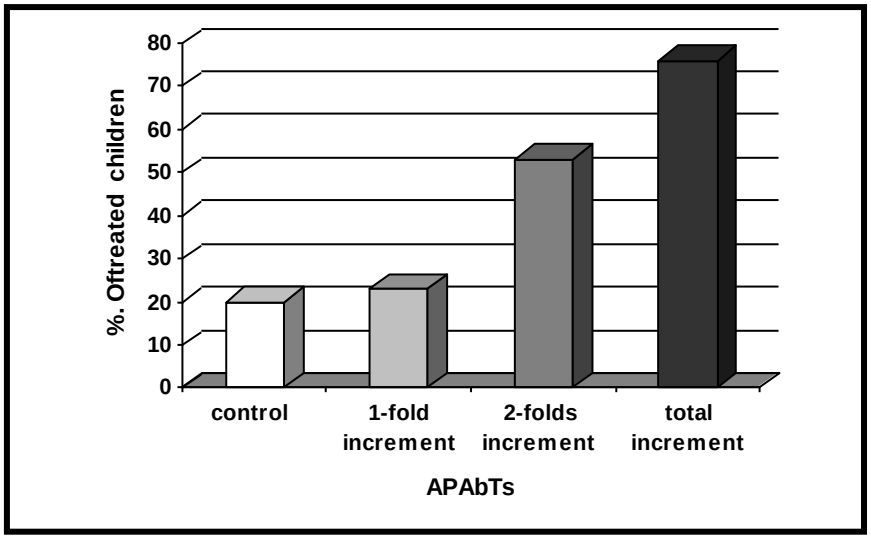

Figure (٩): The Magnitude of Immune Response with ٠.٦ ml/Kg /day of N.S Oil Regardless the Duration.

The magnitude of the immune response induced by using ٠.٣ ml/Kg /day was evaluated in Table(٦) and Figs (١٠,١١and ١٢)for the children who had four and five OPV doses(the same children evaluated in fourth and the fifth subgroups in Table(٤) This group of children had no recent polio vaccination. The same control group enrolled in Table (٥) has been considered. There are nine children who had taken the prescribed oil for a six week duration : six out of them (٦٦٪)had an increase in APAbTs ,two children (٢٢٪)had two folds, and four (٤٤٪) had one fold increment. The other three children (٣٣٪) had no change in their APAbTs .

On the other hand, there are eleven children who had taken the prescribed oil for a three week duration: five (٤٥%)of

them had their APAbTs increased, one (٨%) by two folds and four (٣٦%) by one fold. The other six children(٥٥%)had no change in APAbTs. When the duration of taking the prescribed oil was excluded and the total children re-evaluated ,there are eleven (٥٥%) whose APAbTs had increased, three (١٥%) by two folds ,and eight (٤٠%) by one fold. There are nine children (٤٥%) who had no change in their APAbTs.

In comparison with Table (٥), the important role of the dose of N.S oil is clear, where seventy-six (٧٦٪) percent of the total children taking ٠.٦ ml/Kg /day regardless of the duration had an increase in their APAbTs as compared with fifty five percent (٥٥٪) for the children who had taken half the dose as in Table (٦), .Similarly in respect the magnitude of APATs where fifty two percent (٥٢٪) of the total children who took the full dose (٠.٦ ml/Kg b.w/day) regardless the of the duration have two folds increment in their APAbTs in comparison with fifteen percent (١٥٪) of children who did not taken half the dose.

Finally, although the duration has an important effect on the magnitude of APATs, the dose of N.S oil is more important; where forty-five (٤٥٪) of the children who had taken the full dose (٠.٦ ml /Kg / day) in half the duration (٣ weeks) have two folds increment in comparison with only twenty-two percent (٢٢٪) of children who had taken the half dose (٠.٣ ml/Kg /day) in full duration (٦ weeks).

Table ٦: The Magnitude of APAbTs after taking ٠.٣ ml/kg/day of N.S Oil for three-and six- week Duration

Dose (ml/Kg /day)	Duration (weeks)	Total no. of children	Total no. of children who show increment	%	No. of children had one fold increment	No. of children had two fold increment	no change in titer	%
٠.٣ ml	٦	٩	٦	٦٦.٦	٤	٢	٣	٣٣.٣
	٣	١١	٥	٤٥	٤	١	٦	٥٥٪
Total of ٠.٣ ml		٢٠	١١	٥٥	٨	٣	٩	٤٥٪
Control for ٦ week duration		١٠	٢	٢٠	٢	-	٨	٨٠٪
Control for ٣ week duration		١٠	١	١٠	١	-	٩	٩٠٪

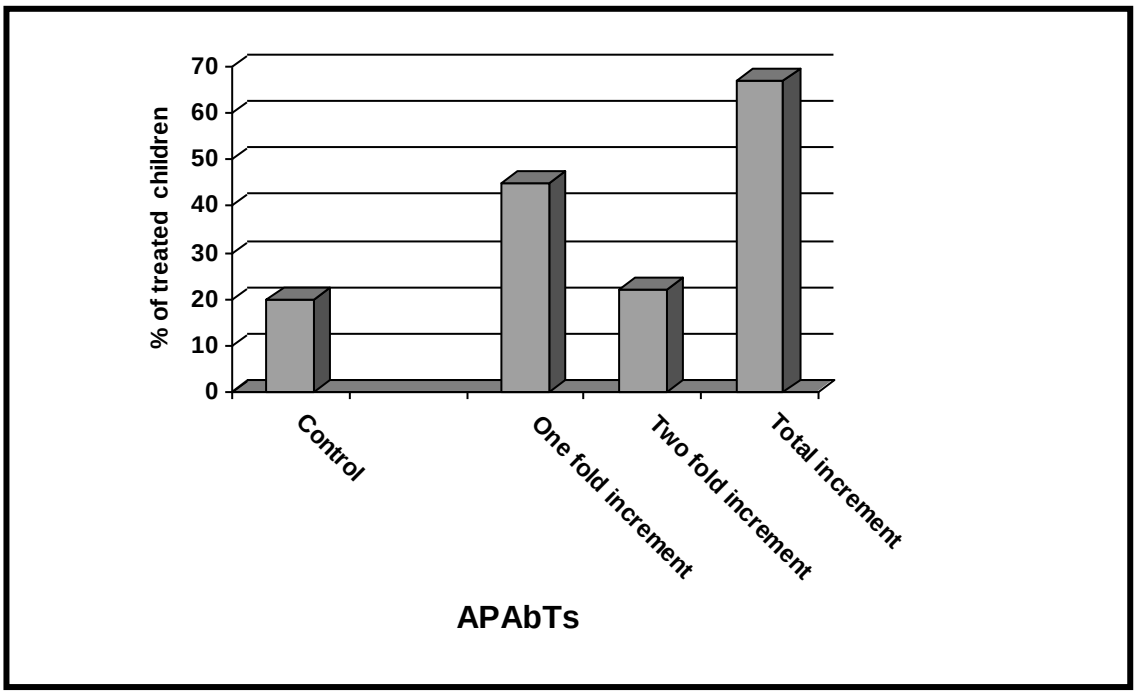

Figure (١٠) : The Magnitude of Immune Response with ٠.٣ ml /Kg /day) for a six week- Duration of N.S Oil Treatment.

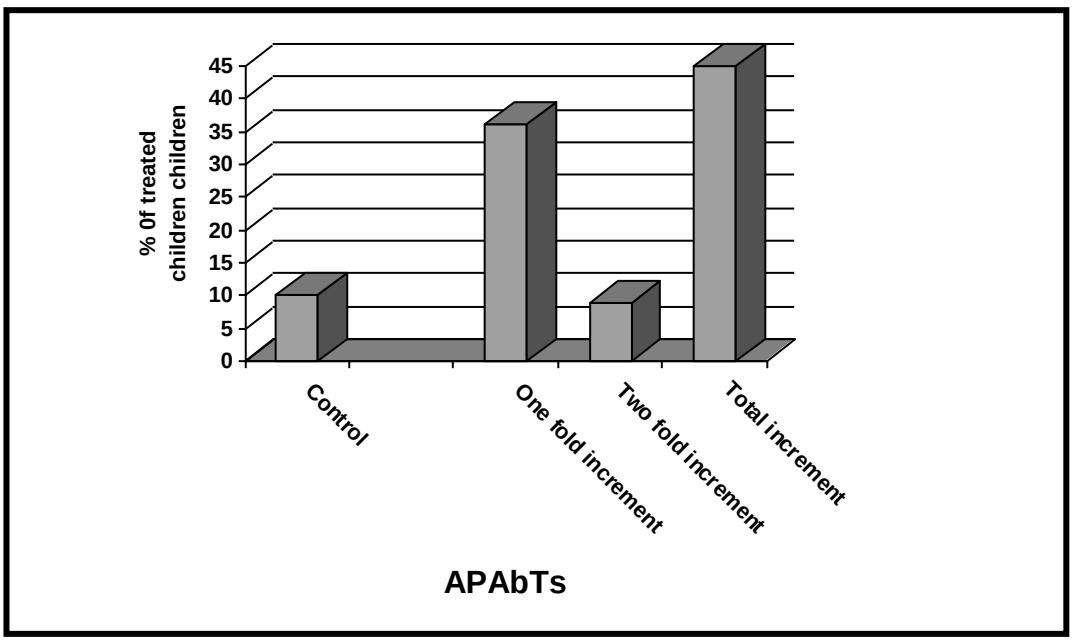

Figure (١١) : The Magnitude of Immune Response with ٠.٣ ml /Kg /day for a three- week Duration of N.S oil Treatment.

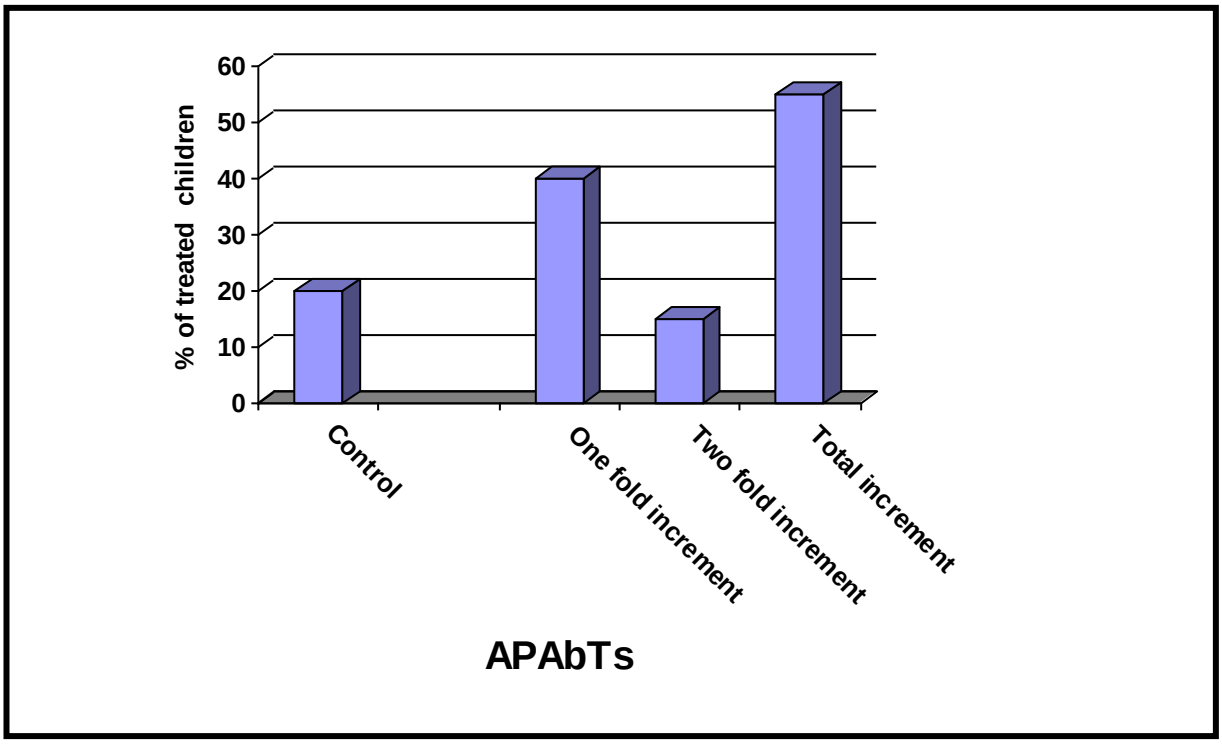

Figure (١٢) : The Magnitude of Immune Response against polio with ٠.٣ ml /Kg /day of N.S Oil Regardless the Duration.

٤.٣. Assessment of N.S Oil Effects on Total Immunoglobulin –class G

The effect of N.S oil on humoral immunity by measuring the total IgG level has been studied in Table (٧) for the children who had taken ٠.٦ ml /Kg /day) of N.S oil for three- or six-week durations.

The range **R** of total IgG before treatment is ٢٨٠-١١٣٣.٧ mg/dL and the mean is ٤٨٨.٣ mg/dL, whereas for the children treated by N.S oil for a three week duration, the **R** of total IgG is ٤١٢.٦-١٣٣٩.٥ mg/dL and the mean is ٩٨٦.٦mg/dL

For the children treated with ٠.٦ ml/ Kg /day of N.S oil for a six week durations the **R** of total IgG is ٤٣٨.٤-١٤٠٨.٥ mg/L and the mean is ١٠٢٥.٣ mg/dL.

From the results above, the enhancing role of NS oil on general humeral immunity appears clearly and this effect correlates with the duration of treatment with N.S oil. This effect might be related to increase the production of antibodies, bone marrow cells and B-cells. It may also be due to increase in the number of memory cells. This consists with what was reported by Cindy, ٢٠٠٠; EI -Kadi and Qundil ٢٠٠٤ and Meral et al, ٢٠٠٣;Sanamin,٢٠٠٤).

Table (٧) : N.S Oil Effect on Total IgG Level

Group	**Range of total IgG level (mg/dL)**	**Mean of total IgG level (mg/dL)**
Before N.S oil	٢٨٠-١١٣٣.٧	٤٨٨.٣
After ٣- week treatment with ٠.٦ ml /Kg /day of N.S oil	٤١٢-١٣٣٩.٥	٩٨٦.٦
After ٦- week treatment with ٠.٦ ml /Kg /day of N.S oil	٤٣٨.٤-١٤٠٨.٥	١٠٢٥.٣

CONCLUSION

١-There is a clear relationship between the number of OPV doses and the specific antibodies titers against polio , where the lowest APAbTs for unvaccinated children is ١:٢٠ and then the APAbTs increases with subsequent OPV doses.

٢-Thereis no difference in specific antibodies titers against polio for children who had two or three OPV doses.

٣-Thers is some correlation between the age of children and the level of APATs ; the lowest is during neonatl period and the range of APATs for children younger than three years apart from neonatal period is ١:٤٠ -١:٣٢٠ whereas that of older children is ١:٤٠ -١: ١٦٠.

٤-N.S oil had important positive role in the enhancement of specific humoral immunity against polio on both total number of responses children and the mgnitude of this immune response,and this positive role depends on :

a-Dose of N.S oil :(٠.٦ ml/kg /day)is the best dose that had achieved the best results in a fixed duration as compared with lower dose (٠.٣ ml/kg /day) that yeilds the lowest result .

b- Duration of treatment : A course ofa six- week duration in a fixed dose of N.S oil produces a higher immune response in comparim with the shorter duration(٣weeks).

c-Number of OPV doses received by children befor N.S oil treatment :The children who have four or more OPV doses had better immune responces in a fixed dose and duration in comparison with the children who have the lowest OPV doses.

٥-N.S oil has a positive role in increasing total immunoglobine – class G (IgG) for children, and this role also correlates with Durtion of treatment.

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RECOMMENDATIONS

In accordance with the results of this study ,and to ensure an increase in childrens specfic immune responses against Polio that have a schedualled vaccintion programme, we rcommended that:

١-The use of N.S oil becomes a part of daily meals for children by adding the oil to milk ,fruit juice or other types of foods.The recommended dose is ٠.٦ml/kg/day .

٢-The use of N.S oil as shown be encourged a part of processed food.

٣-Children are encourage to have N.S oil when they receive OPV doses.

٤-Further reasearch works in this fields against other viral and bacterial infectious disease are encourged as part of the effort to control these disease.

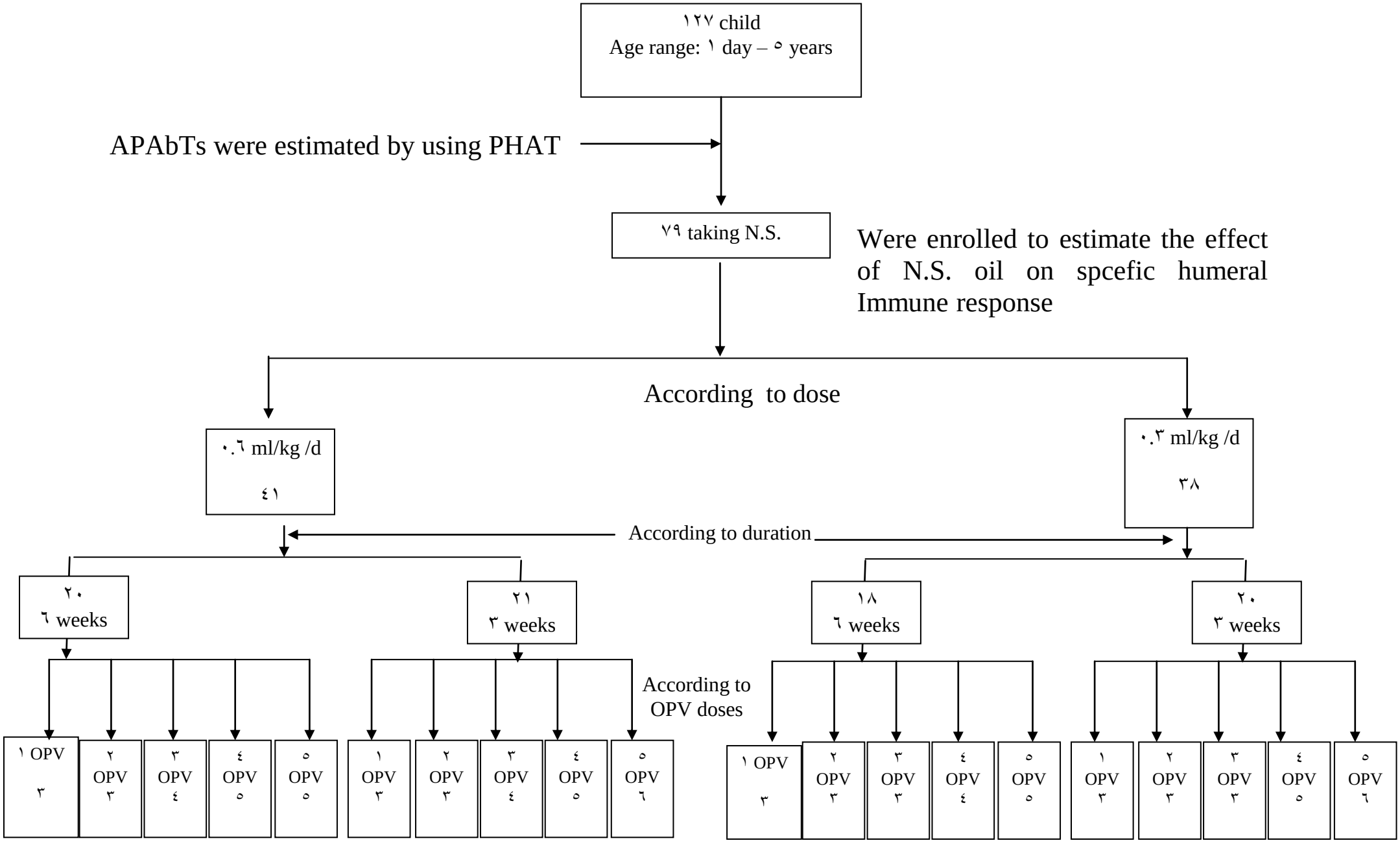

Figure ١ : The Division of Children

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