Diagnosis of Acute Meningococcal Meningitis by Using Of Pcr Versus Conventional Methods In El-Menoufiya Governorate

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Abstract: Back ground: Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore the condition is classified as a medical emergency**.** The conventional methods of diagnosis of bacterial meningitis using bacterial cultures are slow. Also, Antibiotic administration is much more likely to render cultures negative than it is to alter the CSF formula**.** Rapid aetiological diagnosis of bacterial meningitis is crucial for the early targeting of antimicrobial and adjuvant therapy**.** Newer techniques such as PCR detection of bacterial DNA although not widely available, offer the prospect of rapid and sensitive diagnosis**. Aim of the work:** The aim of this study is to evaluate the diagnosis of acute meningococcal meningitis by using the PCR versus the conventional methods in El-Menoufiya governorate. **Subject and Methods**: this study was conducted on 300 patients with symptoms and signs of clinically acute meningitis and 100 persons as control group. This study was performed in Menouf Fever Hospital, El-Menoufiya governorate, Egypt, during the period from March 2010 to February 2012. The methodology of this study was carried out though collaboration with clinical pathology department in faculty of medicine in El-Menoufiya university. The patients were divided according to CSF findings into: **Group (1):** which included 140 patients (88 males 62.8 % and 52 females 37.3 %) with mean age of 8.6 ±9.31 years with acute bacterial meningitis with positive CSF culture ± positive CSF Gram stained smear or positive CSF Latex. **Group (2):** which included 60 patients (40 males 66.7% and 20 females 33.3%) with mean age of 8.8 ± 9.99 years with clinically suspect of acute meningitis with i.e.: Turbid CSF, CSF WBC more than 5/cmm with polymorphnuclear leucocytes predominance, CSF protein more than 45mg/dL &/or CSF glucose < 45mg/dL and negative CSF culture, CSF Gram stained smear and CSF Latex. **Group (3):** comprised 100 patients (65 males 65% and 35 females 35%) with mean age of 8.5 ±9.63 with clinically suspect acute meningitis with i.e.: Clear CSF, CSF pleocytosis: predominant mononuclear cells with negative CSF culture, CSF Gram stained smear and CSF Latex. **Group (4):** which included 100 persons (61 males 61% and 39 females 39%) with mean age of 8.9 ± 9.67 These persons were free from clinical evidences of any CNS diseases including symptoms and signs of meningeal irritation. **Results and conclusions:** In acute bacterial meningitis and suspected acute bacterial meningitis(200 patients); The causative organisms were detected in 140 (70%) patients group (1) by Gram stained smear, culture and latex agglutination test and could not detected in 60 (30 %) patients group (2), the real time PCR test as rapid technique was used to detect meningococcal DNA in 60 CSF samples group (2), which were clinically diagnosed as meningococcal meningitis but not confirmed by either microscopy, culture or Latex, the results showed that 52 were positive and 8 were negative after PCR. So we can conclude that, The PCR is the most effective method for detecting bacteria in clinical practice, especially when Gram stained smear and culture of CSF revealed no organism.

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1. Introduction:

Acute infections of the nervous system are among the most important problems in medicine because early recognition, efficient decision-making and rapid institution of therapy can be lifesaving. Key goals of early management are to emergently distinguish between these conditions, identify the responsible pathogen and initiate appropriate antimicrobial therapy **(Fauci *et al.*, 2012).**

CNS infections are classified by the site of the infection into; Infection of the cerebral cortex (encephalitis), Infection of the meninges (meningitis) and Abscesses usually form in 3 locations within the CNS: The cerebral cortex (Brain abscesses), Between the dura and arachnoid (Subdural abscesses) or Immediately outside dura (Epidural abscesses) **(Southwick., 2007).**

Meningitis can be life-threatening because of the inflammation's proximity to the brain and spinal cord; therefore the condition is classified as a medical emergency **(Tunkel *et al.*, 2004).**

The conventional methods of diagnosis of bacterial meningitis using bacterial cultures are slow. Also, Antibiotic administration is much more likely to render cultures negative than it is to alter the CSF formula **(Kanegaye *et al.*, 2001)**. Rapid aetiological diagnosis of bacterial meningitis is crucial for the early targeting of antimicrobial and adjuvant therapy (Deutch et al., 2006). Newer techniques such as PCR detection of bacterial DNA although not widely available, offer the prospect of rapid and sensitive diagnosis **(Schuurman *et al.*, 2004).**

Aim of the work

The aim of this study is to evaluate the diagnosis of acute meningococcal meningitis by using the PCR versus the conventional methods in El-Menoufiya governorate.

2. Subject and Methods

This study was conducted on 300 patients with symptoms and signs of clinically acute meningitis and 100 persons as control group. This study was performed in Menouf Fever Hospital, El-Menoufiya governorate, Egypt, during the period from March 2010 to February 2012. The methodology of this study was carried out though collaboration between Tropical Medicine department,Faculty of Medicine. AL- Azhar university and Clinical Pathology department, Faculty of Medicine,El-Menoufiya university.

The patients were divided according to CSF findings into:

Group (1): which included 140 patients (88 males 62.8 % and 52 females 37.3 %) with mean age of 8.6 ± 9.31 years with acute bacterial meningitis with positive CSF culture ± positive CSF Gram stained smear or positive CSF Latex.

Group (2): which included 60 patients (40 males 66.7% and 20 females 33.3%)
with mean age of 8.8 ± 9.99 years with clinically suspect acute meningitis i.e, Turbid CSF, CSF WBC more than 5/cmm with polymorphnuclear leucocytes predominance, CSF protein more than 45mg/dL &/or CSF glucose < 45mg/dL and negative CSF culture, CSF Gram stained smear and CSF Latex.

Group (3): comprised 100 patients (65 males 65% and 35 females 35%) with mean age of 8.5 ± 9.63 with clinically suspect acute meningitis i.e, Clear CSF, CSF pleocytosis, predominant mononuclear cells with negative CSF culture, CSF Gram stained smear and CSF Latex.

Group (4): which included 100 persons (61 males 61% and 39 females 39%) with mean age of 8.9 ± 9.67 These persons were free from clinical evidences of any CNS diseases including symptoms and signs of meningeal irritation as control group.

1) All patients and control were subjected to the following:
2) Complete medical history,
3) Full clinical examination,
4) Lab investigations including:
5) Complete blood picture (System Counter K-1000, Japan, Catalogue No; A5873),
6) ESR (by Westergren method),
7) C-reactive protein levels (by nephelometric method),
8) Random blood glucose,
9) Liver function tests and Renal function tests (Integra 400 Auto Analyzer. Germany. Catalogue number; M450045),
10) Blood culture [Diphasic bottles (bio Merieux)]
11) Meningococcal PCR for the CSF samples in suspected bacterial meningitis
patients with negative results of Gram stain, latex or culture.

Inclusion criteria; patients with one of the following were included in the present study ؛

1. Clinically suspect acute meningitis i.e.: Sudden onset of fever (temperature more than 37.5° orally, more than 38.5° rectally or more than 38.0° axillary) and one or more of the following: Stiff neck, Altered consciousness, Other meningeal sign (e.g. kernig's ± Brudzinsk's signs), Petechial/purpural rash (Ministry of Health and Population., 2000).
2. Classic triad of acute meningitis symptoms; boring headache, fever and projectile vomiting
3. Classic triad of acute meningitis signs; positive nuchal rigidity, Kernig's sign and Brudzinski's sign
4. The combination of fever, coma and purpuric rash
5. CSF criteria of acute meningitis; Turbid CSF or CSF WBC >5/cmm, CSF protein > 45mg/dL &/or CSF glucose < 45mg/dL, positive CSF culture, CSF Gram stained smear or CSF Latex.

Exclusion criteria: patients with the following were excluded from the present study؛ Illness of over 10 days duration (generally presumed to be tuberculous meningitis), any patient received antibiotics prior hospital admission, Traumatic CSF punctures, CNS tumor, Hydrocephalus, Cerebrovascular stroke e.g.; cerebral hemorrhage/infarction, subarachnoid or subdural hemorrhage. Encephalopathy e.g.; cholemia, pre-cholemia, renal, respiratory failure, hypertensive, toxic or ischemic encephalopathy.

3. Results:

Tab. (1) shows classification of the studied groups according to CSF analysis results.

Classification / Frequency	Group I	Group II	Group III	Controlgroup	Total
Number	140	60	100	100	400
Percentage	35%	15%	25%	25%	100%

Tab. (2) shows results of age –in years- distribution in the different studied groups.

Groups / Age		Group I n=140	Group II n=60	Subtotal n=200		Group III n=100	Subtotal n=300	GroupIV n=100	Total n=400
Category I; < 1 year	NO	46	8	54	152 = 76%	32	86	33	119
	%	32.9	13.3	27		32	28.66	33	29.75
Category 2; 1 to < 5 years	NO	41	4	45		19	64	18	82
	%	29.3	6.6	22.5		19	21.33	18	20.5
Category 3; 5 to < 19 years	NO	25	28	53		28	81	27	108
	%	17.9	46.6	26.5		28	27	27	27
Category 4; 19 to < 60 years	NO	16	9	25	48 = 24%	12	37	14	51
	%	11.4	15	12.5		12	21.33	14	12.75
Category 5; ≥ 61 years	NO	12	11	23		9	32	8	40
	%	8.5	18	11.5		9	10.66	8	10
Mean (years)		8.6	8.8	8.7		8.5	8.6	8.9	8.7
SD		9.31	9.99	9.65		9.63	9.64	9.67	9.65
Range		2 months to 72 years							
TOS		F value = 3.423, *P* value < 0.05, Significant							

Tab. (3) shows results of gender distribution frequency in the studied groups.

Gender / Groups		Patients n=300				Subtotal	Group IV n=100	Total n=400
		Group I n=140	Group II n=60	Subtotal	Group III n=100			
♂	NO	88	40	128	65	193	61	254
	%	62.8	66.7	64	65	64.3	61	63.5
♀	NO	52	20	72	35	107	39	146
	%	37.2	33.3	36	35	35.7	39	36.5
Ratio		1.69:1	2:1	1.77:1	1.9:1	1.88:1	1.6:1	1.73:1
TOS		F value = 5.142, *P* value < 0.05, Significant						

TOS: Test of significance

Tab. (4) shows results of Clinical Picture in the meningitis studied groups (group I,. group II, and group III).

Groups / Manifestations	Group I n=140		Group II n=60		Subtotal n= 200		Group III n=100	Total =300	
	No	%	No	%	No	%	No=%	No	%
Fever	125	89.2	40	66.6	170	85	95	265	88.3
Meningeal irritation;									
a. Stiff neck	44	31.4	31	51.6	75	37.5	45	120	40
b. Kernig's sign	75	53.5	35	58.3	110	55	56	166	55.3
c. Brudzinski's sign	85	60.7	30	50	115	57.5	55	170	56.6
d. Contra-Brudzinski's sign	80	57.1	35	58.3	115	57.5	54	169	56.3
↑ Intracranial pressure;									
a. Vomiting	90	64.2	5	8.3	137	68.5	70	207	85.6
b. Headache	33	23.5	21	35	45	22.5	50	95	31.6
c. Blurring of vision	60	42.8	25	41.6	85	42.5	30	115	38.3
d. Bulging ant fontanelle	29	20.7	5	8.3	34	17	18	52	17.3
Mental state									
a. Alert	30	21.4	25	41.6	55	27.5	50	105	35
b. Drowsy	60	42.8	20	33.3	80	40	40	120	40
c. Comatosed	50	35.7	15	25	65	32.5	10	75	25
Rash	25	17.8	11	18.3	36	18	12	48	16
Irritability	40	28.5	17	28.3	57	28.5	40	97	32.3
Refusal of feeding	9	6.4	4	6.6	13	6.5	5	18	6
Convulsions	5	3.5	4	6.6	9	4.5	4	13	4.3
CNS Palsies	2	1.4	1	1.6	3	1.5	0	3	1
Death	0	0	1	1.6	1	0.5	0	1	0.33

Tab. (5) shows results of clinical picture in acute meningitis groups related to age.

Manifestations \ Groups	Category 1, 2 & 3 (n =152)		Category 4 & 5 (n =48)		Total =200	
	No	%	No	%	No	%
Fever	132	86.5	38	79.2	170	85
Meningeal irritation;						
a. Stiff neck	47	30.9	28	58.3	75	37.5
b. Kernig's sign	88	57.9	22	45.8	110	55
c. Brudzinski's sign	92	60.5	23	47.9	115	57.5
d. ContraBrudzinski's sign	92	60.5	23	47.9	115	57.5
↑ Intracranial pressure;						
a. Vomiting	110	72.3	27	56.3	137	68.5
b. Headache	29	19.1	16	33.3	45	22.5
c. Blurring of vision	50	31.8	30	62.5	85	42.5
d. Bulging ant fontanelle	34	22.3	0	0	34	17
Mental state (LOC)						
a. Alert	43	28.2	12	25	55	27.5
b. Drowsy	60	39.4	23	47.9	80	40
c. Comatosed	49	32.2	16	33.3	65	32.5
Rash	20	13.2	16	33.3	36	18
Irritability	40	26.3	17	35.5	57	28.5
Refusal of feeding	13	8.5	0	0	13	6.5
Convulsions	7	4.6	2	4.1	9	4.5
CNS Palsies	3	1.9	0	0	3	1.5
Death	1	0.6	0	0	1	0.5

Tab. (6) shows results of the CSF physical examination in the studied groups.

Studied Groups / CSF Physical Ex.			GroupI= 140	GroupII =60	Subtotal	GroupIII =100	Group IV n=100
Aspect	Clear	NO	5	1	6	100	100
		%	3.5	1.6	3	100	100
	Cloudy	NO	60	15	75	4	0
		%	42.8	25	37.5	4	0
	Turbid	NO	75	44	119	0	0
		%	53.5	73.3	59.5	0	0
TOS			X2 = 89.46	*P*-value < 0.001			
Tension	Normal	NO	9	4	13	1	100
		%	6.4	6.7	6.5	1	100
	Increased	NO	48	31	79	30	0
		%	34.3	51.7	39.5	30	0
	Highly increased	NO	83	25	108	69	0
		%	59.3	41.6	54	69	0
TOS			X2 = 93.65, *P*-value < 0.001, Very Highly significant				

TOS: test of significance

Tab. (7) shows results of the CSF chemical examination in the studied groups.

CSF chemical Examination \ Groups			GroupI n=140	GroupII n=60	Subtotal	Group III n=100	Group IV n=100
CSF Glucose mg/dl	normal	NO	0	3	3	96	100
		%	0	5	1.5	96	100
	<45	NO	140	57	197	4	0
		%	100	95	98.5	4	0
	Range		10-45			45-90	
	Mean		18.9	19.5	19.2	68.9	63.2
	±SD		±7.2	±6.8	±7	±7.71	±9.86
TOS			X2 = 87.1, *P*-value < 0.001				
CSF Protein mg/dl	normal	NO	0	2	2	13	100
		%	0	3.33	1	13	100
	>45	NO	140	58	198	87	0
		%	100	96.67	99	87	0
	Range		45-250			10-45	
	Mean		242.1	239.7	240.9	34	33.43
	±SD		±71.9	±69.7	±70.8	±9.49	±8.72
TOS			X^2 = 94.62, *P*-value < 0.001, Very Highly significant				

Tab. (8) shows results of the CSF cytological examination in studied groups.

Groups / CSF cytological Examination			Group I n=140	Group II n=60	Subtotal	Group III n=100	Group IV n=100
CSF-TLC/cmm	<200	NO	9	15	24	70	100
		%	6.5	25	12	70	100
	>200 to <500	NO	21	15	36	30	0
		%	15	25	18	30	0
	>500	NO	110	30	140	0	0
		%	78.5	50	70	0	0
	Mean		1276.95	1329.12	1303	359.3	2.82
	±SD		±51.07	±53.98	±52.52	±312.68	±2.1
Predominant cells			Polymorphnuclear			Mononuclear	
TOS			X2 = 85.57, *P*-value < 0.002, Very Highly significant				

Tab. (9) shows results of comparison between the positive cases in the different diagnostic methods.

CSF Latex / CSF Gram	CSF Latex/ CSF Culture	CSF Gram/ CSF Culture	Blood Culture / CSF Culture	Test	
22.9138	31.8330	22.9138	32.7659	X^2	TOS
< 0.001, Very Highly significant				*P*	

Tab. (10) shows results of detection of Meningococcal DNA by conventional PCR and its sensitivity in CSF of suspected acute bacterial meningitis patients(group II).

j. Sensitivity of PCR test	h. i. Total	g. Conventional PCR Meningococci		e. PCR f. Cases
		l. negative	k. positive	
q. 0.833	p. 60	o. 10	n. 50	m. NO
v. 83.3	u. 100	t. 16.7	s. 83.3	r. %

Tab. (11) shows results of detection of Meningococcal DNA by Real time PCR and its sensitivity in CSF of suspected acute bacterial meningitis patients (group II).

Sensitivity of PCR test	Total	Real time PCR Meningococci		PCR Cases
		negative	positive	
0.867	60	8	52	NO
86.7	100	13.3	86.7	%

Tab. (12) shows the validity of Real time PCR versus conventional PCR Meningococci in diagnosis of acute bacterial meningitis or suspected acute bacterial meningitis.

CSF Real time PCR Meningococci				PCR methods Cases	
Negative; n =8		Positive; n =52			
0		50		Positive n=50	Conventional PCR Meningococci
8		2		Negative n=10	
100	Specificity	96.2	Sensitivity	Validity (%)	
80	NPV	100	PPV		

Tab. (13) shows final detected organisms from 200 cases of acute bacterial meningitis by blood culture, CSF Gram stain, CSF culture, CSF Latex and CSF PCR.

%	NO.	Type	Method / Detected organism
4.5	9	Meningococci	Conventional methods
37	74	Pneumococci	
23	46	H. inflnenzae	
5.5	11	Other bacteria	
26	52	Meningococci	Real time PCR
4	8	No pathogen	
100	200	Total	

4.Discussion

Acute bacterial meningitis is a clinical diagnosis that is established by the affected patient's history, physical examination findings and lab results. The specific infective agents that are involved vary among different patient age groups. Starting treatment early in the course of the disease is crucial. Appropriate antibiotic treatment for the most common types of bacterial meningitis should reduce the risk of death to less than 15%, although the risk is higher among elderly patients (Lutfi Incesu and Anil Khosla., 2009).

Meningococcus is considered to be the leading cause of bacterial meningitis in many regions of the world, causing an estimated 1.2 million cases of bacterial meningitis and sepsis worldwide each year (Ceyhan *et al.*, 2008).

Therefore, The aim of this study was to evaluate the diagnosis of acute meningococcal meningitis by using the PCR versus the conventional methods in El-Menoufiya governorate. For this purpose, 300 patients who were attendants to Menouf Fever Hospital in the period from March, 2010 to February, 2012 with acute clinical meningitis (193 males and 107 females) and their ages ranging between 2 months and 72 years included in this study and subdivided into group I (acute bacterial meningitis), group II (suspect acute bacterial meningitis) and group III (non-bacterial meningitis). In addition to 100 persons whom are free from clinical evidences of any CNS diseases including symptoms and signs of meningeal irritation, of matched age and sex undergoing spinal anaethesia for various non-CNS surgical problems as control group (group IV) (61 males and 39 females).

In the present study, the overall males: females ratio was ≈ 1.73:1 (254/146). This is in agreement with many other studies conducted in Egypt documenting that the male: female ratio ranged from as low as 1.1: 1 to as high as 2.8:1. El-Ramly *et al.* (1996) found in their study that the male: female ratio was 2.8: 1 and their explanation to this finding was related to the Egyptian tradition giving more attention towards the sick male than female.

In our research, the patients' mean age was 8.7 ± 9.65 years with range of 2 months to 72 years, and infants aged less than 1 year represented (29.75%) of cases, in children of the age group 1 to less than 5 years represented (20.5%), while children in the age group 5 to < 19 years represented (27%), in adult from 19 to < 60 years (12.75%) and in older age ≥ 61 years (12.75%). while in the work of Santos., (2005) it was higher as 30.2 ± 15.3 years with range 14 to 90 years.

As regard the results of clinical picture as shown in tables (4), the most frequent complaints in our work (of group I, group II and group III) were fever (89.2, 66.6 and 95% respectively), neck rigidity (31.4%, 51.6% and 45% respectively), positive kerning's (53.5%, 58.3% and 56% respectively), positive Brudzinski's signs (60.7%, 50% and 55% respectively); vomiting (64.2, 8.2 and 70% respectively), and headache (23.5, 35 and 50% respectively) usually severe, bursting in character, unremitting, diffuse or localized and often radiates down the neck into the back).

This was in agreement with Himmelreich *et al.* (2009) who found in most of the patients that on admission, typical symptoms of meningitis were: headache (92.5%), fever (88.2%), nuchal rigidity (80.1%) and (15.6%) died during hospitalization period.

The current work showed that the aspect of the CSF samples was abnormal in (97%): turbid in (59.5%) and cloudy in (37.5) of all acute bacterial meningitis cases. While Dubos *et al.* (2008) reported (89.3%) with turbid or cloudy aspect of CSF by physical examination (naked eye) of all acute bacterial meningitis cases.

While the aspect was cloudy only in 4% in cases of group III as regard CSF finding. These findings agree with Walsh., (1990) who attributed the turbidity of the CSF in some cases of aseptic group to polymorphnuclear cells which may present in the early stages of the aseptic group. On the other hand, Overturf and Hoperich., (1983) stated that CSF is clear in aseptic meningitis.

In the current work the mean CSF WBCs count for all types of bacterial meningitis as shown in table (7) was 1303.0 ± 52.52 (with predominant cells are polymorphnuclear cells) and there was statistically significant difference among the mean WBCs counts of different types being highest in meningococcal meningitis (1892.1 ± 55.31) then pneumococci (1111.2 ± 51.99) and H. influenzae (1099.2 ± 44.95)

Abro *et al.* (2008) reported similar findings to the current study regarding the order of the mean values of CSF leukocytic counts in different etiologic groups as they noted that the highest mean CSF leukocytic count was recorded in meningococcal meningitis cases being 12.500, followed by H. influenzae cases where the mean WBCs count was 11.270 & then pneumococci cases with a mean WBCs count of 10.100.

The current study revealed that the mean value of glucose in CSF of acute bacterial meningitis cases was (19.2 ± 7 mg/dl). Our findings agree with Roos and Tyler., (2005) who reported that CSF glucose is markedly reduced in cases of acute septic meningitis and may reach zero.

In our study, the mean CSF glucose content of meningococcal meningitis cases was (22.3 ±

5.9mg/dl), while it was (15.8 ± 7.3 mg/dl) in H. influenzae & (19.7 ± 8.2 mg/dl) in pneumococcal meningitis cases. The same result was also noted by Abro *et al.* (2008), and Sunit and Arun., (2006) as they found the mean CSF sugar content was (23.5 mg/dl) in meningococcal meningitis cases, (18.2 mg/dl) in pneumococcal meningitis cases and (16.4 mg/dl) in H. influenzae.

In our study, there is statistical significant difference between increased mean CSF protein in acute bacterial meningitis (100%), suspected bacterial meningitis (96.67%), non-bacterial meningitis (87%) and control group (0%). These findings, agree with Kim *et al.* (2003) and disagree with Girgis *et al.* (1993).

In our study, the mean CSF protein content of meningococcal meningitis cases was (293.9 ± 59.6 mg/dl), while it was (237.1 ± 79.1 mg/dl) in H. influenzae cases, and (212.4 ± 76.9 mg/dl) in pneumococcal cases. Dubos *et al.* (2008) reported that the protein level was markedly elevated (220 mg/dl) and that of meningococcal meningitis cases was (74 mg/dl), (70 mg/dl) in H. influenzae cases and (112 mg/dl) in pneumococcal cases.

In our study, the sensitivity of the Gram stain, bacterial culture and Latex in the diagnosis of acute bacterial meningitis were evaluated in 200 studied cases with manifestations of acute bacterial meningitis.

From the present study, as shown in table (9) it can be concluded that Gram stain smear with a 64% diagnostic sensitivity is a test which should always be done when bacterial meningitis is suspected. Greenlee., (1992) reported that Gram stain has a sensitivity of 60 to 90% in experienced hands, depending on the number and type of organisms present. Also, he reported that cultures have a sensitivity of 80 to 90% but drop to 30% in partially treated patients.

In the present work the highest positive Gram stain smears with cases H. influenza was (97.8%) followed by pneumococcal (91.8%) then meningococci (77.8%). This is similar to the work of Escosteguy., (2004) who reported with H. influenzae cases (90.9%), followed by pneumococcal cases (86.7%) & then meningococcal meningitis cases (80.7%). While Silva *et al.* (2008) found that the highest positive yield of Gram stain smears were recorded in meningococcal meningitis cases (93.3%), followed by of pneumococcal meningitis cases (75%) and finally in H. influenzae cases (60%).

In the present study, bacterial culture of CSF samples were positive in 97 (48.5%) of all cases with acute bacterial meningitis. This was in agreement with the work of Abdel Wahab et al., (1992), Adel *et al.* (1996), Kanra *et al.* (1996) and Pusponegoro *et al.* (1998) who reported that the diagnostic sensitivity of the bacterial culture in the diagnosis was 42%, 43.3%, 50.8% and 54.5% respectively. In contrary, Honnas and Petersen., (1998) reported that CSF cultures were positive in 81.3% of all cases. Whereas, in China, Yang *et al.* (1996) found that CSF cultures were positive in 13.3% of the cases.

In the present study, The sensitivity of the Latex for meningococcus was about (88.9%), for pneumococcus (85%), for H. influenzae (97.7%). while Gravis *et al.* (1989) reported that sensitivity of the Latex for meningococcus was about 50%, for pneumococcus ≈ 60%, for H. influenzae ≈ 90% and for group B streptococcus is ≈ 90%.

In the present study as shown in table (9), Highly significant decrease was noted in culture positive cases compared with positive Gram stained smear ($p < 0.001$) this was in agreement with work of Abdel Wahab *et al.* (1992). Also, there was highly significant decrease noted in Gram stained smear positive results compared with positive Latex specimens ($p < 0.001$), this was in agreement with work of Kaplan *et al.* (1990). There was also, a highly significant decrease was in culture positive cases compared with positive Latex specimens ($p < 0.001$), this was in agreement with work of Yang *et al.* (1996) where Latex was more sensitive than bacterial culture ($Chi^2 = 67.6$, $p < 0.005$).

Novel molecular methods offer the opportunity to establish a quick and reliable diagnosis. In the recent past, PCR assays have been developed for the specific detection of bacteria causing meningitis such as meningococci, pneumococci and S. agalactiae (Poppert *et al.*, 2005).

PCR-based assays have been seen as having the potential to provide an early and accurate diagnosis of diseases caused by bacterial pathogens and have improved the rate of microbial detection. Real time PCR is a promising tool for the detection of bacterial DNA from biological fluids (Wu et al., 2008). The nested approach has the highest sensitivity (97%) (Dragon *et al.*, 1993).

Out of 200 cases examined, 140 were confirmed by Gram, culture ± Latex and 60 remained as suspicious but unconfirmed. To confirm the diagnosis of these suspicious cases, in the present study, it has been evaluated the capability of the PCR to detect meningococcal DNA in 60 CSF samples from patients clinically suspected of having meningococcal meningitis and negative results of Gm stain, culture and Latex

In the present study; we use real time PCR for more sensitivity and detection rate. Out of 60 CSF samples; 50 (83.3%) were positive and 10 (16.7%) were negative by conventional PCR meningococci, while 52 (86.7%) were positive and 8 (13.3%) were

negative by real time PCR meningococci. Therefore, the PCR test reduced the number of suspicious cases in this study from 60 to 8, thereby improving the potential for assessment of treatment efficacy.

The failure to detect some bacteria by PCR might be explained by inadequate extraction of DNA which probably reflected low numbers of bacteria in the CSF, explaining the decreased sensitivity of the PCR. PCR can be used for diagnostic purposes when patients have been treated with antibiotics, since both dead and viable microorganisms can be detected (Welinder-Olsson *et al.*, 2007).

PCR of bacterial DNA may augment but not replace culture as a detection method (Saravolatz *et al.*, 2003). Compared with culture, the sensitivity of broad range real time PCR was 86%, and the specificity 98%. Conventional PCR resulted in a sensitivity of 64% and specificity of 98%. PCR was may be a useful supplement (Deutch *et al.*, 2006).

**We can conclude that the use of molecular biology is essential to increase the rate of microbiological diagnosis of meningococcal meningitis. The PCR technique are highly sensitive and specific molecular assay. This is the most effective method of detecting bacteria in clinical practice, especially when Gram stained smear and culture of CSF revealed no organism.

PCR can be used for diagnostic purpose when patients have been treated with antibiotics, since both died and viable microorganisms can be detected, Real time PCR is more rapid and sensitive than conventional PCR and microscopy particularly when antimicrobial therapy has been administered.

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