A Study of immune response against Poliomyelitis vaccine in Children in Hilla City

A Thesis
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دراسة الحالة المناعية عند الأطفال الملتحين بلقاح شلل الأطفال في مدينة الحلة

رسالة
مقدمة إلى مجلس كلية الطب ـ جامعة بابل كجزء من متطلبات نيل درجة الماجستير في الأحياء المجهرية الطبية

من قبل

ديار خليف فليفل الجنابي

بكالوريوس أحياء مجهرية

٢٠٠٦

١٤٢٦

بسم الله الرحمن الرحيم

(وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا)

صدق الله العلي العظيم

(الاسراء: الآية ٨٥)

Summary

In this study, ٢٤٢ serum samples were taken from healthy children at the age of ١ - ٥ years who received oral poliovirus vaccine (OPV) through the routine immunization coverage and national immunization days (NIDs). The information was obtained from parents. Many considerations are taken into account including number of doses received, age, sex, and, type of feeding. Passive haemagglutination test (PHAT) was used to evaluate the anti-poliomyelitis antibodies titers. We use the t test statistical analysis of the results. The following results were obtained:

- There is a positive relationship between the number of OPV doses received and titers recorded. .
- No significant differences were detected between male & female (P>٠.٥).
- In early life, immune response is not at a level that confers the protection. A higher number (٢٢) of children with titer more than ١:٨ occur in the age between ١ – ٤ years. .
- Type of feeding had an effect on the level of antibodies titers.

- Children who had received BCG vaccine show higher immune response (mean of titers = ١٣.١) to poliomyelitis than those who did not receive BCG vaccine (mean of titers = ٩.٧) . No significant difference was found between male and female(P < ٠.٥).

الخلاصة

تم في هذه الدراسة جمع ٢٤٢ عينة دم من أطفال سليمين أعمارهم اقل من خمس سنوات والذين تم إعطاؤهم لقاح شلل الأطفال المضعف عن طريق الفم خلال جدول اللقاحات الروتيني وكذلك خلال أيام اللقاحات الوطنية. تم اخذ المعلومات من أهالي الأطفال وكذلك من خلال بطاقة اللقاحات. في هذه الدراسة عدة اعتبارات تم أخذها بعين الاعتبار منها عدد الجرعات التي أخذها الطفل, العمر, الجنس,ونوع التغذية سواء كانت طبيعية أم اصطناعية أم مختلطة .

في هذه الدراسة تم استخدام طريقة ألتلازن الدموي المنفعل لقياس مستوى عيارية الأجسام المضادة التي تكونت عند الأطفال بسبب اللقاح. تم تحليل النتائج التي أستحصلت إحصائيا بواسطة الاختبار T (t -test) وقد تم الحصول على النتائج التالية:

- هنالك علاقة بين عدد الجرعات المستلمة ومستوى المناعة حيث كلما زادت الجرعات ارتفع عيار الأجسام المضادة .
- لا يوجد فرق معنوي بين الذكور و الإناث ($P < ٠.٥$)
- في بداية حياة الطفل, الاستجابة المناعية تكون غير كافية لمنح الأطفال الحماية الكافية. اكبر عدد من الأطفال اللذين لديهم عيار اكبر من ١:٨ كانوا في الفئة العمرية بين ١و ٤ سنوات.
- نوع التغذية يؤثر على مستوى الاستجابة المناعية حيث إن أعلى عيار سجل عند الأطفال اللذين رضاعتهم طبيعية أدنى عيار عند الأطفال اللذين كانت رضاعتهم اصطناعية.

- الأطفال اللذين استلموا لقاح BCG أظهروا استجابة مناعية (متوسط العيارية هو ١٣.١) أعلى ضد شلل الأطفال من أولئك اللذين لم يستلموا لقاح BCG (متوسط العيارية هو ٩.٧). لا يوجد فرق معنوي بين الذكور و الإناث (P<٠.٥)

List of Contents

List of Tables

List of Figures

Abbreviations

ACIP.	**Advisory Committee on Immunization Practices**
AFR .	**African Region**
BCG .	**Bacillus– Calmette – Guerine**
CDC .	**Center for disease control & prevention**
CNS.	**Central nervous system**
CSF.	**Cerebrospinal fluid**
DPT.	**Diphtheria, pertussis and tetanus**
EMR.	**Eastern Mediterranean Region**
EPI.	**Expanded programs on immunization**
EUR.	**European Region**
IPV.	**Inactivated poliovirus vaccine**
IRES.	**Internal ribosomal entry site**
LMN.	**Lower Motor Neuron**
MMWR.	**Morbidity & mortality weekly report**
NIDs.	**National immunization days**
PHAT.	**Passive heamagglutination test**
SEAR.	**South East Asia Region**
TCID.	**Tissue Culture Infectious Dose**

VP.	**Viral protein**
WHA.	**World Health Assembly**
WHO.	**World Health Organization**
WPR.	**Western Pacific Region**

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Conclusions:

- The level of immunity increases with multiple doses and this indicates the importance of continues vaccination campaigns.
- The main thing we conclude is that the immunity did not reach the sufficient level at which we can say that the vaccination programs perform their role successfully.
- Importance of BCG vaccine in increasing the immune responses.
- There are no differences between male and female in their responses.
- Importance of breast feeding, which provided secretory IgA to the babies.

Recommendations:

- Studies on vaccination sector must be continued and surveillance on acute flaccid paralysis (AFP) cases seems to be essential for preventing poliomyelitis.
- There is a shortage in vaccine availability and its provision , a case which must be resolved.
- Because the immunodeficient persons are at greater risk for VAPP, the children must be tested before they have been vaccinated.

Declaration

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

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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.

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Discussion

The oral poliovirus vaccine (OPV) of Albert Sabin is nearly ideal for use in polio eradication (Sutter et al, ٢٠٠٣, Nathanson and Fine, ٢٠٠٢, Dowdle et al, ٢٠٠٣).

There is a relationship between the number of OPV doses received and the level of APAbs titer. Two studies were carried out by other researchers (Rasha, ٢٠٠٥ and Karim, ٢٠٠١) showed similar results to our results. These results explained by the fact that, the first two doses represents the primary immune response which characterized by slow and low immunity which confirmed.

In the subsequent doses the immune response will be highest than those in the first dose according to the secondary immune response mechanism (Rasha, ٢٠٠٥).

The serological responses to OPV in children of Gambia and Oman after four doses of OPV, provides inadequate serologic protection against poliovirus especially for type ١. This highlights the need for supplemental strategy of NIDs. (WHO, ١٩٩٧)

Al- Obedi found that ٤ OPV doses induced ٨٧.٢٪ of children with detectable neutralizing antibodies against all three types of polioviruses (Al- Obedi, ١٩٩٦).

In Jordan, seroprevalence rates after ٥ doses of OPV showed that ٧٧٪ of children with neutralizing antibody against poliovirus type ٣ (Reichler et al, ١٩٩٧).

Antibody responses to vaccination are influenced by a variety of endogenous factors including genetics, sex, age, and exogenous factors such as stress, nutrition, and infectious diseases (Henk et al, ٢٠٠١). These factors need to be taken into consideration in clinical and epidemiologic studies.

Numerous epidemiologic and clinical studies have noted differences in the incidence and severity of parasitic diseases between males and females. Although in some instances this may be due to gender-associated differences in behavior, there is overwhelming evidence that sex-associated hormones can also modulate immune responses and consequently directly influence the outcome of parasitic infection. (Craig et al, ٢٠٠١). So sex is an important factor which influences the immune response.

In this study , we notice that is no significant difference in the titers between male and female (p>٠.٥) and these results disagree with the results obtained by other researchers (Karim, ٢٠٠١).

Abdul-Karim (٢٠٠٠) found that female had higher antibody level to the ٣-poliovirus serotype than male.

Vaccination response in childhood

Age is an important determinant for the immune response. In infants, maturation of the immune system continues after birth. Neonates are not able to respond to most polysaccharide antigens; children do better after ٢ years of age. Also, the response to protein antigens continues to further maturate during the first years of life. So we classified the children into ٤ age groups as follow:

The table (٣) shows the relation of immunity with age. We can note that the high number of children with titer higher than ١: ٨ occur in age between ١ – ٤ years. These results suggest that in early life, there are no sufficient response because of that the immune system is not developed enough to create protective response and therefore, the vaccine did not play its role in protection (Henk et al, ٢٠٠١).

Nutrition and Efficacy of Vaccination

Protein deficiency can affect immune responses in young children, depending on its severity. Under extreme malnutrition conditions such as marasmic (severe caloric deficiency), and kwashiorkor (severe protein deficiency) impairment of vaccination was found for yellow fever, smallpox, tuberculosis, and polio (Adeiga et al, ١٩٩٤).

Mother milk contains many agents that can help in protection and supports the immunity of body (Goldman, ١٩٩٣). Passive immunity is transferred from mother to infants. The maternally acquired antibodies gradually disappear during the first ٦ months of life (Ahmed and Gray, ١٩٩٤). Mother milk also contains secretory IgA antibodies, which acts on several microbial agents one of them is poliovirus (Goldman, ١٩٩٣).

In this study, we note that the type of feeding had an effect on immune response. The results obtained agreed with those obtained by (Lesourd, ١٩٩٥). Lesourd studied the effect of breast milk and four types of artificial milk on the effect of vaccination. Babies fed breast milk or high-protein cow's milk had an adequate and sustained responses; those fed on formula that was relatively low in proteins

and carbohydrates had high but temporary responses, and those fed on low-protein cow's milk or the soy-based formula had poor responses. Besides protein content, contaminants in the formula may also have had an influence (Lesourd, ١٩٩٥).

The concentration of type ١ and type ٢ IgG neutralizing antibodies in the newborn is approximately equal to those of the mother, type ٣ titers are somewhat lower than those of mother, suggesting differential transplacental transfer of this serotype (Cohen and Wright, ١٩٩١).

In this study, we examined the effect of BCG vaccine on immune responses by increasing the level of titers and note the differences between children who had received BCG and those who had not received BCG vaccine. We find that there are differences as we note in the table ٥.

The immaturity of the immune system increases the susceptibility of young infants to infectious disease and prevents the induction of protective immune response by vaccines. It was reported that mycobacterium bovis Bacillus –Calmette – Guerine (BCG) vaccination induce a potent Th١ response to mycobacterial antigens in newborn (Ota et al, ٢٠٠٢).

BCG vaccine increase the cellular and humoral immune responses to the hepatitis B vaccine, but had only limited influence on the cytokines response to tetanus toxoid and no effect on the antibody response to tetanus and diphtheria toxoid (Ota et al, ٢٠٠٢).The effect of BCG vaccine was apparent at the systemic level, as it increase the antibody response to oral poliovaccine (Ota et al, ٢٠٠٢).

These results demonstrate that BCG vaccine influence the immune response to unrelated antigens in early life, likely through its influence on the maturation of dendritic cells (Ota et al, ٢٠٠٢).

Introduction

Poliovirus, like other enteroviruses, is a member of the family picornaviridae. It is classified into three distinct serotypes (types ١, ٢, and ٣), based on their reaction with reference panels of neutralizing antisera (Melnik, ١٩٩٦). It is characterized by small size (٢٧ to ٣٠ nm in diameter), absence of envelope, and the genome is a single positive strand RNA molecule (Stanley & Walter, ١٩٩٩) .

The word poliomyelitis comes from two Greek words: polio, which means gray, and myelitis, inflammation of the spinal cord. Poliomyelitis can cripple and kill vulnerable individuals, especially children, within days (Stanley & Walter, ١٩٩٩)

Poliomyelitis is an acute viral disease, which ranges in severity from non-specific illness (٩٠ %) to paralysis with permanent disability (EPI, ١٩٩٣). The ratio of inapparent to paralytic infections may be as high as ١٠٠٠ to ١ in children and ٧٥ to ١ in adults, depending on the polio virus type and the social conditions (Sutter et al., ٢٠٠٤).

The number of poliomyelitis cases caused by wild poliovirus infections has been dramatically reduced by the extensive use of two available vaccines: the inactivated poliovirus vaccine (IPV)

developed by Jonas Salk, and the oral poliovirus vaccine (OPV) developed by Albert Sabin. Mass immunization campaigns with OPV, the most widely used vaccine, were a major factor influencing the success of eradication of wild indigenous poliovirus in the Americas (de-Quadros et al. ١٩٩٧). Surveillance for wild poliovirus circulation, through isolation, serotyping and intratypic differentiation of the poliovirus strains as wild or vaccine-related, is essential for global eradication of poliomyelitis (Hull and Dowdle ١٩٩٧).

In May ١٩٨٨, the World Health Assembly of the World Health Organization (WHO) adopted a resolution to eradicate poliomyelitis globally by the year ٢٠٠٠. Expanded Programs on Immunization (EPI) in Iraq began in ١٩٩٥. WHO recommended that infant received four doses of OPV during the first year of life, two booster doses of OPV are recommended at ١٨ months, and from ٤-٦ years of age. (Karim, ٢٠٠١)

NIDs were conducted at the first time in ١٩٩٦ in two rounds included all children < ٥ years of age regardless of immunization status (Wahdan et al, ١٩٩٧).

Protective immunity against poliomyelitis is conferred through immunization or natural poliovirus infection .Poliomyelitis confers type-specific lifelong immunity. Carrier states (asymptomatic

persons excreting poliovirus for more than ٦ months after infection) are rare and have been reported only in immunodeficient persons. (ACIP, ١٩٩٧).

In this study, we measured the immune response against poliomyelitis in children (who are living in Hilla City) with routine immunization and mass campaign of OPV.

Aims of the study

The use of poliomyelitis specific circulatory haemagglutinin to plot the postvaccines immune states in a children population with breast fed, bottle fed, BCG vaccine, and vaccine doses effect.

Literature review.

٢-١- Poliovirus

Polioviruses are part of the genus Enteroviruses and belong to the family Picornaviridea (Pico, implying small, and RNA, the nucleic acid composition). Polioviruses are small icosahedral viruses (٢٧ to ٣٠ nm in diameter); they are nonenveloped and contain a genome of RNA (Stanley & Walter, ١٩٩٩).

Poliovirus is classified into three distinct serotypes (type ١,٢, and ٣) based on their reaction with reference panels of neutralizing antisera (Melnick, ١٩٩٦).

٢-١-١-Composition

The shell of an enterovirus has icosahedral symmetry with ٦٠ subunits (promoters). Most of these are identical, each containing one set of the structural molecules VP١, VP٢, VP٣, and VP٤, the four proteins consist of ٣٠٦, ٢٧٢, ٢٣٨, and ٩٦ amino acids respectively. VP٤ is not exposed at the shell surface and seems to be closely associated with viral RNA (Grandien, ١٩٨٩). The three largest proteins (VP١-VP٣) are similar in core structure; the peptide

backbone of the protein loops back upon itself, forming a barrel of eight strands held together by hydrogen bonds (the beta- barrel).

Between beta barrel and the amino and carboxyl terminal portions of the proteins, the amino acid chain contains a series of loops, which include the chief antigenic sites found on the virion surface; these sites are involved in the neutralizing of virus infection. The smallest protein (VP٤) is functional in the encapsidation of the viral RNA in the mature virion (EPI, ١٩٩٧).

٢-١-٢-Virus replication

Poliovirus employs one of the simplest genetic systems known for proliferation (Pfister et al, ١٩٩٩). The virus enters the cell after attaching to the cellular receptor CD١٥٥ (Mendelsohn et al, ١٩٨٩). Immediately after the virus particle uncoats inside the cell, the genomic RNA is translated under the control of the internal ribosomal entry site (IRES) into a single polypeptide (Jang et al, ١٩٨٨ , Pelletier & Sonenberg, ١٩٨٨).

The polyprotein is then processed into functional proteins by two viral proteinases (Pfister et al, ١٩٩٩). With the aid of viral proteins, most notably the RNA-dependent RNA polymerase and the genome-linked protein along with cellular components, the viral

RNA is transcribed into minus-strand copies that serve as templates for the synthesis of new viral genome (plus-strand RNA). Newly synthesized plus-strand RNA can serve as messenger RNA for more protein synthesis, engage further in RNA replication, or be encapsidated by an increasing pool of capsid proteins (Wimmer et al, ١٩٩٣ , Xiang et al, ١٩٨٧). In suitable tissue culture cells (for example, HeLa cells), the entire replication cycle is complete in only ٦ to ٨ hours and yields ١٠٤ to ١٠٥ progeny virions per cell (Jeronimo et al, ٢٠٠٢)

٢-١-٣-History

Poliomyelitis has occurred sporadically from ١٦٠٠ to ١٣٠٠ BC. However, epidemic poliomyelitis is a modern disease related to improved sanitation and human hygiene. In the eighteenth century, the first description of poliomyelitis was given by Underwood while in the nineteenth century, the first epidemic of poliomyelitis occurred in Island of St. Helena (Samuel et al, ١٩٩٨). 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 (Stanely and Walter, ١٩٩٩). The first major polio epidemic reported in the United States occurred in Vermont

during the summer of ١٨٩٤. In ١٩٠٥, Wickman recognized that asymptomatic infection and transmission occurred via the gastrointestinal tract *(*Jublet & Lipton, ١٩٨٩*)*.

The most important development in the history of poliomyelitis was the introduction of polio vaccines. They decreased the incidence of paralytic poliomyelitis in the United States to fewer than ١٠ cases per year *(*CDC, ١٩٩٣ ; Weibel & Benor, ١٩٩٦*)*. Recent developments have included the cloning and sequencing of several strains of the three types of poliovirus(Racaniello & Baltimore, ١٩٨١. Stanway et al, ١٩٨٤) and the resolution of the viral structure to ٢٩ nm by x-ray crystallography (Hogle et al, ١٩٨٥). These techniques have made it possible to determine the precise viral coat amino acids that induce antibody responses (Wiegers & Demick, ١٩٩٢) and the location and the amino acid sequence of the site on the virus for cellular attachment (Palmenberg, ١٩٨٩,Hogle et al, ١٩٨٥,Rossman & Palmeberg, ١٩٨٨). The poliovirus receptor on the cell membrane has been identified and is a member of the immunoglobulin superfamily (Mendelsohn et al, ١٩٨٩).

٢-٢-Poliomyelitis :General Considerations

Poliomyelitis is an infectious disease caused by three types (serotypes ١, ٢, and ٣) of poliovirus, which is an enterovirus (a type of virus that inhabits the intestinal tract). The three serotypes are not cross-protective, which means that the individual must develop immunity to each type for complete protection against the disease (ACIP, ١٩٩٧ ; Grabenstein, ١٩٩٧).In countries where poliomyelitis is endemic, the disease often is caused by poliovirus serotype ١, less frequently by poliovirus serotype ٣, and least frequently by poliovirus serotype ٢. (ACIP, ١٩٩٧).

Poliomyelitis can be transmitted directly by fecal-oral contact or indirectly by contact with infectious saliva or feces (or by contaminated sewage or water). (Knolle, ١٩٩٥ , van der Avoort et al, ١٩٩٥). Polioviruses enter the mouth and replicate in the oropharynx and intestinal tract (ACIP, ١٩٩٧ ; Grabenstein, ١٩٩٧). From there, the viruses are carried by the blood stream into the central nervous system(CNS), resulting in cell destruction of the motor neurons of the anterior horn and the brain stem (ACIP, ١٩٩٧, Grabenstein, ١٩٩٧). However, The exact mechanism by which the CNS becomes infected remains uncertain and controversial. A study involving

transgenic mice expressing the human poliovirus receptor suggested that poliovirus spreads from muscle to CNS by means of peripheral nerve muscle fibers, rather than directly from the blood stream. (Ren & Racaneillo, ١٩٩٢).

Motor function of the individual is therefore impaired while the sensory function remains unaltered. (ACIP, ١٩٩٧ , Grabenstein, ١٩٩٧). Paralytic symptoms usually occur ٧ to ٢١ days from the time of initial infection (range is from ٤ to ٣٠ days). The period of communicability starts after viral replication, continuing as the virus is excreted in oral secretions and feces. Communicability ends when replication and excretion of virus cease, which usually occur ٤ to ٦ weeks after infection. More than ٩٠٪ of susceptible contacts become infected after household exposure to the wild poliovirus (ACIP, ١٩٩٧).

٢-٢-١-Clinical Symptoms and Signs

About ٩٥٪ of poliomyelitis infections are asymptomatic; these inapparent cases are still considered infectious (Grabenstein, ١٩٩٧).

Abortive (minor illness) type of poliomyelitis occurs in about ٤ to ٨٪ of infections and its manifestations include fever, headache, sore

throat, listlessness, anorexia, vomiting ,and abdominal pain (Joyce, ٢٠٠٠).

Neurologic examination is normal. The illness lasts from a few hours to about ٢ to ٣ days and is clinically indistinguishable from other nonspecific viral infections; it can be suspected clinically during an epidemic. The major illness types include nonparalytic and paralytic poliomyelitis. Nonparalytic poliomyelitis has more severe systemic manifestations than the abortive type, and with positive signs of meningeal irritation that make it clinically indistinguishable from aseptic meningitis caused by other enteroviruses (Modlin, ١٩٩٥).

Paralytic poliomyelitis can be classified as spinal, bulbar, or spino-bulbar disease (Joyce, ٢٠٠٠). The development of paralysis is rapid (about ٢ to ٤ hours), usually accompanied by fever and muscle pain, rarely progresses after the patient's temperature has returned to normal, and usually completed by ٣ days (Joyce, ٢٠٠٠). Spinal paralysis is usually asymmetric affecting one or more limbs. Deep tendon reflexes are absent or diminished. Bulbar paralysis is a serious form of poliomyelitis. It involves the medulla oblongata

which contains an important collection of nerve cells dealing with vital functions such as respiration and swallowing (ACIP, ١٩٩٧).

Many patients recover some muscle function after the acute episode. Prognosis can be firmly assessed usually within ٦ months after the onset of paralytic manifestations (ACIP, ١٩٩٧).

٢-٢-٢-Epidemiology

٢-٢-٢-١-Poliomyelitis seasonality

The transition of poliomyelitis to the epidemic phase was first seen in societies in cooler climates with advanced systems of hygiene and sanitation (Melnick, ١٩٩٦). Most of outbreaks in countries with temperate climates occurred at the end of spring through early autumn consistent with the classical seasonality of poliomyelitis reported during the prevaccination era. However, ongoing transmission during the winter months was not uncommon, especially in South Africa in ١٩٨٢, Bulgaria in ١٩٩١,and Jordan in ١٩٩٢, when peak incidence rates occurred. Outbreaks in tropical areas were reported in all times of the year, occasionally with peaks during the rainy season (Peter et al, ١٩٩٧).

٢-٢-٢-٢-Incidence

The incidence of paralytic poliomyelitis is peaked in the United States in ١٩٥٢ with more than ٢٠,٠٠٠ cases. Because of the introduction of the killed IPV (Salk vaccine) in ١٩٥٤ and the live attenuated OPV (Sabin vaccine) in ١٩٦١, the incidence decreased to less than ١٠ cases per year in the United States (Weibel & Benor, ١٩٩٦. ,Strebel et al, ١٩٩٢.). There is still a relatively high occurrence of the disease in Asia and Africa *(*Ramia et al, ١٩٨٧ ;CDC, ١٩٩٣*)*.

Poliomyelitis eradication was certified in the Americas in ١٩٩٤, the last case being reported from Peru in September ١٩٩١ (Robbins & de Quados, ١٩٩٧). In the European region, six virologically confirmed cases were reported in ١٩٩٧, all from south-eastern Turkey (Hull et al, ١٩٩٩). West and central Africa remain heavily endemic, with the Democratic Republic of the Congo and Nigeria serving as major reservoirs of wild poliovirus. South Asia is the other major global reservoir with Afghanistan, Bangladesh, India, Nepal and Pakistan remaining heavily endemic. Wild poliovirus, type ٢, was identified in ١٩٩٧ in only three countries -- Afghanistan, India and Pakistan (Hull et al , ١٩٩٩).

٢-٢-٢-٣-Route of Transmission

Human is the only known reservoir for member of the human enterovirus group, and a close human contact appears to the primary avenues of spread. Poliovirus is primarily spread by fecal-hand-oral transmission from one host to another. Poliovirus is transmitted from person to person; this virus can be spread to others by droplets from the upper respiratory tract during the early days of infection and more commonly, infected persons pass large numbers of virus particles through their feces, from where they may be spread directly or indirectly (Melnick, ١٩٩٦) .The virus is shed in oral secretions for several weeks and in the feces for several months (Kroon et al, ١٩٩٥).

It is often introduced into the household by small children who are not toilet trained and spreads in a family very rapidly, infecting most members in ٤ to ٥ days (Kroon et al, ١٩٩٥). Household spread depends on prior immunity, household size, and sanitary hygiene conditions (Melnick, ١٩٩٠). Transmission is related also to environmental factors such as sanitation, level of hygiene, crowded conditions, geography, the season, and host characteristics.

٢-٢-٣-Pathology and Pathogenesis.

The pathogenesis of poliovirus infection indicates that prevention through immunization can be accomplished by inhibiting replication at and dissemination from the gastrointestinal tract, by inhibiting viremia that follows, or by doing both (Stanley & Walter, ١٩٩٩).

As previously noted, polioviruses and other enteroviruses are spread by fecal-hand-oral transmission. After replication in the oropharynx and intestinal mucosae, the virus replicates in the submucosal lymphatic tissue (Wolnisky et al, ١٩٨٢), leading to a primary viremia, followed by replication in nonneural target tissues, and secondary viremia and CNS invasion. The exact route the poliovirus takes to enter the CNS is unclear, but viremia is required for CNS invasion (Kornreich et al, ١٩٩٦, Lipton & Jubelt, ١٩٩٣).

After exposure to poliovirus by way of the oral cavity, the virus attaches and enters the specific cells that express the poliovirus receptor (Mendelsohn et al, ١٩٨٩).The virus replicates locally at the site of virus implantation (e.g., tonsils, intestinal M cells , and Peyer patches of the ileum) or at the lymph nodes that drain these tissues.

The host range of poliovirus and tissue tropism is determined by the expression of the poliovirus receptor, which belongs to the immunoglobulin superfamily (Mendelsohn et al, ١٩٨٩).

There is evidence to suggest that poliovirus enters the neuraxis at areas where the blood-brain barrier is defective, such as the area postrema and another possibility is that the virus reaches the neuromuscular junction during the viremia, entering the distal axon and transported by retrograde axonal transport to the CNS (Roivainen et al, ١٩٩٣)

٢-٢-٤-Differential Diagnosis

Paralytic poliomyelitis may be confused with Guillain- Barré syndrome; in the latter, (a) the muscle weakness is more symmetric and ascending, with onset over a longer period of time (several days to ١ week) (Modlin, ١٩٩٥ ; Friedrich, ١٩٩٧), and with loss of sensation in about ٨٠٪ of cases; (b) paresthesia (which is an abnormal touch sensation such as burning or prickling often occurring in the absence of external stimulus) is common; and (c) CSF findings consist of high protein content with normal or minimal pleocytosis (presence of a greater than normal number of cells in the CSF) (Modlin, ١٩٩٥).

Other than Guillain-Barré syndrome, atypical/typical presentation of poliomyelitis may be mistaken for other clinical entities such as transverse myelitis (an inflammation of the spinal cord) (Friedrich, ١٩٩٧), traumatic neuritis, infection caused by other enteroviruses (notably enterovirus ٧١) (Hull et al, ١٩٩٤); coxsackieviruses A٧ (Grist & Bell, ١٩٨٤); A٩, or A٢٣ [Echovirus ٩]; or group B coxsackieviruses) (Gear, ١٩٨٤), or other paralytic conditions (Sabin, ١٩٨١) (e.g., injury of the spinal column resulting from periostitis/osteomyelitis, snake or tick bites, schistosomiasis [blood fluke infection], chemical poison, or following administration of anesthesia and certain drugs) (Gear, ١٩٨٤).

٢-٣-Post-Polio Syndrome

After ٣٠–٤٠ years, ٢٥٪–٤٠٪ of the persons who contracted paralytic polio during childhood can experience muscle pain and exacerbation of existing weakness or develop new weakness or paralysis. This disease entity, called post-polio syndrome, has been reported only in persons infected during the era of wild poliovirus circulation. Risk factors for post-polio syndrome include

a- the passage of more time since acute poliovirus infection

b- the presence of permanent residual impairment after recovery from the acute illness.

c- being female (Ramlow et al, ١٩٩٢).

٢-٤-Vaccine-Associated Paralytic Poliomyelitis (VAPP)

Cases of VAPP were observed almost immediately after the introduction of live, attenuated poliovirus vaccines (Terry, ١٩٦٢ ; Henderson et al, ١٩٦٤). Before the sequential IPV-OPV schedule was introduced, ١٣٢ cases of VAPP were reported during ١٩٨٠–١٩٩٥ (CDC, ١٩٩٧).

Fifty-two cases of paralysis occurred among otherwise healthy vaccine recipients, ٤١ cases occurred among healthy close contacts of vaccine recipients, and ٧ cases occurred among persons classified as community contacts (i.e., persons from whom vaccine-related poliovirus was isolated but who had not been vaccinated recently or had been in direct contact with vaccine recipients).

Additional ٣٢ cases occurred among persons with immune system abnormalities who received OPV or who had direct contact with an OPV recipient. The overall risk for VAPP is approximately one case in ٢.٤ million doses of OPV vaccine distributed, with a

first-dose risk of one case in ٧٥٠,٠٠٠ first doses distributed. Among immunocompetent persons, ٨٣٪ of cases among vaccine recipients and ٦٣٪ of cases among contacts occurred after administration of the first dose (Strebel et al ١٩٩٢ ; Prevots et al, ١٩٩٤).

Among persons who are not immunodeficient, the risk for VAPP associated with the first dose of OPV is sevenfold to ٢١-fold higher than the risk associated with subsequent dose (Prevots et al ١٩٩٤).

Immunodeficient persons, particularly those who have B-lymphocyte disorders that inhibit synthesis of immune globulins (i.e., agammaglobulinemia and hypogammaglobulinemia), are at greatest risk for VAPP (i.e., ٣,٢٠٠-fold to ٦,٨٠٠- fold greater risk than immunocompetent OPV recipients) (Sutter & Prevots, ١٩٩٤). Since implementation of the sequential IPV-OPV schedule in ١٩٩٧, five cases of VAPP with onset in ١٩٩٧ and two cases with onset in ١٩٩٨ were confirmed.

Three of these cases were associated with administration of the first or second dose of OPV to children who had not previously received IPV, and one of the ١٩٩٨ cases was associated with administration of the third dose. Although these data suggest a decline in VAPP after the introduction of the sequential schedule,

continued monitoring with additional observation time is required to confirm these preliminary findings because of potential delays in reporting (Prevots, ١٩٩٨).

٢-٥-Polio eradication

After the widespread use of poliovirus vaccine in the mid-١٩٥٠s, the incidence of polio declined rapidly in many industrialized countries. In the United States, the number of cases of paralytic polio reported each year declined from>٢٠,٠٠٠ cases in ١٩٥٢ to <١٠٠ cases in the mid-١٩٦٠s (Strebel et al, ١٩٩٢). In ١٩٨٨, the WHA resolved to eradicate polio globally by ٢٠٠٠ (WHA, ١٩٨٨). This global resolution followed the regional goal to eliminate polio by ١٩٩٠, set in ١٩٨٥ by the countries of the Western Hemisphere. The last case of polio associated with wild poliovirus isolation was reported from Peru in ١٩٩١, and an International Certification Commission in ١٩٩٤ (CDC, ١٩٩٤) certified the entire Western Hemisphere as free from indigenous wild poliovirus.

The following polio eradication strategies, which were developed for the Americas, were adopted for worldwide implementation in all polio-endemic countries (Hull et al, ١٩٩٤):

- Achieve and maintain high vaccination coverage with at least three doses of OPV among infants aged <١ year.
- Develop sensitive systems of epidemiologic and laboratory surveillance,
- Administer supplemental doses of OPV to all young children (usually those aged<٥ years) during National Immunization Days (NIDs) to rapidly decrease widespread poliovirus circulation.
- Conduct mopping-up vaccination campaigns (i.e., localized campaigns that include home-to-home [or boat-to-boat] administration of OPV) in areas at high risk to eliminate the last remaining chains of poliovirus transmission.

In ١٩٩٨, global coverage with at least three doses of OPV among infants aged <١ year was ٨٠٪. All WHO regions reported coverage rates of>٨٠٪, except the African Region (AFR), where coverage improved from ٣٢٪ in ١٩٨٨ to٥٣٪ in ١٩٩٨ (CDC, ١٩٩٧). Also in ١٩٩٨, a total of ٩٠ countries conducted either NIDs (٧٤ countries) or Sub-National Immunization Days (١٦ countries) (CDC, ٢٠٠٠).

These ٩٠ countries provided supplemental doses of OPV to approximately ٤٧٠ million children aged <٥ years (i.e., approximately three-quarters of the world's children aged <٥ years) (CDC, ١٩٩٧). In ١٩٩٩, NIDs were conducted in all ٥٠ polio-endemic countries. NIDs in the AFR targeted approximately ٨٨ million children aged <٥ years (CDC, ١٩٩٧). Synchronized NIDs were conducted in ١٨ countries of the European Region (EUR) and Eastern Mediterranean Region (EMR), vaccinating ٥٨ million children aged <٥ years. Another ٢٥٧ million children aged <٥ years were vaccinated in December ١٩٩٨ and January ١٩٩٩ in countries of the EMR (Pakistan), South East Asia Region (SEAR) (Bangladesh, Bhutan, India, Myanmar, Nepal, and Thailand), and Western Pacific Region (WPR) (China and Vietnam) (CDC, ١٩٩٨ ; CDC, ١٩٩٩). NIDs in India reached١٣٤ million children, representing the largest mass campaigns conducted to date. Each round of NIDs in India was conducted in only one-day (CDC, ١٩٩٨).

Mopping-up campaigns have been conducted widely in the countries of the Americas (including Brazil, Colombia, Mexico, Peru, and several countries in Central America) and more recently in the Mekong delta area encompassing Cambodia, Laos, and Vietnam

in ١٩٩٧ and ١٩٩٨, and in Turkey in ١٩٩٨ (CDC, ١٩٩٩). These supplemental immunization activities have been successful in decreasing the number of reported polio cases globally from ٣٥,٢٥١ in ١٩٨٨ (when the polio eradication target was adopted) to ٦,٢٢٧ in ١٩٩٨, a decrease of ٨٢٪ (CDC, ١٩٩٧). This decrease in incidence is even more remarkable considering the progress in implementing sensitive systems for AFP surveillance, which substantially increased the completeness of reporting of suspected or confirmed polio cases (CDC, ١٩٩٧).

To conduct virological surveillance, a global laboratory network has been established that processes stool specimens in WHO accredited laboratories, with both quality and performance monitored closely (CDC, ١٩٩٧).

Concurrent with the decline in polio incidence, the number of polio-endemic countries has decreased from >١٢٠ in ١٩٨٨ to approximately ٥٠ in١٩٩٨. Approximately ٥٠٪ of the world's population resides in areas now considered polio-free, including the Western Hemisphere, WPR (which encompasses China), and EUR.

Two large endemic areas of continued poliovirus transmission exist in South Asia and Sub-Saharan Africa (CDC, ٢٠٠٠). Priority

countries targeted for accelerated implementation of polio eradication strategies include seven reservoir countries (Bangladesh, Democratic Republic of the Congo, Ethiopia, India, Nepal, Nigeria, and Pakistan) and eight countries in conflict (Afghanistan, Angola, and Democratic Republic of the Congo, Liberia, Sierra Leone, Somalia, Sudan, and Tajikistan) (CDC, ١٩٩٧). Progress in these countries will be essential to achieve the goal of global polio eradication by the end of ٢٠٠٠(CDC, ٢٠٠٠).

٢-٦-ORAL POLIOVIRUS VACCINE (OPV)

٢-٦-١-Background

Routine production of OPV in the United States has been discontinued. However, an emergency stockpile of OPV for polio outbreak control is maintained. Because OPV is the only vaccine recommended controlling outbreaks of polio, this section describes OPV and indications for its use (CDC, ٢٠٠٠).

٢-٦-٢-Immunogenicity

After complete primary vaccination with three doses of OPV, >٩٥٪ of recipients develop long-lasting (probably lifelong) immunity to all three poliovirus types. Approximately ٥٠٪ of vaccine recipients develop antibodies to all three serotypes after a

single dose of OPV (McBean et al, ١٩٨٨). OPV consistently induces immunity of the gastrointestinal tract that provides a substantial degree of resistance to re-infection with poliovirus. OPV interferes with subsequent infection by wild poliovirus, a property that is important in vaccination campaigns to control polio epidemics.

Both IPV and OPV induce immunity of the mucosa of the gastrointestinal tract, but the mucosal immunity induced by OPV is superior (Onorata et al ١٩٩١). Both IPV and OPV are effective in reducing pharyngeal replication and subsequent transmission of poliovirus by the oral-oral route.

٢-٦-٣-Use of OPV for Outbreak Control

OPV has been the vaccine of choice for polio outbreak control. During a polio outbreak in Albania in ١٩٩٦, the number of cases decreased ٩٠٪ within ٢ weeks after the administration of a single dose of OPV to >٨٠٪ of the population aged ٠–٥٠ years. Two weeks after a second round of vaccination with OPV, no additional cases were observed (Prevots et al, ١٩٩٨).

Rapidly implemented mass vaccination campaigns resulting in high coverage appears to have been similarly effective in interrupting wild poliovirus outbreaks in other countries (Patriarca et

al, ١٩٩٧). European countries that rely solely on IPV for routine poliovirus vaccination (e.g., the Netherlands and Finland) have also used OPV for primary control of outbreaks (CDC, ٢٠٠٠).

During the ١٩٩٢–٩٣-polio outbreak in the Netherlands, OPV was offered to members of a religious community affected by the outbreak (who were largely unvaccinated before the outbreak) and other persons living in areas affected by the outbreak. IPV was given to immunized persons outside the outbreak areas to ensure protection in this population (Oostvogal et al, ١٩٩٤). During the ١٩٨٤–٨٥ polio outbreak in Finland, ١.٥ million doses of IPV initially were administered to children <١٨ years for immediate boosting of protection (Hovi et al, ١٩٨٦).

Later, approximately ٤.٨ million doses of OPV were administered to ٩٥٪ of the population. In contrast, mass vaccination with IPV exclusively had little impact on outbreaks and has rarely been used since OPV became available (Patriarca et al, ١٩٩٧).

٢-٦-٤-Advantages & Disadvantages of (OPV)

OPV is used for routine immunization and for global eradication of polio (Ghendon & Robertson, ١٩٩٤). WHO recommends that infants receive four doses of trivalent live OPV, at birth and at ٦, ١٠,

and ١٤ weeks of age, respectively. If a dose of OPV is not given at birth, then the fourth dose should be given at the time of the measles immunization contact, or at any other contact with the health care system during the first year of life. There should be an interval of at least ٤ weeks between any two doses. (WHO, ١٩٩٥) These OPV doses are part of the basic routine immunization coverage recommended by the EPI to protect children against major causes of morbidity and mortality in childhood, especially in endemic countries (Hull et al, ١٩٩٧).

٢-٦-٤-١-Advantages of OPV

OPV offers the following advantages: (WHO, ١٩٩٥)

١. It rapidly induces a long-lasting immunity.

٢. It is easy to administer, requiring no needle or syringe.

٣. It induces a high degree of gastrointestinal immunity, suppressing excretion of wild poliovirus.

٤. It induces a high level of population immunity (herd immunity), thereby reducing transmission of wild poliovirus.

٥. It is less expensive.

OPV has the ability to induce secretory immunity in the intestinal mucosa, which is the primary site of viral replication

(Ghendon & Robertson, ١٩٩٤). Person-to-person spread of the vaccine virus may help protect unimmunized persons or boost the immunity of those already vaccinated (Heymann et al, ١٩٨٧ , WHO, ١٩٩٧).

OPV induces herd immunity in two ways: (a) OPV recipients may shed the live attenuated vaccine virus that can infect (and protect) their contacts, and (b) when OPV recipients are exposed to the wild poliovirus, shedding of the virus through feces and pharynx is reduced (Murdin et al, ١٩٩٦). The ease of administration (oral), which results in simplified logistics (operations) and improved safety of mass immunization campaigns, low cost, and availability make the OPV ideal for use in both developing and industrialized countries (Hull et al, ١٩٩٧).

٢-٦-٤-٢-Disadvantages of OPV

OPV has certain limitations:

١. Suboptimal seroconversion rates after three doses reported in tropical developing countries.

٢. Poor thermostability of the vaccine.

٣. Extremely rare occurrence of vaccine-associated paralytic poliomyelitis (VAPP) in vaccine recipients and their contacts (Friedrich, ١٩٩٧, Wright & Karson, ١٩٩٥).

High degrees of seroconversion have been attained with the use of two or three doses of OPV in temperate, industrialized countries; the seroconversion rate after three doses of OPV is reported to be greater than ٩٠٪ in response to all three types of poliovirus (McBean et al, ١٩٨٨). In tropical countries, however, seroconversion after three doses averages only ٧٣٪, ٩٠٪, and ٧٠٪ for types ١, ٢, and ٣, respectively (Patriarca et al, ١٩٩١).

Suboptimal seroconversion may be due to the following factors: interference among the three strains of vaccine virus, high levels of maternal antibodies, a seasonal effect which is probably related to interference from other enteroviruses, and diarrhea. To enhance seroconversion in developing countries, a variety of approaches have been considered, including increasing vaccine potency, revaccinating infants who had diarrhea at the time of the previous dose, providing supplemental doses of OPV in routine programs or NIDs, which are usually held at the time of the year when seasonal effects are favorable (dry cooler months and usually low incidence

of diarrhea), and administering the vaccine to children at older ages to reduce the effect of passively acquired antibodies from the mother (Maldonado et al, ١٩٩٧, Deming et al, ١٩٩٧). IPV has been proposed to resolve the issue of suboptimal seroconversion with the use of OPV (Patriarca et al, ١٩٩١). IPV, however, produces inadequate secretory intestinal immunity and will not eradicate polio in developing countries when used alone (Ghendon & Robertson, ١٩٩٤). Maternal antibodies reduce the seroconversion response to IPV and thus may not immunize infants in countries where polio is endemic.

Mixed OPV/IPV schedules (which are used in some developed countries) provide improved systemic immunity, but intestinal secretory immunity does not differ from that provided by OPV alone in developing countries (WHO,١٩٩٧). Because of the cost and the complexity of administration, mixed OPV/IPV schedules are not considered suitable for routine immunization in developing countries (Hull et al, ١٩٩٧).

In rare instances, administration of OPV has been associated with subsequent paralysis in healthy recipients and their contacts. This may be caused by the attenuated poliovirus in the vaccine

reverting to virulence by mutation. The risk of vaccine-associated paralytic poliomyelitis (VAPP) is extremely small, occurring at a rate of ١ case for every ٢.٥ million OPV doses administered or ١ case in ٧٠٠,٠٠٠ first doses administered (Strebel et al, ١٩٩٢) compared with an incidence of ٢ to ٥ cases of paralytic poliomyelitis due to the wild poliovirus for every ١٠٠٠ non-immunized children in highly endemic countries (Hull et al, ١٩٩٧).

٢-٧-Precautions and Contraindications

٢-٧-١-Immunodeficiency

Infection with poliovirus posses an increased risk for persons with primary B cell immunodeficienties and ,in these persons, infection with wild virus or vaccine strains may develop in an atypical manner, with an incubation period longer than ٢٨ days, a high mortality rate after a long chronic illness, and unusual lesions in the central nervous system (WHO, ١٩٩٣)

Among vaccine - associated cases in immunologically abnormal persons in the United States types ٢ and ١ were the polioviruses most commonly isolated from stool specimens (Strebel et al. ١٩٩٢) .

Prospective and retrospective studies in both developing and industrialized countries report no serious adverse events in over ٤٠٠ HIV-infected children who received live attenuated oral polio vaccine (OPV) (Onorato & Markowitz,١٩٩٢).

٢-٧-٢-Injections

Several studies have shown that injections (for antibiotics or other vaccines) increase susceptibility to polio. In fact, researchers have known since the early ١٩٠٠s that paralytic poliomyelitis often started at the site of an injection (WHO, ١٩٩٣). When diphtheria and pertussis vaccines were introduced in the ١٩٤٠s, cases of paralytic poliomyelitis skyrocketed (Lindsay et al, ١٩٨٦). Children who received DPT (diphtheria, tetanus, and pertussis) injections were significantly more likely than controls to suffer paralytic poliomyelitis within the next ٣٠ days (Sutter et al, ١٩٩٢). Injections must be avoided in countries with endemic poliomyelitis (Wyatt, ١٩٩٠) . Health authorities believe that all “unnecessary” injections should be avoided as well (Wyatt et al, ١٩٩٢).

٢-٧-٣- Malnutrition

Data on the risk of infection with wild poliovirus in malnourished children are not available (WHO, ١٩٩٣) . Following

a dose of OPV, serum neutralizing antibody titers were similar in malnourished and well-nourished children; however, in malnourished children, secretory IgA antibody has been detected significantly less often, at lower levels, and with a delayed appearance (Chandra, ١٩٨١).

٢-٧-٤- Physical activity

Early studies showed that for persons who developed 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 did not relate to subsequent paralysis (WHO, ١٩٩٣).

٢-٧-٥-Pregnancy

Although no adverse effects of OPV have been documented among pregnant women or their fetuses, vaccination of pregnant women should be avoided (CDC, ٢٠٠٠). However, if a pregnant woman requires immediate protection against polio, she can receive OPV in accordance with the recommended schedules for adults (CDC, ٢٠٠٠). Clinical data suggest that both the incidence and the severity of poliomyelitis may increase in pregnant women (Harjulehto et al, ١٩٩٣).

٢-٧-٦-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 at greater risk of paralysis.

Materials and Methods

٣-١-Materials.

٣-١-١-Serum samples

Two-handred and forty two (٢٤٢) blood samples were collected from healthy children in Babylon Maternity and Children hospital whom ages were ranging from less than ١ year to more than ٥ years. These samples were then used to obtain their serum by centrifugation at ٢٥٠٠ rpm.

٣-١-٢--Equipment and materials used:

Equipments and tools.

Name of equipment	Type	Origin
Centrifuge	Heareus	Austria
Refrigerator	Heareus	Austria
Incubator	Heareus	Austria
Autoclave	Heareus	Austria
Automatic micropippete	Oxford	USA

Biological reagents

Trivalent attenuated oral poliovaccine was used as an antigen to neutralize the antibodies that might be found or present in serum samples which we had collected.

Composition of OPV

Aqueous suspension in Earle medium with ٠.٥٪ lactalbumin of attenuated poliomyelitis virus types ١, ٢, and, ٣, grown in *Cercopithecus aethiops* kidney tissue cultures and stabilized with ١M magnesium chloride.

Each ٠.١ ml dose contains: not less than: ١.٠٠٠.٠٠٠ **T.C. I. D.٥٠** (tissue culture infectious dose) of type ١, ١٠٠.٠٠٠ **T.C.I. D. ٥٠** of type ٢, and ٦٠٠.٠٠٠ **T.C. I. D.٥٠** of type ٣.

The OPV manufactured in the United States contains approximately threefold to tenfold the minimum dose of virus necessary to meet these requirements consistently. Each dose of ٠.٥ ml also contains<٢٥ mg each of streptomycin and neomycin. No adjuvant or preservatives were used (Sutter et al., ٢٠٠٤)

٣-١-٤-Chemical reagents

١- Trisodiume citrate

٢- Glucose

٣- NaCl

٤- Tannic acid powder

٣-١-٥- Others

١- Syringes and needles.

٢- Cotton.

٣- Plane tubes.

٤- Centrifuge tube.

٥- Microtiter plate.

٣-٢ – Methods

After the data were collected, they have been classified into ٤ age groups as follow:

Study groups	Age(year)	No.
Group ١	**Less than ١ year**	٤٦
Group ٢	١-٢	٦٨
Group ٣	٢-٤	٧٠
Group ٤	**More than ٤ years**	٥٨
Total		٢٤٢

٣-٢-١- Preparation of solution and reagents.

٣-٢-١-١-Alsever's solution

Alsever's solution is an isotonic, anticoagulant blood preserving solution that permits the storage of whole blood at refrigeration temperature for about ١٠ weeks (Talib, ١٩٩٦). This solution was prepared by dissolving ٢٤.٦ gm glucose (BDH company); ٩.٦ gm trisodium citrate (BDH company); and ٥.٠٤ gm sodium chloride (BDH company) in ١٢٠٠ ml distilled water; the pH was then adjusted to ٦.١ with ١٥٪ citric acid.

٣-٢-١-٢-Tannic acid solution

The solution was prepared by dissolving ٠.٥gm of tannic acid powder ($C_{٧٦}H_{٥٢}O_{٤٦}$; BDH company; M.W = ١٧٠١.٢٢) in ٢٠ ml distilled water.

The volume was then completed to ١٠٠ ml with distilled water to obtain final tannic acid concentration of ٠.٥٪. This solution was used to tan RBCs (i.e. to mope the Age found on sheep erythrocyte surface).

٣-٢-١-٣- Preparation of tanned erythrocyte

Sheep blood (١٠ ml), obtained from animal under sterile conditions, was mixed with ٣٠ ml of Alsever's solution. The resulting mixture was kept at ٤ degree centigrade. From this mixture, ٣ ml was taken in a sterile centrifuge tube and centrifuged at ٢٥٠٠ rpm for ٥ minutes. After using sterile Pasteur's pipette, the supernatant was discarded and the deposit was resuspended in ١٠ ml of normal saline and centrifuged at ٢٥٠٠ rpm for ٥ minutes. The supernatant was discarded again and the deposit was resuspended in ١٠ ml of normal saline and mixed thoroughly.

From this mixture, three ml was taken in a sterile glass tube into which three ml of ٠.٥٪ tannic acid solution was added, mixed and placed in the water bath at ٣٧ 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 was added and gently mixed

٣-٢-٢- Passive Haemagglutination test (PHAT)

According to the modified methods of (Gravey et al., ١٩٧٧), PAHA used to determine the level of antibodies against poliovirus in sera of children after the immunization programs.

Procedure:

Tanned sheep erythrocytes (٣ ml) were mixed with one ml of the poliovaccine and left at room temperature for ١٠ minutes. After centrifugation at ٢٥٠٠ rpm for five minutes, the supernatant was discarded and to the deposit ٣ ml of normal saline was added. Then centrifugation at ٢٥٠٠ rpm for ٥ minutes. The supernatant was discarded and to the deposit ٢ ml of normal saline was added and mixed. Microtitration plates were used to estimate anti-poliovirus antibodies titer in children sera, according to the method of Garvey et al. (١٩٧٧).

The procedure was carried out as follows:

١- Fifty microliter of normal saline was dispensed in each well of the microtitration plate wells.

٢- Fifty microliter of children sera was added to the first well and mixed. Then serial dilutions were prepared by transferring fifty

microliter from the first well to the second well, after being mixed thoroughly, fifty microliter was transferred to the third well and so on. From the eleventh well fifty microliter was discarded. Serial dilutions thus was obtained from the first well to the eleventh well: ١: ٢, ١: ٤, ١: ٨, ١: ١٦, ١: ٣٢, ١: ٦٤ accordingly.

٣- To the twelfth well, fifty microliter of tanned sheep erythrocytes covered with the poliovirus was added (the latest well represened the negative control well which was lacking the presence of anti-poliovirus antibodies).

٤- The microtitration plate was shaken gently for about ٢ minutes and then incubated at ٣٧ degree centigrade for ٢ hours. Then the presence or absence of haemagglutination was recorded. PHAT titer was represented by the latest dilution that gave a positive result.

Results

A total of ٢٤٢ serum samples were obtained from normal children of known age, sex, type of feeding, and vaccination status. The samples were then tested by the method of passive haemagglutination test to evaluate the level of immune response against poliovaccine given during routine programs or through mass vaccination campaign. We came up with the following results:

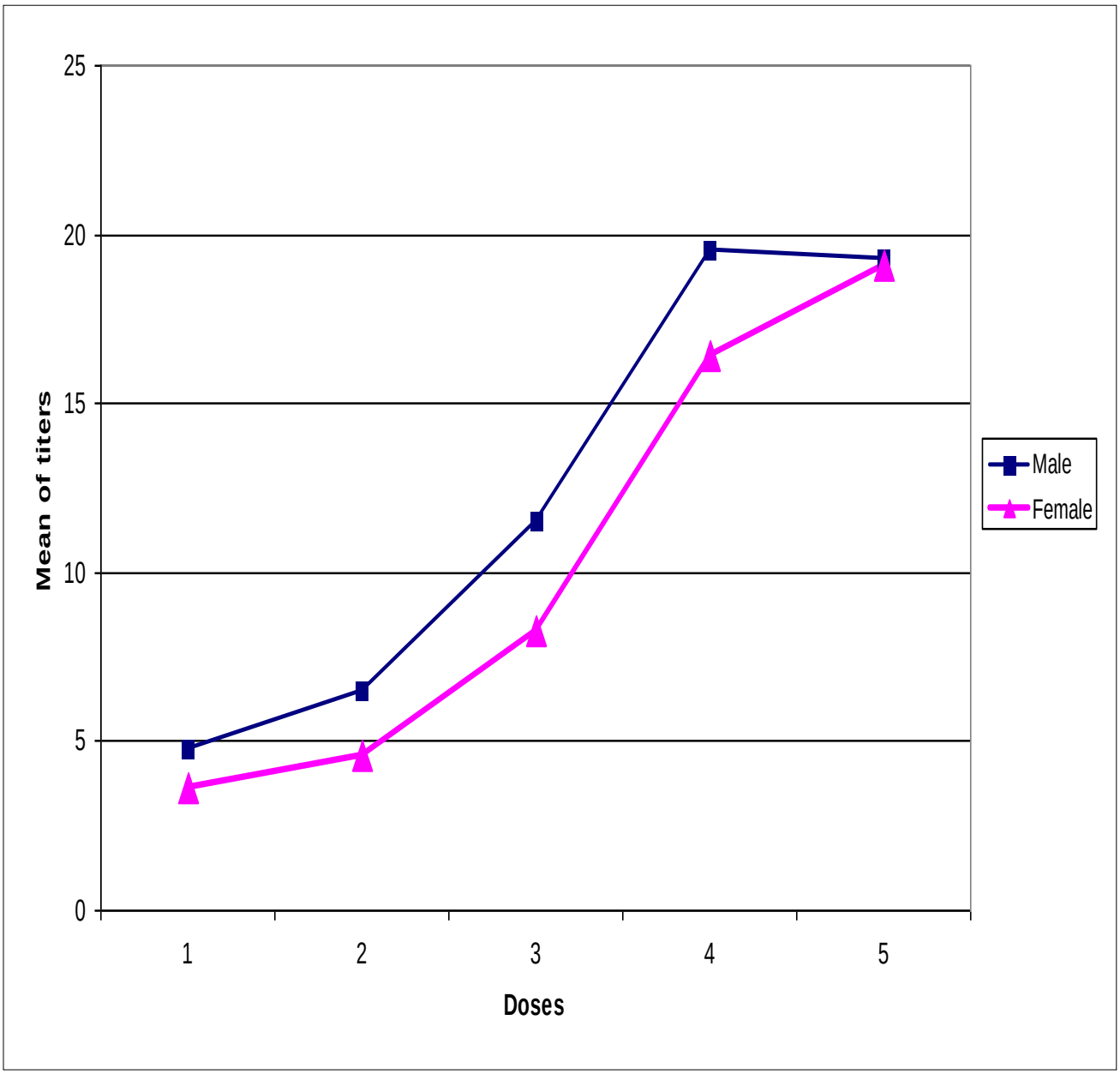
A – Number of doses and the level of Ab response.

Low titers were found even in those who received many doses of OPV as shown in the following table:

Table (١). Show the mean of antibody titers with progressing in vaccine doses number.

Vaccine doses	Antibody titers of age groups			
	< ١	١-٢	٣-٤	>٤
١	٣.٨	٣.٢	٦.١	٤.٥
٢	٥.٨	٥.٥	٧	٦.٣
٣	٨.٣	١٢.٣	١٢.٢	١٠.٦
٤	٠	١٨.٩	١٦.٧	١٧.٨
٥	٠	١٧.١	٢١.١	١٩.٥

There ia a relationship between the number of OPV doses received and the level of anti-polio antibodies titers as shown in the figure ١ :



(Fig.١): Relationship between number of doses and the level of Abs titers.

B – Antibody response in respect to sex.

We found that there is no significant difference between male and female in antibody response to OPV doses as shown in table (٢).

Table (٢) show the differences in immune responses between male and female

Sex	***Male***			***Female***		
Age (year)	No.	%	Mean of Titers	No.	%	mean of Titers
<١	٢١	٨.٧	٦.٣	٢٣	٩.٥	٥.١
١-٢	٣٧	١٥.٣	٨.١	٢٩	١١.٩	١١.٤
٣-٤	٣٥	١٤.٤	١٤.٣	٣٠	١٢.٤	٩.٨
> ٤	٣٩	١٦.١	١٠.٤	٢٨	١١.٥	١١.٣

C - Vaccination response in childhood.

Table (٣) shows the immune responses according to the age groups mentioned in table ١.

Age (Year)	*Titers*					*Total*
	١:٢	١:٤	١:٨	١:١٦	١:٣٢	
< ١	١١	١١	١٥	٥	٥	٤٧
١- ٢	٨	١٩	٧	٢٢	٩	٦٥
٣- ٤	١	١٦	٢٢	١٤	١٠	٦٣
> ٤	٩	١٨	١٨	١٣	٩	٦٧
Total	٢٩	٦٤	٦٢	٥٤	٣٣	٢٤٢

١-less than ١ year

Children less than ١ year show low level of antibodies (١: ٨ in maximum) and no significant differences between male and female were detected (P < ٠.٥) as shown in the Figure ٢ :

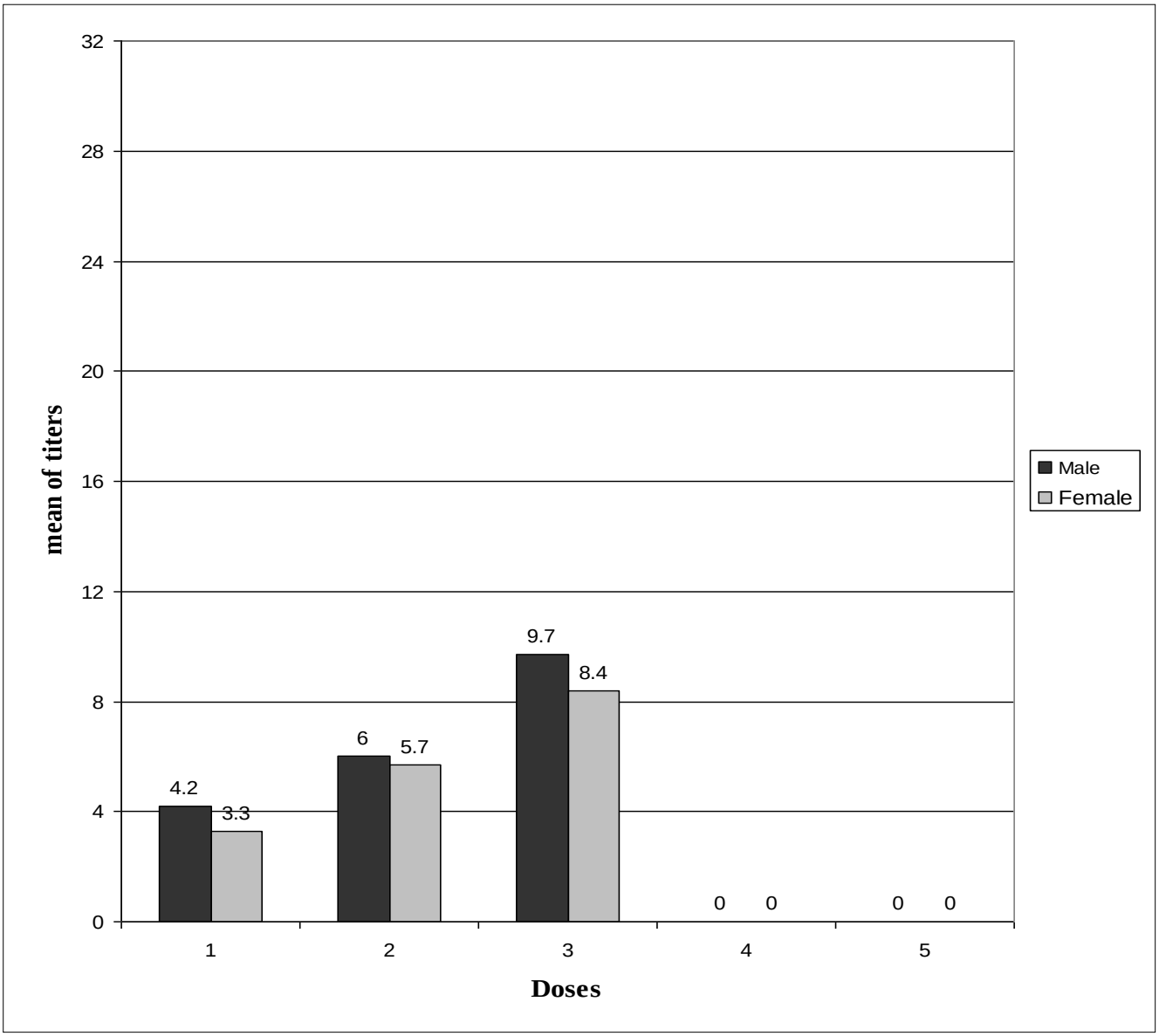


fig ٢:The immune response against poliomyelitis in children less than one year

٢- from ١-٢ years

Figure ٣ explains the immune response in children from ١- ٢ years. Titers (mean of titers) in this figure are higher than those in figure ١(reach to ١: ٢٠) and we can note the rise in level of immunity with progress in vaccination schedule (dose ١,٢…٥).

There are significant differences (P> ٠.٥) between doses ١&٢ and doses ٤ & ٥ but these differences are not found between male and female.

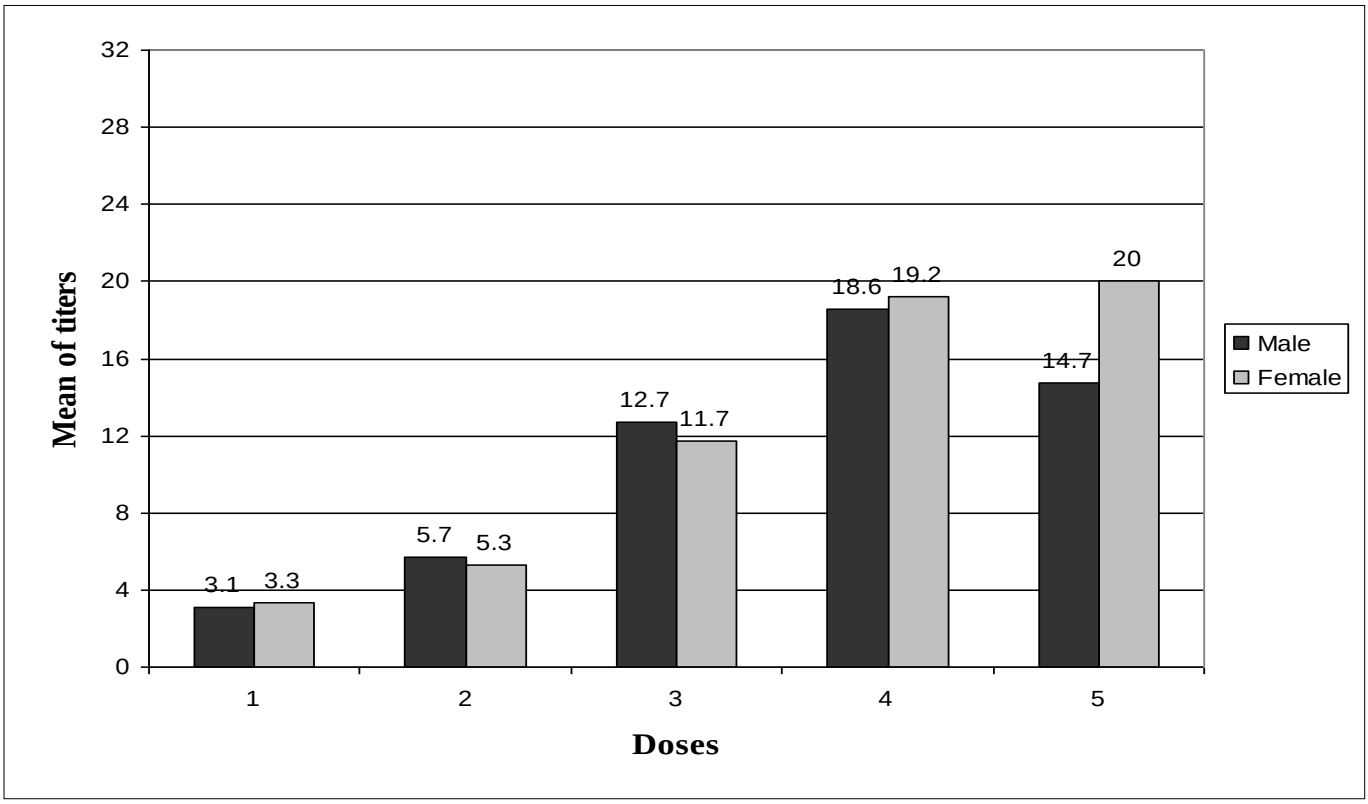


Fig ٣:The immune response against poliomyelitis virus in children ١-٢ years

٣- from ٣-٤ years

Children from ٣-٤ years and children of more than ٤ years do not differ from those shown in figure ٣. No significant differences between male and female (P< ٠.٥) were found. (Fig ٤&٥)

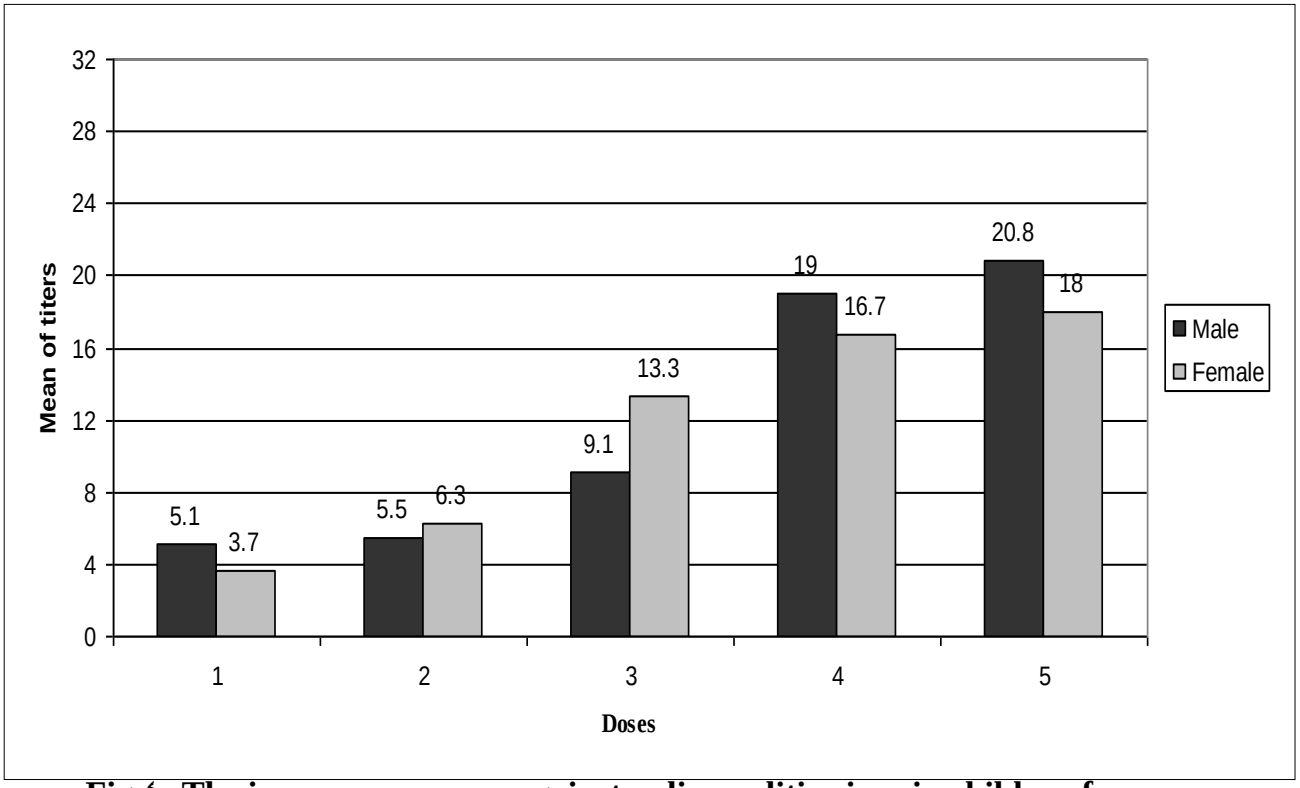


Fig ٤: The immune response against poliomyelitis virus in children from ٣-٤ years

٤- more than ٤ years.

Figure ٥ explain the immune response in children of more than ٤ years group by the fact that with progress in vaccination, the immune response began to rise as we note in the ٤ and ٥ doses. There are significant difference (P > ٠.٥) between dose ٤

and dose ٣ and between dose ٥ and dose ٤. No significant difference between male and female (P < ٠.٥).

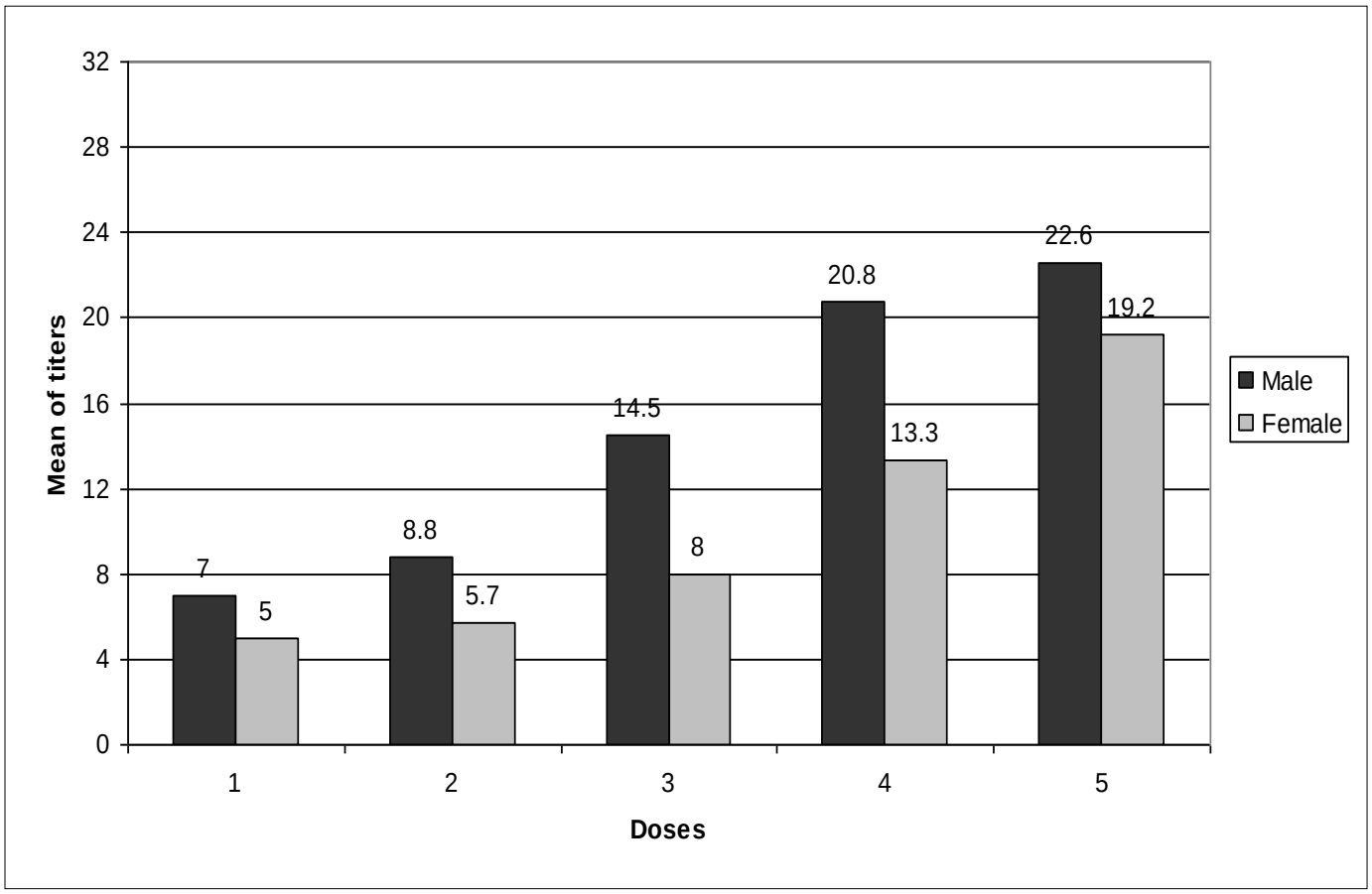


Fig ٥:The immune response against poliomyelitis virus in children more ٤ years

D- Feeding type effect

In this study we found that the type of feeding also affects the level of immunity, table (٤). Children who depend on breast-feeding show significant difference (P > .٠٥) in the level of

antibodies from children who do not depend on breast-feeding (bottle & mixed feeding).

When we look at the table below, we can note that the highest mean of titers was found in the sector of children with breast feeding if it is compared with the results obtained from children with bottle and mixed feeding. Mean of titers were measured with progress in doses number and we can note that in each dose, breast fed children had the higher number.

No significant difference was found between male and female (P>٠.٠٥).

Table (٤) show the effect of type of feeding on the immune responses against poliovaccine.

Type of feeding	Mean of titers				
	١ Dose	٢ Dose	٣ Dose	٤ Dose	٥ Dose
Breast feeding	٥.٧	٧.٦	١١.٨	١٦.٢	٢١
Bottle	٣.٤	٥.٤	١٠.١	١٢.٣	١٣.٨
Mixed	٣.٧	٦.٨	١١.٧	١٢.٨	١٦.٢

In the following figures, we explain the previous results:

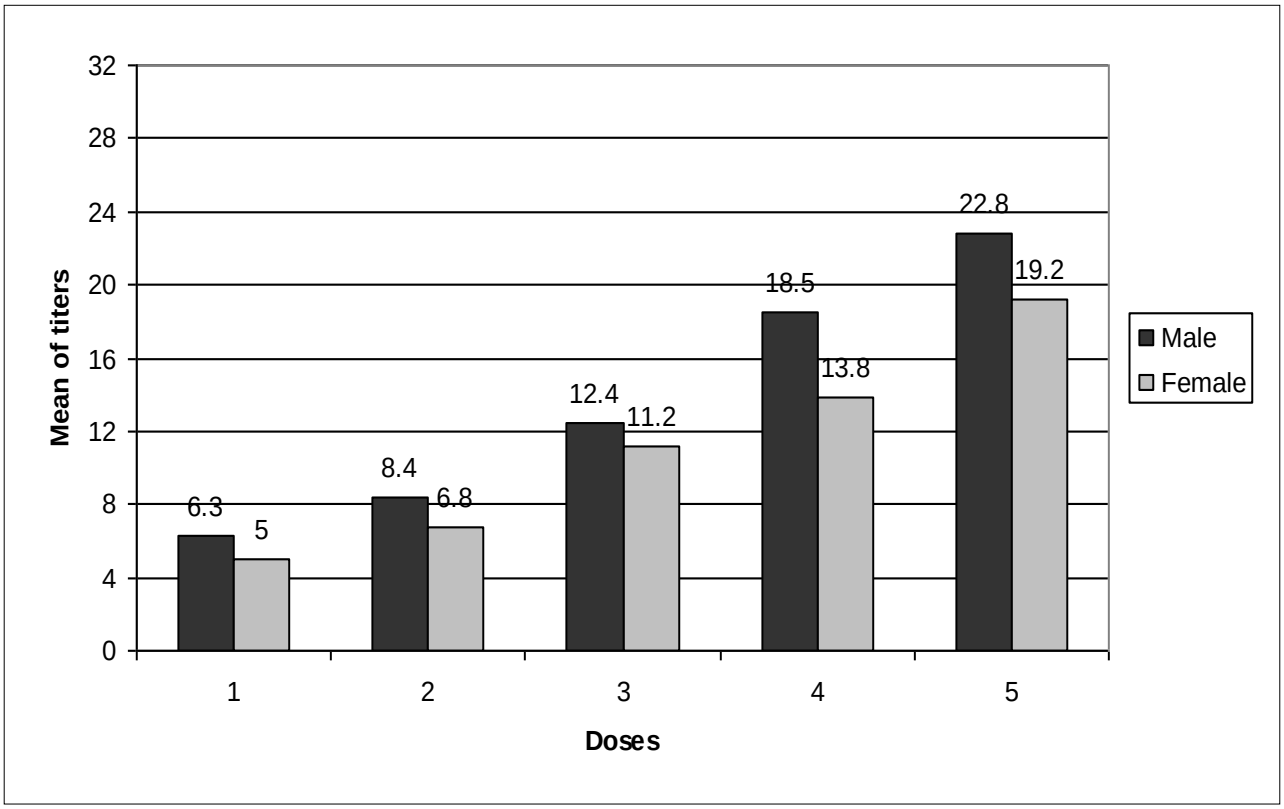


Fig ٦: The immune response against poliomyelitis in children who depend on breast feeding with different doses of vaccine.

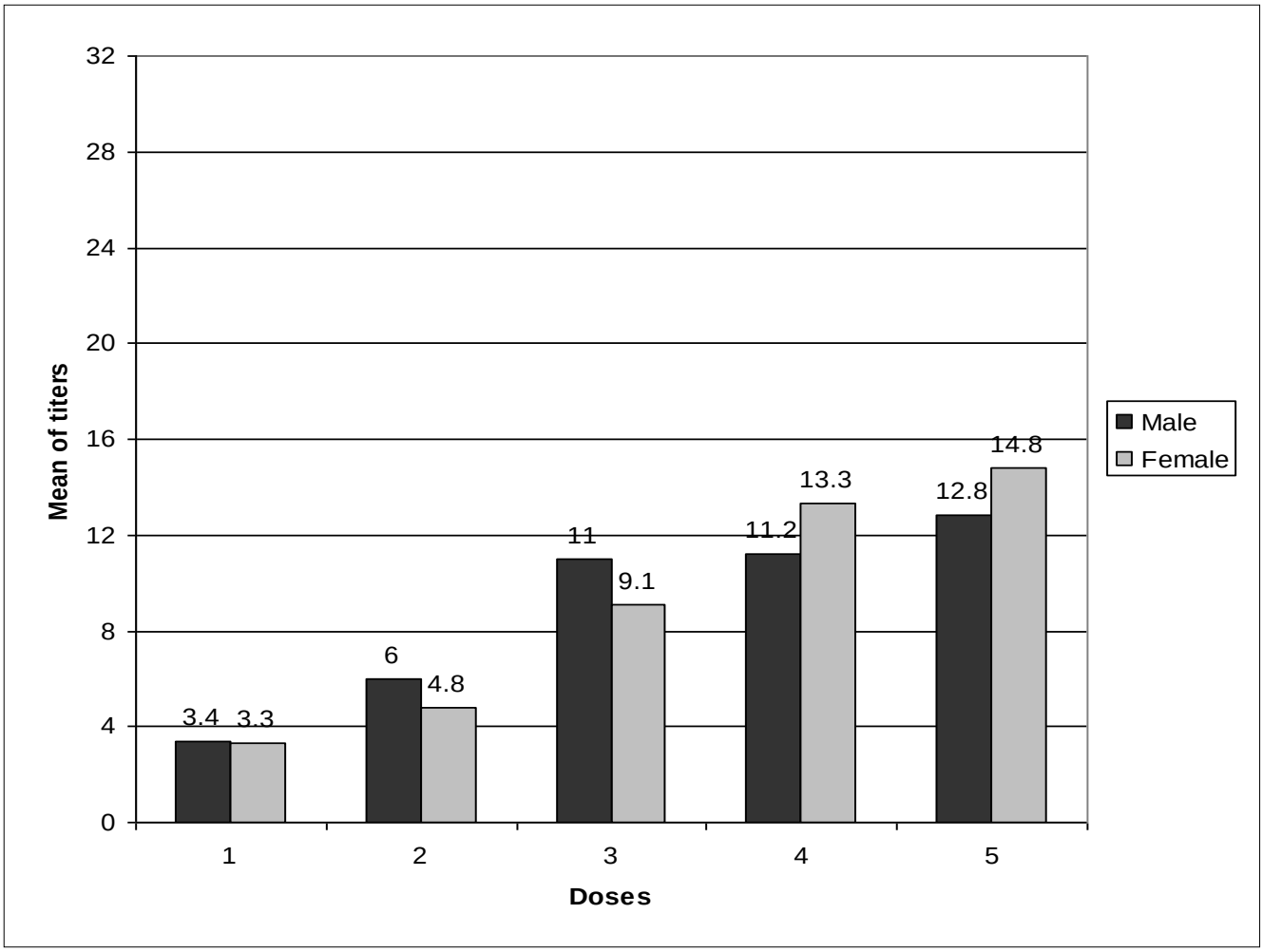


Fig ٧: The immune response against poliomyelitis in children who depend on bottle feeding with different doses of vaccine.

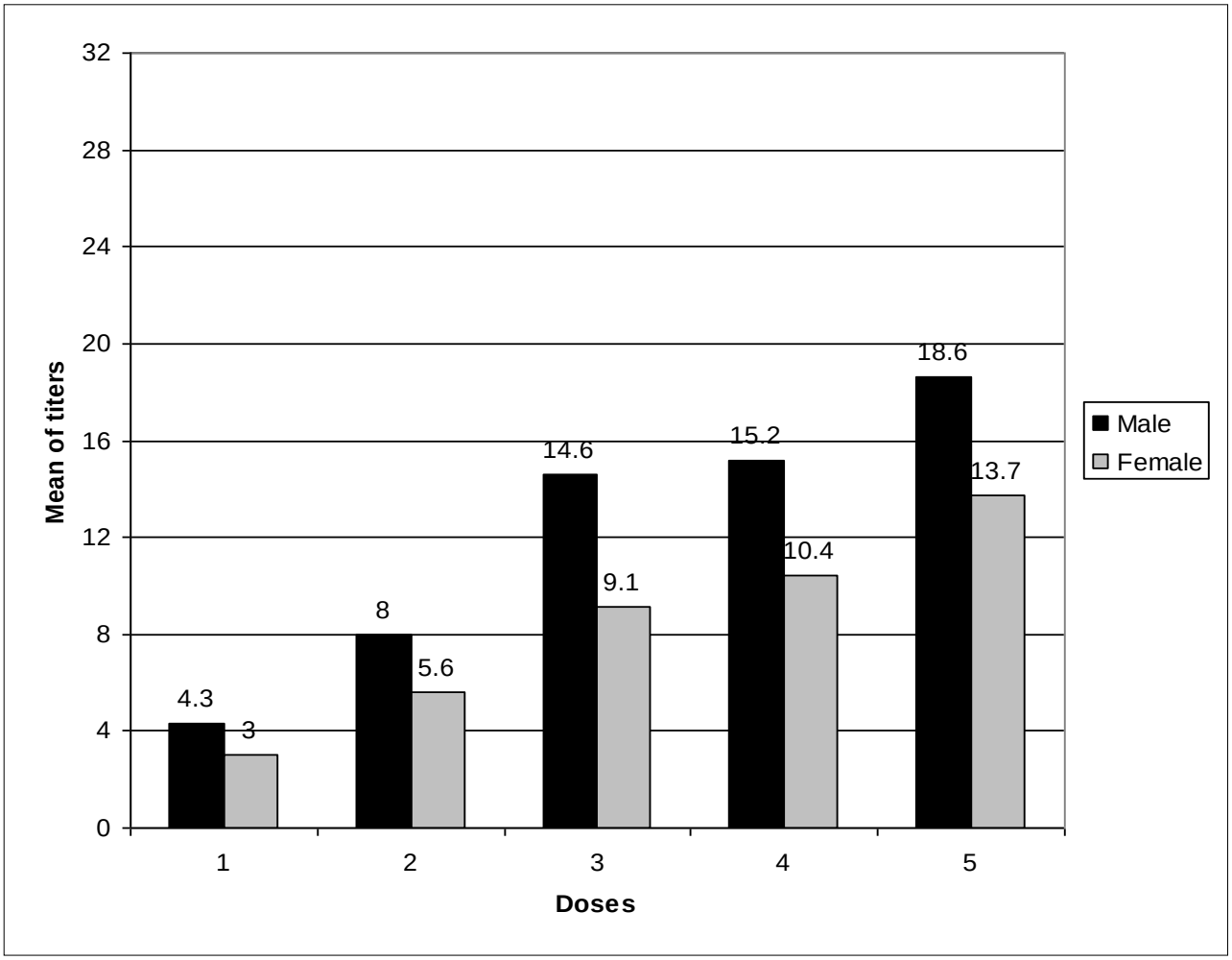


Fig.٨ : The immune response against poliomyelitis in children who depend on mixed feeding with different doses of vaccine.

E- Role of BCG vaccine

BCG vaccine was found to act as an enhancing factor for immune response. we found that the children who had received BCG vaccine had higher level of antibodies if it is compared with the children who did not receive the same vaccine as we note in the table ٥.

Table (٥) shows the impact of BCG vaccine as immunity enhancing factor.

Sex	BCG +		BCG -	
	No.	Mean of titers	No.	Mean of titers
Male	٨٩	١٤.٨	٤١	١٠.٦
Female	٦٣	١١.٤	٤٩	٨.٩

In the figures ٩ & ١٠ we can note the differences in antibody response between BCG + children and BCG - children. The immune response in children who receive OPV vaccine for one time and BCG + (M= ٤.٥ & F= ٦) do note differ from those with one dose of OPV and BCG – (M =٤ & F= ٤.٢). From the second dose we began

to note the difference (M= ١١.٢ & F= ٩.٧) for BCG + and (M= ٧ & F= ٥.٤) for BCG-.

Third dose give the following results : (M =١٣.٢ & F =١٢) for BCG+ and (M = ٨.٢ & F= ١٠.٤) for BCG-. In the fifth dose, we record that the children with BCG+ were (M= ٢١.٥ & F= ٢٠.١) and the children with BCG- were (M= ١٨.٢ & F= ١٦). In addition, we did not found significant differences between male and female (p < ٠.٥).

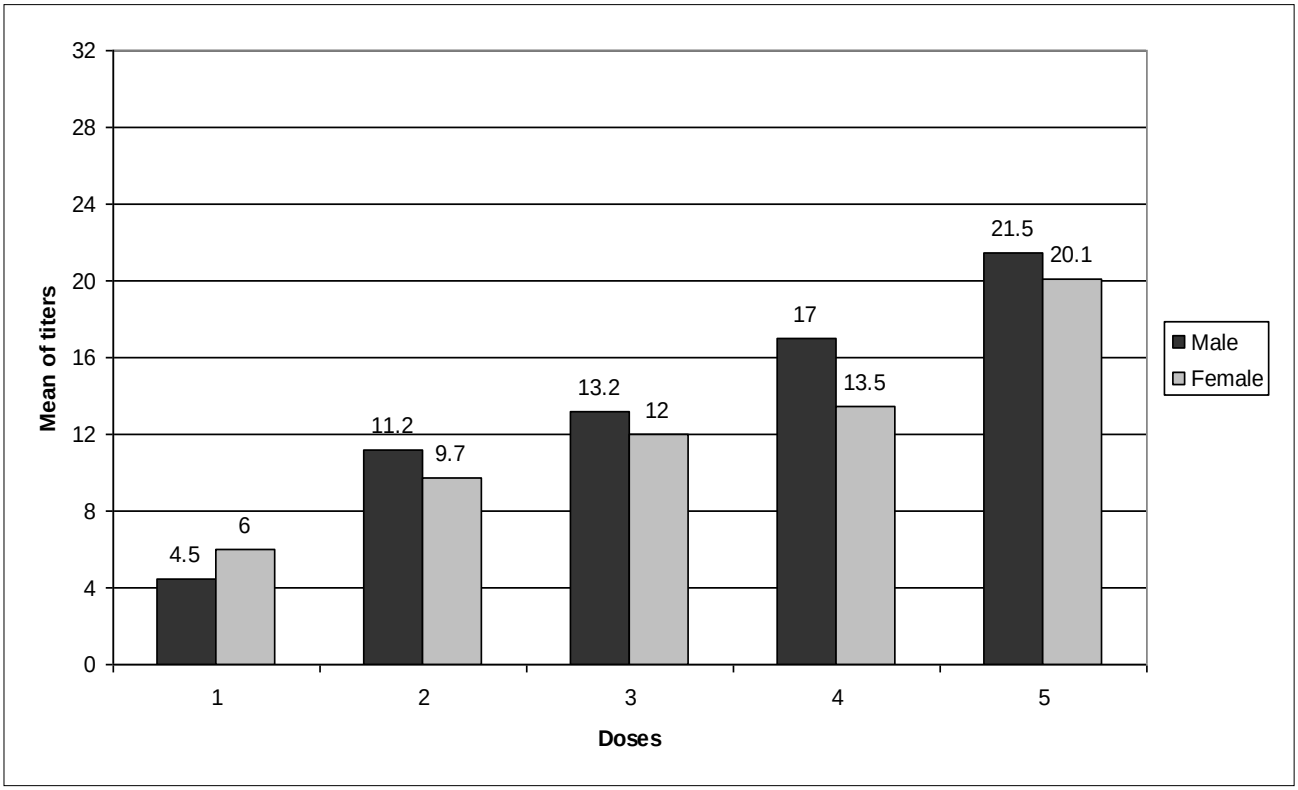


Fig. ٩: The immune response against poliomyelitis in children who had received BCG vaccine.

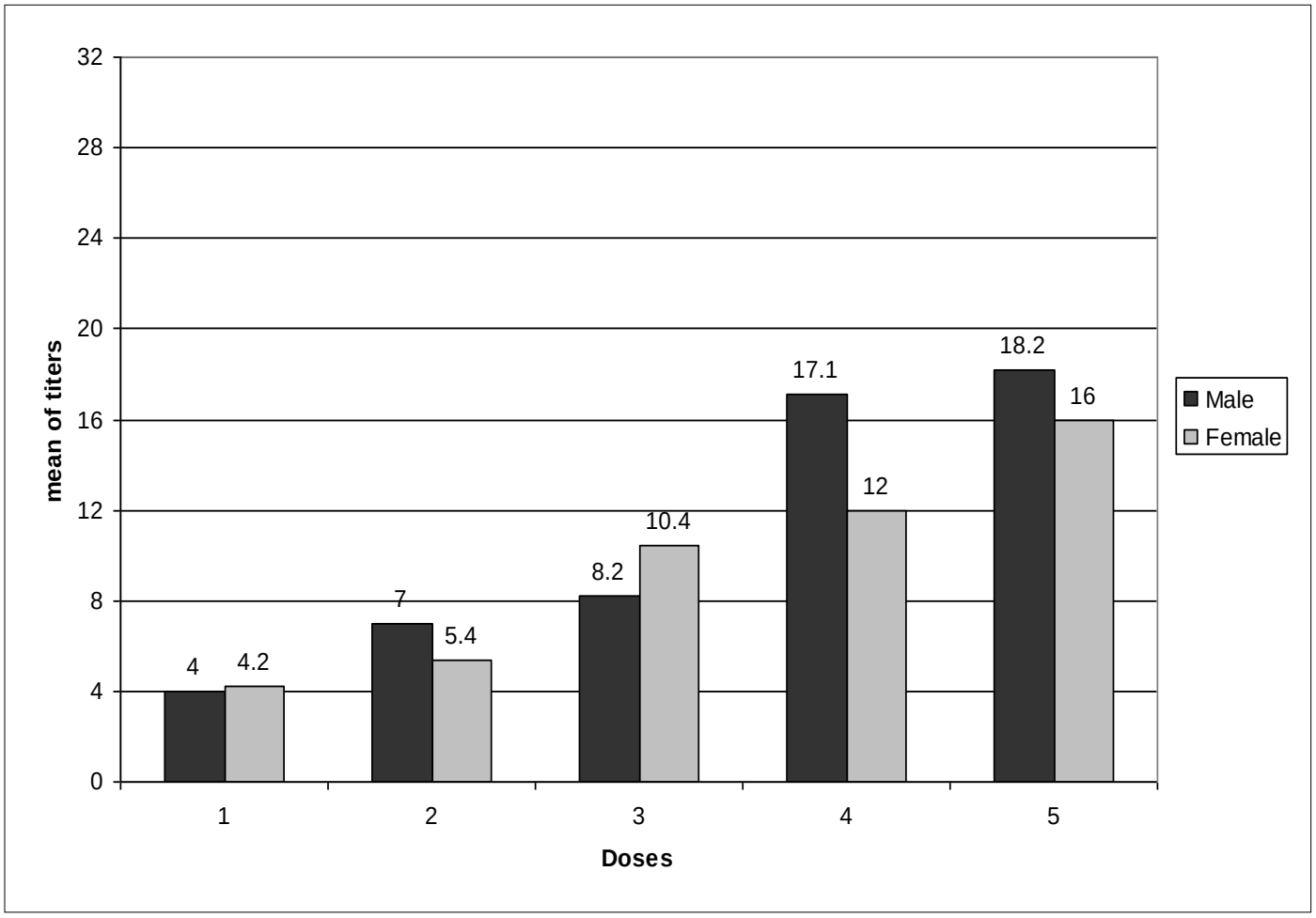


Fig. ١٠: The immune response against poliomyelitis in children who had not received BCG vaccine

Type of feeding	Mean of titers				
	١ Dose	٢ Dose	٣ Dose	٤ Dose	٥ Dose
Breast feeding	٥.٧	٧.٦	١١.٨	١٦.٢	٢١
Bottle	٣.٤	٥.٤	١٠.١	١٢.٣	١٣.٨
Mixed	٣.٧	٦.٨	١١.٧	١٢.٨	١٦.٢

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