Incidence Of Meningococci Infection In Gaza Strip

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الملخص

مدى انتشار ميكروب النيسيريا في عدوى التهاب السحايا بين أطفال قطاع غزة

أجريت الدراسة لمعرفة مدى انتشار ميكروب النيسيريا بين أطفال قطاع غـزة. 1048 عينه سائل نخاع شوكي فحصت وذلك بعمل مزرعة لهذا الميكروب، و كذلك بالفحص المباشـر بواسطة صباغة الميكروب. 95 (8%) عينه أظهـرت نتـائج إيجابيـة لهـذا الميكـروب. 10 (0.9%)أظهرت نتائج ايجابية للميكروب في الدم (Meningococcemia). أظهـر الفحـص بصبغة جرام نتائج ايجابية في 110 (10%) من عينات سائل النخاع الـشوكي. نـسبة النتـائج الايجابية كانت الأعلى في الأطفال حديثي الولادة، و كذلك الفئة العمرية 1-4 سنوات. العوامـل الاجتماعية مثل انخفاض المستوى الاقتصادي كان لها دور في حدوث العدوى. أظهرت النتـائج أن هناك علاقة قوية بين حالات ال Meningococcemia وانخفاض عدد كرات الدم البيـضاء، و كذلك نسبة انخفاض الكالسيوم كذلك نقص في البروتينات من النوع C . فحـص الـسلالات السيرولوجية أظهر أن الأنواع السائدة في قطاع غزة هي W135 وB. نـوع B يوجـد فـي المنطقة الشمالية وغزة و رفح، بينما نوع W135 كان السائد في منطقة خان يونس.

الكلمات المفتاحية: ميكروب النيسيريا، التسمم الدموي بالنيسيريا، التهاب السحايا.

ABSTRACT

A total of 1048 CSF samples examined from clinically diagnosed meningitis patients were subjected to microscopic and cultured. 95 (8%) samples were showed positive for *Neisseria meningitidis* and 10 (0.9%) samples showed positive for meningococcemia. Gram positivity was observed in 110 (10%) of the CSF samples. The percentage of bacterial isolates was highest in new borne and infants and in patients of 1-4 years age group. The low socioeconomic status was the main risk factor for development of infection. There was high significant associations between meningococcemia and leucopenia, thrombocytopenia, hypocalcaemia and protein C deficiency. The only serogroup of *N. meningitidis* which found in

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Gaza Strip were serogroups B and W135. serogroup B was more prominent in North Gaza, Gaza and Rafah, while serogroup W135 was more prominent in Khanyounes.

Key wards: Neisseria meningitides, Meningococcemia, Miningitis.

Introduction:

Meningitis is the inflammation of meninges, the membranes that cover the brain and spinal cord **(Jacobs, 2000)**. The most common etiological agents of bacterial meningitis in children are *H. influenzae* type b, *N. meningitidis* and Strep. *pneumoniae,* where group *B streptococcus, E. coli* and *Listeria monocytogenes* are the most common causative agents in neonates **(Clark, 1994)**, the uncommon pathogens are *Staph aureus* and *Pseudomonas aerogenos. Mycobacterium tuberculosis* is the less frequent cause of bacterial meningitis **(Lever, 1996)**. Many viruses have the ability of causing meningitis such as *Echovirus, Coxsachievirus* type A and *Herpes simple virus* type I and II, *Epstien-Barr virus*, *HIV, Varicella Zoster virus* and *Cytomegalovirus* **(Bouckenooghe and Shandera, 2000)**. In addition to *Cryptococcus neoformans* and *Candida,* which are the most common fungi isolated from CSF **(Imanagha, *et al* 1998)**. Meningococcal disease is a contagious bacterial infection caused by meningcococci (*Neisseria meningitidis)* with high case fatality rate. The mode of spread from person to person by direct contact through respiratory droplets from infected patients or carriers. *Neisseria meningidtis* is a common inhabitants of the mucosal membranes of nose and throats where it usually causes no harm up to 5-10% of a population may be asymptomatic carriers **(Nelson, 1996)**.

Meningococcal disease occurs worldwide as endemic infection **(Fernandez, *et al* 1999)**. Strains of B and C causes the majority of infections in industrialized countries, while strains of serogroups A and to a lesser extent C dominate in third world countries **(ElBushr, *et al* 2000, and Cizman, *et al* 2001)**. The incidence of meninigococcal disease during the last 30 years varies from 1-3/100,000 in most industrialized nations to 10-45/100,000 in some third-world countries. These different attack rates reflect the different pathogens properties of *N. meningitidis* strains, different socioeconomic, environmental and climatological conditions **(Caugant, 1998)**.

Sub-saharan Africa has a special epidemiological pattern, this region designated the meningitis belt was first described by Lebeyssonniae in 1963 and comprised 10 countries, i.e.; Burkina Faso, Ghana, Togo, Benin, Niger,

Nigeria, Chad, Cameroon, Central African, Republic, and the Sudan **(Lapeyssonnie, 1963)**, later Ethiopia,Mali, Guinea, Senegal and the Gambia were added to form what is presently denoted the expanded meningitis belt **(Riedo, *et al* 1995)**. In this region, meningococcal disease caused by serogroup A occurs in yearly recurrent waves, during epidemic peaks, the disease incidence may approach 1000/100,000 inhabitants. *Neisseria N. meningitidis* was classified into seven clonal complexes **(Caugant, 1998)**. The largest outbreaks, which originated in Northern China and spread to the south and later globally, were caused by two clones of serogroup A (subgroup I and III) **(Achtman, 1995, and Jones, 1995)**. Group B isolates with ET-5 characteristics were discovered in China in 1974 and in China, Japan, Thailand, Spania, Cuba, Chile and Brazil in 1980s. Clonal complexes, identified as ET-5, lineage III, cluster A4, and ET-37 **(Zhu and Xul, 1995)**.

Neisseria meningitidis causes both endemic and epidemic disease principally meningitis and *meningococcemia* **(CDC, 1985)**. As a result of the control of *Haemophilus influenza* type 6 infections *N. meningitidis* has become the leading cause of bacterial meningitis in children and young adults in the United States, with an estimated 2600 cases each year **(CDC, 1993)**. In a multistate surveillance conducted during 1989-1991 in USA, the incidence of meningococcal disease peaks in late winter to early spring. Attack rates are highest among children 3-12 months of age and then steadily decline among older age groups. Serogoup B organisms accounted for 40% of all cases and serogroup C for 45%. Serogroups W-135 and Y are strains that could not be serogouped accounted for most of the remaining cases **(CDC, 1993)**. Other data indicated the proportion of cases caused by serogroup Y strains is increasing **(CDC, 1996)**. Serogroup A, which rarely causes disease in the United States is the most common cause of epidemics in Africa and Asia. In the United States localized outbreak of serogroup C disease and a statewide group B epidemic have recently been reported **(Jackson, *et al* 1995, and CDC, 1994)**.

Antibiotics are the cornerstone of treatment sulfonamides decreased mortality to 10% **(Zhu and Xul, 1995)**. In the 1950 and 1960 sulfonamide resistance necessiteated a switch to penicillin or chloramphenicol. Decreased susceptability (MIC $\geq$ 0.25mg/liter) has been reported in several countries, this decreased sensitivity is caused by a reduced affinity to penicillin binding protein type 2 **(Soez-Nieto, *et al*, 1992)**. Occasionally penicillin resistance due to plasmid-related β-lactamase production occurs **(Dillow, *et al* 1983)**. Chloramphincol resistance has also been reported **(Galimand, *et al* 1998)**.

Materials and Methods

Specimens: A total of 1048 CSF specimens submitted to the pediatric hospitals in Gaza Strip were examined for bacterial agents by each test.

Microbiological examinations:

The following samples were collected

1- Cerebrospinal fluid (CSF)

All CSF specimens were inoculated in Columbia base agar with 5% sheep blood, chocolate agar and in supplemented peptone broth, all plates and tubes were incubated at 35^0C in addition Chocolate agar was maintained in an atmosphere of 5 to 8% CO_2 . Daily examination of the media for bacterial growth was. performed for 3 days Peptone broth was subcultured after 2 days onto chocolate agar **(Popovic et al. 1999)**.

2- Blood culture

Blood specimens were withdrawn from the patients for blood culture and inoculated in bottles containing Trypticase Soy Broth. The blood culture bottles were incubated at 35^0C and subcultured on solid media (blood agar, MacConckey agar and Chocolate agar) after 24 to 48hr and at 7 days **(Cheesbrough, 1993)**

3- Skin biopsy

Samples from skin rash were collected, smear were prepared and stained with Gram stain **(Thomas et al. 1999)**.

Identification of *N. meningitidis*

Purified isolates of *N. meningitidis* were identified by using Kovac's oxidase test, carbohydrate fermentation reactions and API, NH system.

Specific antiserum for the determination of serogroups of *Neisseria meningitidis* was applied for the isolated colonies.

Serogroups A, B, C, W135 and Y are the most cause of meningococcal disease **(Frasch, 1987)**

Antibiotic susceptibility test

All the isolated and purified strains were tested for the antibiotics susceptibility test. The method used was the disk diffusion method which principally depends on the determination of minimum inhibition concentration and the inhibition zone. The calibrated inoculum of *N. meningitidis* was inoculated into Muller Hinton media and the antibiotic

disks were placed on the surface of plates. Inhibition zones were determined after incubation at 37^0C for 24hr **(Henry, 1996)**.

Protein C examination:

The method used in the determination of protein C is called VIDAS protein C, manufactured by Biomeriux company. VIDAS protein C is an automated quantitative test for use on the VIDAS analyzer for the quantitative measurements of protein C in human plasma using the ELFA technique (Enzyme Linked Fluorescent Assay). The assay test comprised two step enzyme immunoassay method with final fluorescent detection **(Biomeriux, 2001)**

The Results:

The total number of samples examined for meningitis were 1048. 95 (8%) samples were positive for *N. meningitidis* and 10(0.9%) samples showed positive for meningococcemia. 58% of the cases were females, while 42% of them were males (table 1)

Table (1) : Relationship Between Type of Diagnosis and Sex

	Meningitis		**Meningococcemia**		**Both**		**Total**	
	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
Male	18	58	13	41	9	28	40	42
Female	13	42	19	59	23	72	55	58
Total	31	100	32	100	32	100	95	100

75% of the cases were living in towns, 21% in Refugee camps and 4% in villages table (2)

Table (2): Distribution of Cases By Type of Diagnosis and Residency Area

	Meningitis		Meningococcemia		Both		Total	
	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
North	9	29	12	38	12	37	33	34
Gaza	13	42	13	41	14	44	40	42
Midzone	4	13	7	22	2	6	13	14
Khanyounis	4	13	0	0	2	6	6	6
Rafah	1	3	0	0	2	6	3	3
Total	31	100	32	100	32	100	95	100

The mean age of cases was 2.9 years, 79% of the cases were <5 years, table (3)

Table (3): Distribution of Cases By Age Groups

Age group year	Meningitis		Meningococcemia		Both		Total	
	No of cases	%	No of cases	%	No of cases	%	No of cases	%
<2	11	36	19	59	15	47	45	47
2-<5	11	36	9	28	10	31	30	32
5-<9	7	23	3	9	6	19	16	17
9+	2	6	1	3	1	3	4	4
Total	31	100	32	100	32	100	95	100

Table (4) showed that 60% of meningococcal disease cases were low in family income, 36% were have a moderate family income and 4.0% were have high family income.

Table (4) Distribution of Cases By Family Income

Income level	Frequency	Percentage
High	4	4.0
Moderate	34	36
Low	57	60

Table (5) showed the distribution of cases by the methods of diagnosis 51% of the meningitis cases were diagnosed by CSF culture, 40% of the meningococcemia cases were diagnosed by the blood culture, 46% of the total cases were diagnosed by Gram stain, 28% of the cases showed hypocalcaemia, 5% of the meningitis cases showed protein C deficiency.

Table (5) Distribution of Cases By The Technique of Diagnosis

	Meningitis		Meningococcemia		Both		Total	
	No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
CSF culture	27	87	0	0	13	41	40	51
Blood culture	0	0	15	47	19	59	34	40
Gram stain	24	77	0	0	9	29	33	46
Hypocalcaemia	3	10	19	59	5	16	27	28
Protein identification	2	5	17	46	18	49	37	100

Table (6) showed the serological types of N. meningitides. 77% of the strains were belong to B serogroup while 23% of the strains were W135 serogroup.

Table (6) Distribution of Cases By *N. meningitidis* Serogroups

Serogroup	Frequency of cases	Percentage
Group B	44	77
W135	13	23

Table (7) showed the antibacterial susceptibility and resistant strains of N. meningitidis to a panel of antibiotics used in the treatment of meningococcal cases. 88% of N. meningitidis were susceptible to penicillin G, 99% susceptible to ampicillin, and 52% were susceptible to Sulfanamide N. meningitidis strains showed 100% susceptible to chloramphenicol, ceftriaxon, cefotaxime rifampicin, and ciprofloxacin.

Table (7) Frequency of N. meningitidis Susceptibility For Antibiotics

	Percentages of susceptible strains	Percentages of resistant strains
Penicillin G	88	12
Ampicillin	99	1
Sulfonamide	52	48
Chloramphencol	100	0
Ceftriaxone	100	0
Cefotaxime	100	0
Rifampicin	100	0
Ciprofloxacin	100	0

Discussion:

In this study the aim of the survey was to determine the incidence of the Neisseria meningitidis with its different forms. The study population consists of 95 children diagnosed as meningococcal disease by means of confirmed laboratory results. The results of the study revealed that 58% of the cases were females and 42% of these were males. The distribution of cases by residency were varied. 42% of the cases were living in Gaza town, 29% in North zone, 13% both in Mid zone and Khanyounis area and 3% in Rafah, these results confirmed that the crowdness especially in the north and Gaza town was high the crowdness index in Refugee camps >3 this factor may play an important role in the transmission of infection, 75% of the cases were living in towns while 21% in refugee camps and 4% in villages. Approximately more than one quarter of cases had a history of upper

respiratory tract infections perceeding the meningococcal infection which confirmed that the transmission of infection from carriers for pathogens or cross infections from cases. On the other hand about 75% of these were exposed to passive smoking through household contact. The study results revealed that 15% of these cases were underweight and 83% of them were normal weight therefore the risk factor of low weight was neglected. It was concluded that no single factors involved deeply in the development of infection, so the characteristics of the pathogens like invassivenes and virulence was high. Meningococci pass through the mucosal epithelium via phagocytic vacuoles as a result of endocytosis **(Virj, et al 1996)**. During invasion, several bacterial factors modulate metabolism of the mucosal cell **(Rudel, et al 1996)**, binding of pili and class 5 OMPs to their receptors transduces a signal to the host cell Por B, a class 2/3 OMP may translocate into the target cell membranes and affect the maturation of phagosomes **(Virj, et al 1992)**. Meningococci can survive and proliferate in the blood stream by virtue of particular bacterial virulence factors or incompleteness of the host defense. Members of the Neisseriaceae have developed a mechanism for acquiring iron from human transferin by using transferin binding proteins **(Pettersson, et al 1997)**. The menincococcemia cases were present in significant number among the recorded and confirmed cases, once a viable meningococi have reached the blood stream different disease manifestations can develop. In some patients probable those with low degrees of bacteremia meningococi are cleared spontaneously leaving behined so called transient meningococcemia characterized by short febrile flue like episode **(Gedde-Dahl, et al 1990, and Sullivan and Lascoleat, 1987)**, when the bacteremia is not cleared, clinically overall disease develops. In these cases, the ultimate clinical presentation is determined by bacterial properities such as Endotoxin released and by host characterizations such as hostimmune status. Endotoxin release is a strain specific virulence factor. During growth and lysis of meningococci endotoxin is released in the form of vesicular outer membrane structures consistency of up to 50% of LOS and outer membrane protein, lipids and capsular polysaccharide **(Devoe and Gilchrist, 1973)**. The results showed 5 out of 95 cases died from meningococcal diseases with case fatality rate 5.0%, it is important to mention that 4 out of 5 deaths were females, and one case were male, all the five deaths were less than 5 years old, two of them were less than 2 years old. The incidence of meningococcal disease begins to increase in December and reach its peak in February then return to reach its normal range. It was noticed that there is no recorded cases in June this could be attributed to that in summer season dry hot climate the incidence of

respiratory disease declined. Prompt bacteriological diagnosis in patient with fulminant meningitis syndrome (FMS) was carried out with a gram stain for the skin lesion biopsy specimen or CSF **(Periappuram, et al 1995)**. 46% of the specimen examined showed positive gram stain. Early diagnosis of meningitis and recognition of a patient at risk are crucial for the timely start of life saving antibiotics and antishock therapy. The antimicrobial biogram in this study showed that there were strains of N. meningitidis resistant to penicillin G (12.0%) and ampicillin (2%). Although the penicillin is the drug of choice to treat meningococcemia and meningococcal meningitis **(Virj, et al 1996)**, this result was in agreement with results recorded in several countries (Spain, Greece, Switzerland, United Kingdom, and USA) **(Jackson, et al 1994)** which showed that strains of meningococcemia had been resistant to penicllin G and required a treatment with other antibiotics such as the third generation of chphalosporins. Chloramphincol and cephalosporins results showed that all strains of N. meningitidis were susceptible. For patients infected with penicillin resistant strains, broad spectrum cephalosporins (e.g.; ceftriaxone) are recommended **(Galimand, et al 1998)**, also chloramphenicol is useful as alternative therapy in the case of meningcococcal disease **(Virj, et al 1996)**. Rifampicin is the drug of choice for chemoprophylaxis where there is no resistance strains for rifamoicin was recorded. According to CSF and blood culture this is a congruent with other studies which indicated for the same conclusion **(Schwentker, et al 1937, and VanEsso, et al, 1987)**. The results of this study showed that culture yielded small number of isolates this may be due to pretreatment patients since many physician relay on clinical diagnosis.

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