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RESEARCH ARTICLE

COMPARISON BETWEEN TURBIDIMETRIC AND PYROGALLOL RED METHODS FOR THE ESTIMATION OF PROTEIN LEVELS IN CEREBROSPINAL FLUID AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET KENYA

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ABSTRACT

There are a number of methods currently used for measuring proteins in cerebrospinal fluid which incorporate turbidimetry, colorimetry and electrophoresis. For a long time turbidimetry using 3% Trichloroacetic acid (3%TCA) was in routine use, however colorimetric methods like pyrogallol red are emerging and being embraced. This study was aimed at comparing cerebrospinal fluid protein measurement using 3% TCA turbidimetry andpyrogallol red colorimetry to determine whether the methods have co-relation in the values obtained. The study was carried out at the Moi Teaching and Referral Hospital where, CSF of 120 patients were randomly collected and protein level measurement done in parallel using the two methods (3% TCA turbidimetry andpyrogallol red). correlation coefficient (r) between pyrogallol red and 3% TCA was 0.876375with a p value of 0.00 at 5% α level and a confidence interval of 0.83 - 0.91 which thus showed there is variation in CSF protein values obtained using pyrogallol red and 3% TCA. Accuracy and precision were determined based on the control materials run from which it was observed that for both normal and high controls, pyrogallol red showed better accuracy and precision as compared to 3%TCA which thus makes use of pyrogallol red the preferred method for more reliable results.

INTRODUCTION

Cerebrospinal fluid (CSF), is a clear, colorless, bodily fluid, that occupies the subarachnoid space and the ventricular system around and inside the brain and spinal cord. In essence, the brain "floats" in it (Pezzlo, 1998). The CSF occupies the space between the arachnoid matter (the middle layer of the brain cover, meninges), and the pia matter (the layer of the meninges closest to the brain). It constitutes the content of all intra-cerebral ventricles, cisterns, and sulci, as well as the central canal of the spinal cord (Gripshover *et al.,* 2000). It acts as a "cushion" or buffer for the cortex, providing a basic mechanical and immunological protection to the brain inside the skull and serves a vital function in cerebral auto regulation of cerebral blood flow. The CSF is produced at a rate of 500 ml/day. Since the brain can contain only 135 to 150 ml, large amounts are drained primarily into the blood through arachnoid granulations in the superior sagittal sinus. Thus the CSF turns over about 3.7 times a day. This continuous flow into the venous system dilutes the concentration of larger, lipid-insoluble molecules penetrating the brain and CSF (Eeg-Olofson *et al.,* 1981).

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The CSF contains approximately 0.3% plasma proteins, or approximately 15 to 40 mg/dL, depending on sampling site. The blood CSF barrier is a physical barrier, consisting of different anatomical structures, for the diffusion and filtration of macromolecules from blood to CSF. The integrity of these barriers and CSF bulk flow determines the protein content of the CSF (Reiber, 1995). In newborns, CSF protein concentrations are high, but decrease gradually during the first year of life, and are maintained at low levels in childhood. In adults, CSF protein concentrations increasewith age (Eeg-Olofson *et al.,* 1981). The CSF to serum albumin concentration quotient (*Q*alb*)* can also be used to evaluate blood–CSF barrier integrity. Posture and physical activity may influence the CSF protein concentration, resulting in higher CSF protein concentrations in inactive, bed-ridden patients (Seyfert *et al.,* 2002). Elevated CSF protein concentrations can be found in the majority of patients with bacterial, cryptococcal and tuberculous meningitis (Negrini *et al.,* 2000). A concentration of >1.5 g/l is specific but insensitive for bacterial meningitis as compared to a variety of other inflammatory diseases (Lindquist *et al.,* 1988). In viral neuroinfections CSF protein concentrations are raised to a lesser degree (usually <0.95 g/l) (Negrini *et al.,* 2000). Non-infectious causes for an increased CSF protein and sometimes with an increased cell count include subarachnoidal hemorrhage, central nervous system (CNS) vasculitis, and CNS neoplasm (Jerrard *et al.,* 2001).

Elevated total protein concentration with normal CSF cell count (albuminocytologic dissociation) is a hallmark in acute and chronic inflammatory demyelinating polyneuropathies but protein levels may be normal during the first week (Seneviratne, 2000). CSF is obtained with relative ease by lumbar puncture (LP) prior to analysis, a procedure performed by a medical doctor through insertion of a needle between the fourth and fifth lumbar vertebrae and drawing out the fluid. A study carried out in India using ninety C.S.F. samples to determine a sensitive and economical method for estimating CSF proteins in which excellent co-relation was observed with modified BCG method and routine pyrogallol red method. The proposed modified bromocresol green (BCG) method is applicable to automated as well as manual measurements (Pushpa Durgawale *et al.,* 2005).

Shephard and Whiting in Turkey developed an automated turbidimetric method using benzalkonium Chloride and compared it with conventional automated methods. From their study, they came up with a new turbidimetric method that correlated better with the pyrogallol method than did the benzethonium chloride and Coomassie Brilliant Blue methods; was more precise than the Coomassie Brilliant Blue and benzethonium chloride methods; had better precision and recovery in the critical decision concentration range; had satisfactory albumin-globulin response and analytical range; used less expensive and accessible chemicals and was suitable for automation (Fatma Meric *et al.,* 2004). Most clinical laboratories are replacing their manual precipitation techniques for the determination of cerebrospinal fluid protein with automated assays such as pyrogallol red molybdate or benzethonium chloride turbidimetric assays.

However, some clinical laboratories lack access to automation due to cost implications and thus this study is aimed at determining whether CSF protein estimation using 3% Trichloroacetic Acid (TCA) manual method yields results correlating to the pyrogallol red method.

MATERIALS AND METHODS

Ethical approval for the study was sought from Moi Teaching and Referral Hospital and Moi University Institution Review and Ethical Committee (IREC). Formal Approval Number: FAN: IREC 00086. Non blood stained CSF of 120 patients received in the Biochemistry laboratory were utilized forthe study. The protein level of each C.S.F was measured using the two methods; 3% Trichloroacetic Acid (TCA) and pyrogallol red.Pyrogallol red was combined with proteins in an acidic solution to form a bluish-purple colored complex, which was read spectrophotometrically between 600- 650nm and in 3% TCA Proteins were precipitated by 3% TCA producing turbidity whose concentration is directly proportional to the amount of CSF proteins present in the sample. Pathological and normal controls were used to check accuracy and precision during the entire testing process. The controls used were:

- PNPUC (Preci-normal protein control for urine and cerebrospinal fluid)
- PPUC (Preci-pathological protein control for urine and cerebrospinal fluid)

Accuracy and precision of pyrogallol red and 3% TCA were determined based on the control materials run

RESULTS

Table 1. Concentrations of CSF samples using pyrogallol red and TCA

SAMPLE NO.	PYROGALLOL(mg/dl)	TCA(mg/dl)
1	29.1	80
2	13.6	50.1
3	22	72
4	36.3	191.7
5	10.7	31.7
6	24.9	55.9
7	13.9	15.4
8	22.4	46.2
9	104.4	137.2
10	5.7	1.9
11	11.7	31.1
12	27.2	58.3
13	35.1	136.3
14	16.3	52.7
15	134.1	142.9
16	0.5	11.4
17	46.7	82.8
18	15.8	39.2
19	5.5	11.5
20	7.7	10.7
21	22.2	60.3
22	7.2	24.3
23	7.6	43.1
24	24.1	73.4
25	118.5	408.8
26	38.4	22.4
27	17	9.1
28	14.2	15.7
29	121.8	204.1
30	10.1	54.2
31	23.8	37.7
32	137.6	188.1
33	111.5	235.8
34	127.7	241.1
35	5.7	5.7

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36	9.8	10.6
37	13.6	17.3
38	26.4	52.9
39	18.6	14.1
40	15	14.1
41	17.3	33.1
42	32.1	73.6
44	7.7	16.1
45	12.7	27.9
46	34.8	91.1
47	15.5	54.2
48	7.9	30
49	17.7	31.1
50	13.2	19.4
51	31.8	34.4
52	37.7	41.1
53	3.4	3.7
54	134.6	142.9
55	19.8	27.7
56	134.3	283.6
57	97.1	189.3
58	0.4	0
59	18.4	22.1
60	103.5	116.2
61	47.2	54.6
62	9.7	16.4
63	101.6	254.9
64	34.7	56.7
65	33.5	90.5
66	14.2	54.2
67	82.8	232.8
68	94.5	156.6
69	114	304
70	15.5	63.6
71	108.2	134.2
72	8.2	12.1
73	15.6	23.5
74	33.4	49.6
75	96.1	156.8
76	7.2	19.4
77	84.2	235.8
78	55.1	76.8
79	112.4	79.4
80	116	174.9
81	11.2	32.4
82	41.5	53.7
83	21.2	35.2
84	133.4	135.7
85	123.5	154.3
86	21.4	34.8
87	29.9	56.1
88	37.3	78.9
89	39.5	54.7
90	3.6	19.2
91	29.2	40.6
92	173.5	276.7
93	66.4	79.4
94	61.4	99
95	181.2	256.8
96	40.08	56.2
97	2.8	9.5
98	16.1	26.9
99	82.2	132.9
100	106.1	243.4
101	30.4	46.3
102	27.8	45.4
103	190.3	250
104	43.2	74.6
105	23.9	76.3
106	83	175.5
107	170.9	295.5
108	116.9	174.3
109	28.1	37.6
110	147.8	234.9
111	20.9	56.4
112	39.5	90.3
113	196.3	267.9
114	22.4	87.1
115	65.9	45.7
116	77	116.8
117	102	179.4
118	26.9	45.3
119	6.4	19.2
120	25.8	32.3

Table 2. Mean and SD values for pyrogallol PNPUC control

Pyrogallol PNPUC control						
+3SD	+2 SD	+1 SD	Mean	-1 SD	-2 SD	-3 SD
23	22	21	20	19	18	17

Table 3. Mean and SD values for pyrogallol PPUC control

PYROGALLOL PPUC CONTROL						
+3SD	+2 SD	+1 SD	Mean	-1 SD	-2 SD	-3 SD
159	158	157	156	155	154	153

From Table 2

Pyrogallol PNPUC A

Ctual value: 20 Calculated mean: 18.3
Accuracy: 20–18.3= 1.7 Therefore (1.7/20x100%=8.5) 100%-8.5 = 91.5%accuracy

Pyrogallol PPUC

Actual value: 156Calculated mean: 159.5
Accuracy: 156–159.5= -3.5 Therefore (3.5/156x100%=2.24) 100%-2.24 = 97.76%accuracy

TCA PNPUC Actual value: 20calculated mean: 22.8
Accuracy: 20–22.8= -2.8 Therefore (2.8/20 x 100%=14) 100%-14 = 86%accuracy

TCA PPUC Actual value: 156calculated mean: 183.2

Accuracy: 156-183.2= -27.2 Therefore (27.2/156x100%=17.44) 100%-17.44 = 82.56%accuracy

Pyrogallol normal control had an accuracy of 91.5% as compared to TCA normal control which had an accuracy of 86%. Pyrogallol high control had an accuracy of 97.7% while TCA high control had an accuracy of 82.56%. From the above analysis of control material run using pyrogallol red and 3%TCA, it was observed that for both normal and high controls, pyrogallol red showed better accuracy as compared to 3%TCA.

Precision

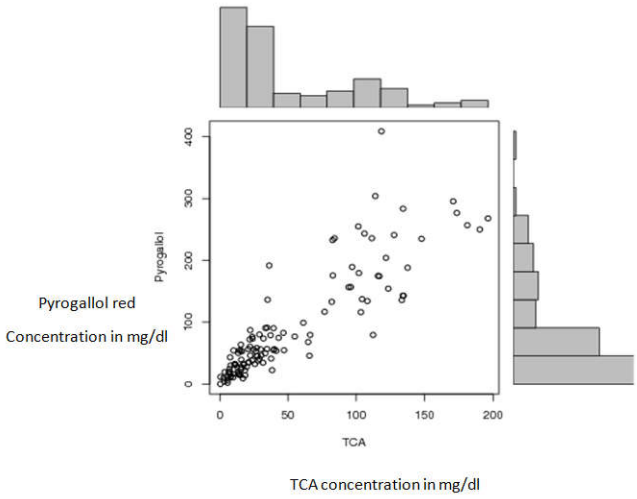
From table 2, the calculated SD values were as follows:

Pyrogallol normal control had an SD of 1.6 while TCA normal control had an SD of 2.1.
Pyrogallol high control had an SD of 8.4 while TCA high control had an SD of 9.3

From the above, it was thus observed that pyrogallol red has better precision than 3% TCA in the analysis of CSF protein levels.

Table 4. Summary of data analyzed

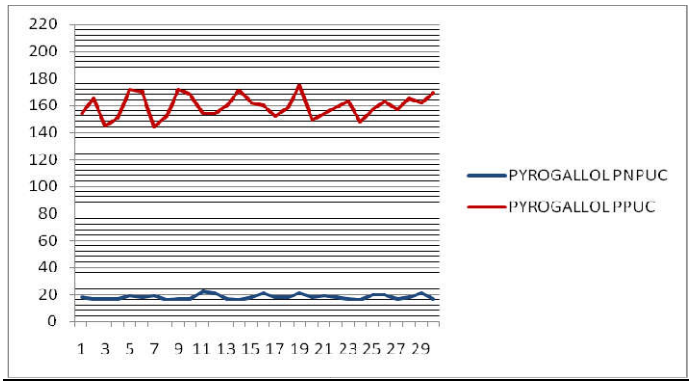
Variable Y	Pyrogallol
Variable X	TCA
Sample size	120
Correlation coefficient r	0.876375
Significance level	p = 0.00
95% Confidence interval for r	0.83 - 0.91



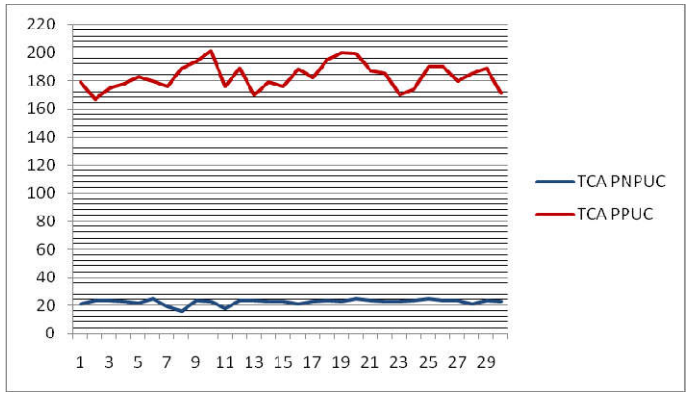
Graph 1. Linear graph showing values obtained using pyrogallol red and TCA

Table 5. Summary of mean and SD of control materials run

	Pyrogallol pnpuc	Pyrogallol ppuc	TCA pnpuc	TCA ppuc
Mean	18.3	159.5	22.8	183.2
SD	1.6	8.4	2.1	9.3



Graph 2. Levvey Jennings chart showing pyrogallol red controls results



Graph 3. Levvey Jennings chart showing TCA controls results

Table 6. Mean and SD values for TCA PNPUC control

TCA PNPUC CONTROL						
+3SD	+2 SD	+1 SD	Mean	-1 SD	-2 SD	-3 SD
23	22	21	20	19	18	17

Table 7.Mean and SD values for TCA PPUC control

TCA PPUC CONTROL						
+3SD	+2 SD	+1 SD	Mean	-1 SD	-2 SD	-3 SD
159	158	157	156	155	154	153

DISCUSSION

Analysis of collected data using Pearson correlation coefficient gave an r value of 0.876375 and a confidence interval of 0.83 - 0.91. The assigned P value at the onset of the study was P=0.05. From the data analyzed, the P value obtained from the samples analyzed concurrently using the two reagents was 0.00, thus P value is less than 0.05 meaning the difference in the values obtained using two reagents i.e. pyrogallol red and 3%TCA are significantly different. A p value of 0.00 thus gives credence to reject the null hypothesis and the alternative hypothesis accepted.

Thus there is variation in CSF protein values obtained using pyrogallol red and 3% TCA. From graph 1, the linear graph plotted using pyrogallol as the Y-variable and TCA as the X-variable demonstrates the lack of linearity as evidenced by the amount of scatter visualized in the graph. This therefore suggests that the variation in concentrations is significant between pyrogallol red and 3%TCA. In a study carried out in India, randomly selected 90 samples of C.S.F. were usedfor protein determination by the modified BCG andpyrogallol red method for comparison. Concentrationsof various C.S.F. samples were compared. Co-relationanalysis was performed and confirmed by 'paired ttest.

Data was analyzed statistically by SPSS 10.1Version software package.Out of 90 samples analyzed 36 were within normalrange by pyrogallol red method. Same samples weresimultaneously run for protein measurement withmodified B.C.G. method. All 36 samples valuesobtained by kit method (pyrogallol red) whencompared with modified B.C.G. values the mean forpyrogallol red was 28.61 mg % and for B.C.G. it was28.63 mg % with standard deviation 8.043forpyrogallol red and 7.665 for B.C.G (Pushpa Durgawale *et al.,* 2005), this thus shows that pyrogallol red method has good linearity.

Based on the above findings, the study concluded that;

- There is variation in CSF protein values estimated using 3% TCA and pyrogallol red
- CSF protein estimation using 3% TCA gives higher protein values as compared to pyrogallol red.
- Pyrogallol red has more accuracy in estimating CSF protein levels as compared to 3% TCA
- Pyrogallol red has a higher precision in estimating CSF protein levels as compared to 3% TCA

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