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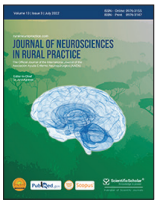

Original Article

Cost estimation for spinal muscular atrophy diagnosis using multiplex ligation-dependent probe amplification and droplet digital polymerase chain reaction

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ABSTRACT

Objectives: Diagnosing spinal muscular atrophy necessitates determining the copy number of the *SMN1* gene and the number of *SMN2* gene copies, which correlate with disease severity. The present study aims to conduct a detailed cost-effectiveness analysis to determine *SMN1* and *SMN2* exon seven-copy numbers using droplet digital polymerase chain reaction (ddPCR) and multiplex ligation-dependent probe amplification (MLPA).

Materials and Methods: Financial data were meticulously gathered from the institute's operational test facility from January 2022 to December 2022. The annual costs for capital assets, operational expenses, consumables, and other supportive facilities were calculated. Probabilistic sensitivity analysis (PSA) was also conducted to evaluate statistical uncertainty.

Results: The copy numbers of *SMN1* and *SMN2* gene exon seven were concordant between ddPCR and MLPA, with a significant correlation in test outcomes. The kappa coefficient was 1.000 and $P = 0.0001$. The annual capital and operational costs for ddPCR were INR 13,64,400 ($16,056) and INR 65,37,000 ($76,920), respectively. The MLPA's annual capital and operational costs were INR 17,64,400 ($20,760) and INR 89,82,000 ($105,670). The calculated cost per test for ddPCR was INR 1,646 ($20), while for MLPA, it was INR 5,970 ($70). Furthermore, based on 10,000 simulations, the PSA determined that the ddPCR-based diagnosis is up to 83.6% cost-effective. It supports its integration into clinical practice for better patient outcomes and optimized healthcare costs.

Conclusion: Utilizing ddPCR to determine the copy number of *SMN1* and *SMN2* gene exon seven offers cost-efficiency and time-saving advantages compared to alternative methods.

Keywords: Copy number, Cost estimation, Droplet digital polymerase chain reaction, Multiplex ligation-dependent probe amplification, Probabilistic sensitivity analysis, Spinal muscular atrophy

INTRODUCTION

Spinal muscular atrophy (SMA) is a genetic disease categorized by the progressive weakness and atrophy of voluntary muscles. It is mainly caused by mutations in the *SMN1* gene, which produces the SMN protein crucial for motor neuron survival.[1] While treatment options are historically limited, advancements in gene therapy and molecular-based treatments have revolutionized the management of SMA.

SMA is an autosomal recessive disorder caused by the mutation in the *SMN1* gene located on chromosome 5q13, resulting in reduced production of the SMN protein.[2] The severity of SMA is associated with the number of copies of the *SMN2* gene, which produces a limited amount of functional SMN protein. Mutations affecting splicing can also contribute to SMA pathology due to abnormalities in the *SMN2* gene.[1]

Diagnosing SMA requires determining the copy numbers of both the *SMN1* and *SMN2* genes, which can impact the disease severity.[3,4] Various technologies, including multiplex ligation-dependent probe amplification (MLPA), quantitative polymerase chain reaction (PCR), next-generation sequencing (NGS), array comparative genomic hybridization, and droplet digital PCR (ddPCR), are available for detecting copy number variations (CNVs) in these genes.[5,6] Each technology has its strengths and limitations regarding sensitivity, specificity, cost, and the type of information it provides.

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Early detection of SMA is crucial due to its rapid progression, and timely intervention can significantly enhance patient outcomes. Screening for SMA holds significant importance for several reasons, including facilitating early interventions, identifying carriers, enabling informed family planning and healthcare decisions, providing prognostic insights, and allocating healthcare resources to support individuals affected by SMA.[7]

Early screening for SMA is supported by a multifaceted scientific rationale, which considers disease pathogenesis and emerging therapeutic strategies. Molecular investigations into SMA pathophysiology have identified potential targets for precision medicine, emphasizing the importance of early detection.[3] In addition, the clinical adoption of early screening initiatives offers the potential to reduce the burden of SMA by preventing disease progression and improving patient outcomes.[7,8]

In this study, we aim to highlight the effectiveness of a low-cost test for identifying CNVs in the *SMN1* and *SMN2* genes as a diagnostic tool for SMA.

MATERIALS AND METHODS

Patient selection, blood sample collection, and molecular testing

Detailed information regarding the study participants and the workflow followed is consistent with the procedures described in a previously published study.[9] Deoxyribonucleic acid (DNA) was isolated from the blood samples, and its quality was assessed using the Qubit 4 Fluorometer. CNV was detected using two technologies: ddPCR and MLPA. The study analyzed a total of 72 samples using both ddPCR and MLPA. The study followed the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. The outcomes of these tests, including their accuracy and precision, were evaluated.

Detailed cost analysis

Financial data were meticulously collected by the Health Technology Assessment team from the operational test facility at the All India Institute of Medical Sciences, Jodhpur, India, from January 2022 to December 2022. We employed standard cost calculation methodologies to evaluate the expenses incurred per test for the *SMN1*/*SMN2* genes using ddPCR and MLPA techniques. This investigation involved a comprehensive analysis of both capital and operating costs associated with each method. A detailed breakdown of expenses, categorized under specific heads, is provided to establish a carrier screening laboratory for SMA diagnosis. The following sections provide a detailed description of these expenses. The capital assets cost included the building cost (a research laboratory devoted to SMA testing), equipment (Sanger sequencer with eight capillaries, PCR, ddPCR, other small equipment, and a DNA isolation instrument), furniture, and fixtures. The operating cost included the human resources for instrumentation operation and results in interpretation (scientist/technician). The consumables cost included the kits for test procedures, plastic, glassware, sample collection and processing consumables, report printing cost, etc. Furthermore, other costs included instrument maintenance and electricity costs.

The annualized cost of capital assets

In this study, the annual cost of capital assets was calculated by dividing the procurement cost of machines by their estimated lifespan, which was assumed to be 5 years. Equipment costs from the institute's finance department included various small instruments essential for SMA diagnosis, such as freezers, deep freezers, air conditioners, tables, chairs, and other necessary items. Building costs were estimated by considering the monthly rent for laboratory space. For a comprehensive understanding of the costs and detailed cost analysis, please refer to Supplementary File 1.

Operating cost

The operational expenses primarily included the monthly salaries of specialized scientific and technical personnel overseeing laboratory operations and maintaining equipment maintenance. Their responsibilities ranged from sample collection and DNA isolation to preparing PCR reactions, operating instruments, and analyzing data. Salaries were determined following standard government norms for technical and scientific staff. It is important to note that the SMA diagnosis test is a crucial step preceding DNA isolation from blood samples for subsequent procedures.

Operational expenditure also covered consumables associated with capital items, such as kits, reagents, chemicals, and electricity. Information on these expenses was gathered from various suppliers and vendors over 6 months, aligning with the instrument requirements for SMA testing, including ddPCR and Sanger DNA sequencing using SeqStudio. The monthly electricity consumption cost was calculated by multiplying daily power consumption by the unit cost. This comprehensive approach ensures a thorough understanding of operational costs.

Test throughput

The SMA diagnostic protocol involves detection using ddPCR and SeqStudio-based methodologies. This study found that a single facility can perform approximately 400 tests per month using ddPCR and 150 tests per month using SeqStudio. The cost analysis for SMA diagnosis using ddPCR and SeqStudio spans the financial year from 2022 to 2023, assuming a 5-year

lifespan for instruments, small equipment, furniture, and fixtures, with 100% depreciation accounted for.

The per-test cost for SMA diagnostic testing (SeqStudio/ddPCR) was calculated using the following formula:

$$\frac{\text{Total capital cost} + \text{Total operating cost}}{\text{Total test of SMA conducted}}$$

Statistical analysis

The precision of the ddPCR test was evaluated by calculating the mean, standard deviation, and coefficient of variation (CV).[9] The performance of ddPCR and MLPA test results was compared using weighted k-statistics. The classification for k-statistics accuracy was as follows: 0.81 – 1.0 (perfect), 0.61–0.80 (good), 0.41 – 0.60 (moderate), 0.21 – 0.40 (weak), and <0.20 (poor). A $P < 0.05$ was considered statistically significant. Statistical analysis was conducted using the Statistical Package for the Social Sciences software.

Cost-effectiveness analysis using probabilistic sensitivity analysis (PSA)

Our study calculated the incremental cost-effectiveness ratio (ICER) as the incremental cost per additional case detected of SMA using the ddPCR method compared to the MLPA method in India. There is an official willingness-to-pay (WTP) threshold in India, which was set at the gross domestic product per capita in 2024 at $2,411. To evaluate the robustness of the base-case result, we performed a PSA and scenario analysis. To assess how results fluctuate across a spectrum of input parameter values, we conducted multiple sensitivity analyses by individually varying the following parameters while keeping all other variables constant: (1) The cost associated with each testing method and (2) the number of tests performed using each method. In addition, we performed PSA, wherein all parameters were simultaneously varied over 10,000 iterations.

RESULTS

In comparing ddPCR and MLPA test results, as outlined in the primary study, it was found that the copy number of the *SMN1* and *SMN2* gene exons seven was concordant between the two methods.[9] The comparison of the MLPA and ddPCR test results yielded significant correlations for the *SMN1* and *SMN2* genes. This was evidenced by a Kappa coefficient of 1.000 and a $P < 0.0001$ for both *SMN1* and *SMN2* gene exon seven, indicating substantial agreement between the two methods. Furthermore, the reliability of ddPCR test results compared to MLPA was 100% for all CNVs in both *SMN1* and *SMN2* gene exon seven. The result indicates that ddPCR yielded consistent and accurate results across all tested CNVs, demonstrating its reliability as a diagnostic tool for SMA.

Moreover, the repeatability of ddPCR tests was evaluated, with the CV calculated for CNV in *SMN1* and *SMN2* gene exon seven. The results showed a CV of <4% for both genes, indicating high repeatability and precision of ddPCR testing across different CNVs. The findings suggest that ddPCR is a reliable and consistent method for detecting CNVs in the *SMN1* and *SMN2* genes, offering comparable performance to the gold standard method of MLPA.[9]

In addition, the interpretation of ddPCR test results is also straightforward. 2-D amplitude plot shows four clusters representing the droplets: Blue cluster (top left), FAM-positive droplet with the only mutant template (*SMN1*/*SMN2* gene), orange cluster (top right), double-positive droplets with both templates inside, gray cluster (bottom left), negative droplets with no template (*RPP30* gene), green cluster (bottom right), hexachlorofluorescein (HEX) positive droplets with only wild-type template. We found zero to four copies of the *SMN1* or *SMN2* gene in the studied population at our tertiary care center, as presented in [Figure 1a-e].

SMA diagnosis cost analysis through ddPCR

The annual capital and operational costs associated with ddPCR-based SMA testing are presented in Table 1. The building and maintenance expenses for the ddPCR facility in the current year were estimated at INR 1,34,400. The total annualized cost of equipment required to establish the ddPCR laboratory for SMA screening was calculated to be INR 11,00,000. This equipment cost included maintenance expenses, ensuring comprehensive coverage for maintenance over a 5-year period. The annual cost of small instruments, furniture, and other fixtures essential for laboratory operations was also determined to be INR 1,30,000. Human resource expenses for the ddPCR facility were estimated at INR 21,00,000/year.

Furthermore, the yearly expenditure on consumables, kits, chemicals, and reagents necessary for conducting 4800 tests amounted to INR 42,57,600. Furthermore, the shared annual electricity cost for the ddPCR facility was calculated to be INR 1,80,000. For detailed calculations, please refer to Supplementary File 1.

Cost analysis of SMA diagnosis using the MLPA method

In the case of MLPA, the Sanger DNA sequencing test incurred laboratory and maintenance costs totaling INR 1,34,000 for the current year, 2023–2024. The annual expenditure for the MLPA laboratory facility's equipment reached INR 15,00,000, with embedded equipment maintenance costs ensuring comprehensive maintenance for 5 years and operating the laboratory with essential small instruments, furniture, and fixtures incurred an annual cost of INR 1,30,000. Human resource costs for the MLPA

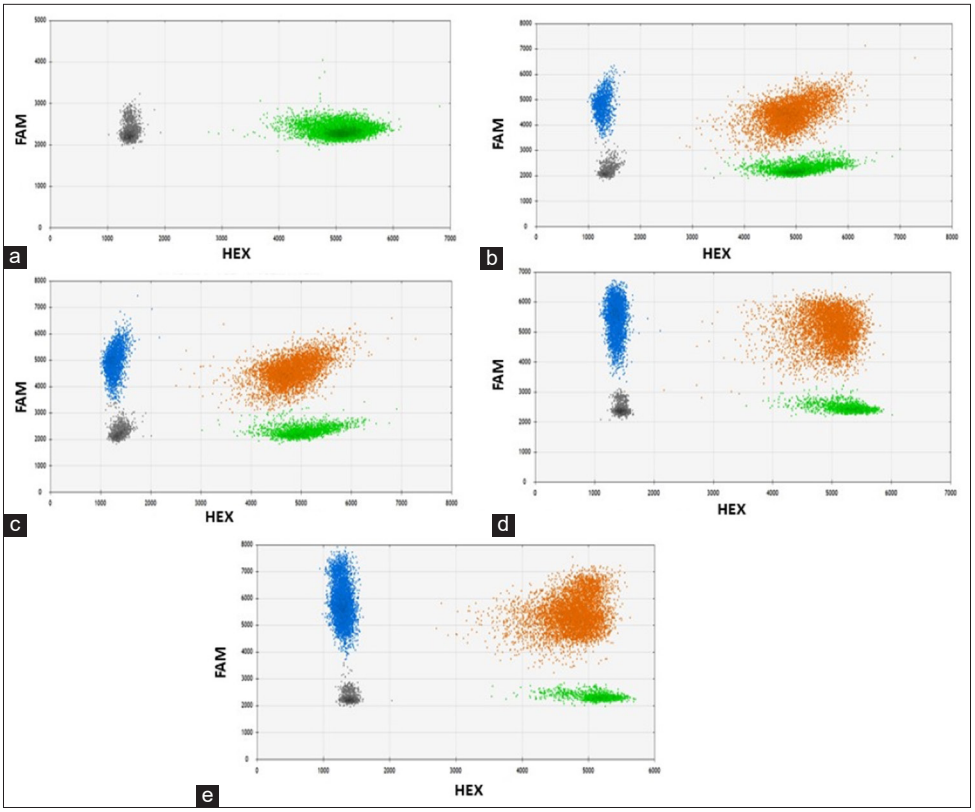

Figure 1: The 2D amplitude plot of droplet fluorescence for the *SMN1*/*SMN2* and *RPP30* (housekeeping gene) in the droplet digital polymerase chain reaction assay. The X-axis represents the fluorescence amplitude of the HEX channel (*RPP30* gene), while the y-axis represents the fluorescence amplitude of the FAM channel (*SMN* gene). Each droplet is categorized into one of four clusters: Double-negative (gray, bottom left), *SMN*-positive (blue, top left), *RPP30*-positive (green, bottom right), and double-positive for *SMN*/*RPP30* (orange, top right). (a) Sample with zero copies of the *SMN* gene, (b) 1 copy of the *SMN* gene, (c) 2 copies of the *SMN* gene, (d) 3 copies of the *SMN* gene, and (e) 4 copies of the *SMN* gene.

laboratory facility were estimated at INR 21,00,000/year. The yearly expenses for consumables, test kits, and reagents/chemicals to conduct 1800 tests were INR 66,42,000. The annual electricity cost amounted to INR 2,40,000.

Detailed price breakdowns for SMA testing through the MLPA method are presented in Table 2. In comparison, the capital and operating costs for SMA diagnosis through ddPCR were INR 13,64,400 and INR 65,37,600, respectively. For MLPA, these costs were INR 17,64,400 and INR 89,82,000, respectively. Further details on the cost of SMA testing through ddPCR and MLPA methods are available in the supplementary file. Please refer to the supplementary file for a detailed breakdown of the costs.

The costs associated with ddPCR and MLPA testing methods may decrease with an increase in the number of tests conducted. There is limited evidence regarding the cost of these genetic tests in low and middle-income countries. However, within governmental and private healthcare settings, charges typically range from approximately INR 2,000 to INR 7,000 per test. It is worth noting that international prices vary and are influenced by the country's infrastructure and laboratory capabilities.

Tables 1 and 2 comprehensively summarize the capital and operating costs associated with SMA diagnosis through ddPCR/NGS test services. The ddPCR SMA test is conducted 4800 times yearly, incurring an annual cost of INR 79,02,000 ($92,265). This total cost is allocated as follows: 15% to capital expenses and 85% to operating expenses, as illustrated in Figure 2a. In contrast, the annual cost for SMA diagnosis through MLPA services is INR 1,07,46,400 ($126,428) for 1800 tests, with 16% allocated to capital costs and 84% to operating costs [Figure 2b]. The expenditures for the ddPCR and MLPA SMA testing facilities comprise two significant components, accounting for over 75% of the total cost.

Conversely, expenditures for ddPCR and MLPA testing facilities primarily consist of three critical elements,

Table 1: The annual cost for SMA testing using the ddPCR method provides monthly and yearly breakdowns for comprehensive analysis.

Type of cost	Monthly	Annually
Capital cost (INR)		
Building rent along with maintenance	11,200	1,34,400
Equipment	91,667	11,00,000
Other small equipment, furniture, and other fixtures	10,833	1,30,000
Total	1,13,700 ($1,338)	13,64,400 ($16,056)
Operating cost (INR)		
Human resources	1,75,000	21,00,000
Consumables kits, reagents, etc.	3,54,800	42,57,600
Electricity	15,000	1,80,000
Total	5,44,800 ($6,410)	65,37,600 ($76,920)
Total (1+2) (INR)	6,58,500 ($7,748)	79,02,000 ($92,965)
Test conducted	400	4800
Per test cost	Approx. INR 1,646 ($20)	

SMA: Spinal muscular atrophy, ddPCR: Droplet digital polymerase chain reaction

Table 2: The annual cost associated with SMA diagnosis using the MLPA method provides monthly and yearly breakdowns for comprehensive analysis.

Type of cost	Monthly	Annually
Capital cost (INR)		
Building rent along with maintenance	11,200	1,34,400
Equipment	1,25,000	15,00,000
Other small equipment, furniture, and other fixtures	10,833.33	1,30,000
Total	1,47,033 ($1,730)	17,64,400 ($20,760)
Operating cost (INR)		
Human resources	1,75,000	21,00,000
Consumables kits, reagents, etc.	5,53,500	66,42,000
Electricity	20,000	2,40,000
Total	7,48,500 ($8,806)	89,82,000 ($1,05,670)
Total (1+2) (INR)	8,95,533 ($10,536)	1,07,46,400 ($1,26,428)
Test conducted	150	1800
Per test cost	Approx. INR 5,970 ($70)	

SMA: Spinal muscular atrophy, MLPA: Multiplex ligation-dependent probe amplification

accounting for more than 95% of the total cost: equipment costs, workforce costs, and the cost of consumables [Figure 2b]. Upon examining the unit costs, the annual capacity for SMA tests through ddPCR is 4800, resulting in a unit cost of INR 1,646 ($20). In contrast, for SMA tests through the MLPA method with an annual capacity of 1800, the unit cost is INR 5,970 ($70). The utilization of the MLPA method entails a larger laboratory footprint, an increased requirement for furniture and fixtures, additional personnel for data analysis, and higher electricity consumption, all of which contribute to the observed cost variations. This information is summarized in Figures 2a and b. The cost-effectiveness of SMA testing using ddPCR and the elevated cost of testing using MLPA is consistent with conclusions from earlier studies.

PSA, cost-effectiveness analysis between ddPCR, and MLPA

The results from the PSA for both diagnostic methods are detailed in Figure 3. The total cases detected using ddPCR and MLPA methods, the total costs associated with each technique, and their respective ICERs are described in Table 3. The cost-effectiveness acceptability curve derived from the PSA results provides insight into the probability that ddPCR will be a cost-effective intervention. Specifically, if a health policymaker's WTP per appropriately diagnosed child is zero, there is a 60% probability that ddPCR could be the preferred option. As the WTP increases, the country's GDP per capita rises significantly to 83.6%.

DISCUSSION

SMA is primarily caused by mutations in the *SMN1* gene on chromosome 5 q13.2, with disease severity linked to the number of *SMN2* gene copies.[2] MLPA has emerged as a significant advancement in SMA diagnostics, effectively detecting copy number changes in the *SMN* gene through a single reaction. Despite its benefits, MLPA's complex procedures pose financial challenges, prompting interest in alternative diagnostic approaches.[10] Despite its benefits, MLPA's complex procedures pose financial difficulties, prompting interest in alternative diagnostic approaches. The potential of the ddPCR platform in SMA diagnosis is promising, offering simplicity, promptness, and cost-effectiveness compared to MLPA. With comparable sensitivity and specificity, ddPCR minimizes the risk of false positives.[9,11] Furthermore, ddPCR-based SMA testing incurs lower equipment and reagent costs, reducing overall detection expenses. Its multiplexed capability enables simultaneous

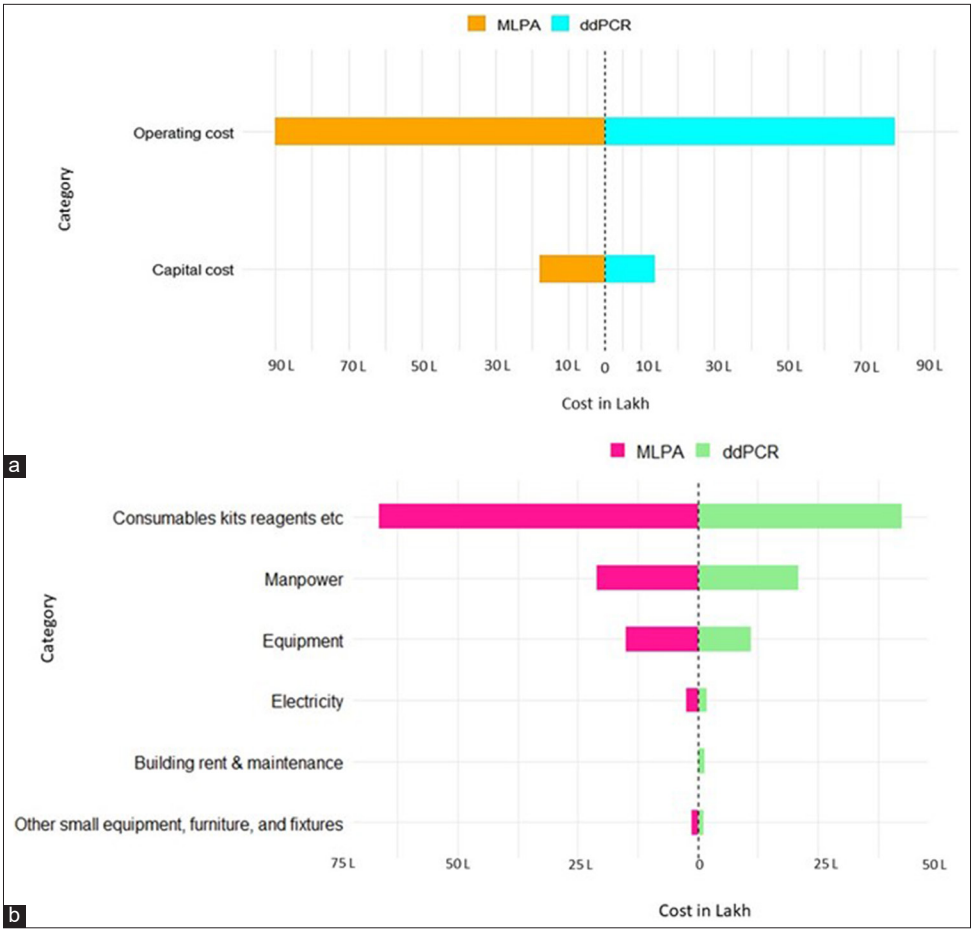

Figure 2: (a) Percentage distribution of operating and capital costs for spinal muscular atrophy (SMA) testing conducted through droplet digital polymerase chain reaction (ddPCR) and multiplex ligation-dependent probe amplification (MLPA), (b) Detailed breakdown of costs associated with SMA testing using ddPCR and MLPA.

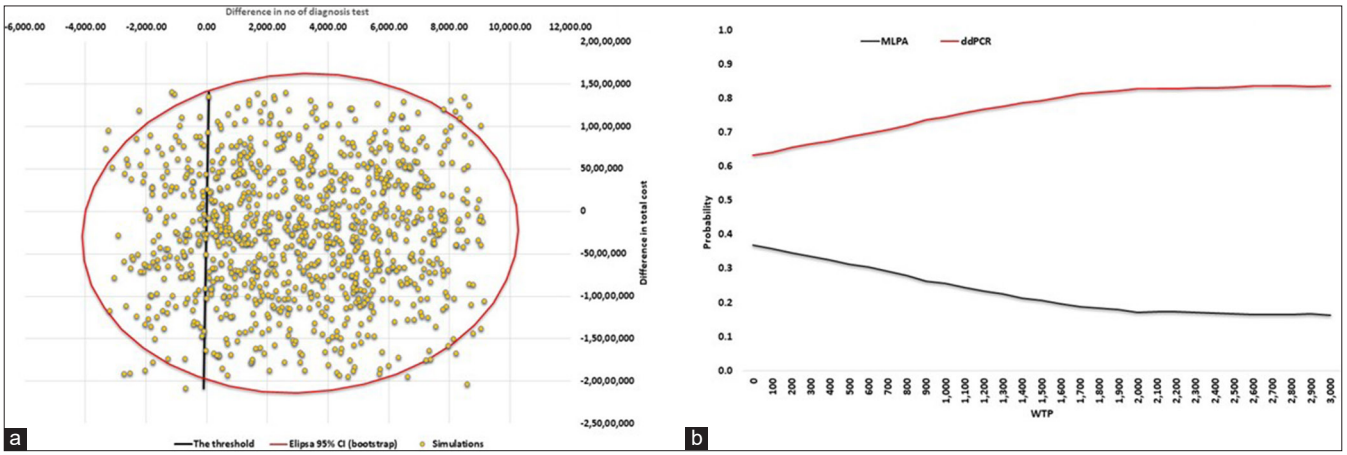

Figure 3: Probabilistic sensitivity analysis cost-effectiveness (a) scatter plot of incremental societal cost in India and an incremental number of cases detected of spinal muscular atrophy by droplet digital polymerase chain reaction versus multiplex ligation-dependent probe amplification and (b) cost-effectiveness acceptability curve (incremental cost-effectiveness ratio, per case detected). WTP: Willingness to pay.

Table 3: Range of uncertainty of the endpoints of economic analysis (non-parametric method).

Parameters	Methods	Average	95% CI		Median	IQR	
Case detected	ddPCR	4,842.57	275.85	9,405.99	4,867.43	2,500.22	7,075.85
	MLPA	1,756.94	130.96	3,483.58	1,723.91	870.12	2,637.51
	Difference	3,085.62	−2,040.35	8,493.27	3,065.52	721.77	5,376.39
Total cost	ddPCR	78,83,864.60	3,51,027.45	1,54,09,569.99	79,25,345.38	37,58,257.64	1,19,65,174.74
	MLPA	1,04,65,144.73	4,46,136.01	2,08,61,991.11	1,02,57,690.74	50,24,719.30	1,59,40,847.08
	Difference	−25,81,280.13	−1,75,42,350.06	1,17,48,642.77	−25,98,446.61	−79,59,823.30	32,61,794.85
ICER		Domination	−23,884	24,681	−462	−2,518	1,358

ddPCR: Droplet digital polymerase chain reaction, MLPA: Multiplex ligation-dependent probe amplification, ICER: Incremental cost-effectiveness ratio, CI: Confidence interval, IQR: Interquartile range

detection of *SMN1* and *SMN2* genes, maintaining accuracy while being cost-effective.[12] In contrast, MLPA requires sophisticated laboratory setups, highly trained personnel, and substantial investments, resulting in higher costs per test and limited feasibility outside centralized laboratories.

SMA, a severe neurological disorder, has seen hope through gene replacement therapies like Zolgensma.[13,14] However, despite government support and crowdfunding efforts to bridge the financial gap, the high costs associated with patient care make it inaccessible to many.[14,15] Under the rare disease policy, the Indian Government provides targeted support for SMA patients, although with certain restrictions.[16,17] Moreover, crowdfunding initiatives have become instrumental in supplementing treatment costs, demonstrating a collaborative effort to address patients' financial challenges with SMA.

Research indicates that the carrier frequency of SMA ranges from 1 in 40 to 1 in 70, Globally.[18] Due to its severity and high carrier frequency, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology recommend population-based carrier screening for SMA at the time of conception. Early screening of pregnant women for *SMN1*/*SMN2* gene variants holds promise as an effective strategy to prevent SMA-affected births.[19] ddPCR is a high-throughput, time-saving, and cost-effective method for detecting CNVs in the *SMN1* and *SMN2* genes.[9] This technique could be utilized for carrier screening during prenatal or preconception stages, aiding in identifying at-risk pregnancies and facilitating informed reproductive decisions.

Newborn screening for SMA has been implemented in the United States and Europe as a public health initiative to enable early detection and timely interventions, potentially improving patient outcomes.[20,21]

Recent guidelines emphasize the importance of identifying *SMN1* heterozygous deletions, which represent the majority of SMA carriers.[22] ddPCR-based molecular testing provides a cost-effective and time-saving approach for efficiently detecting carriers. While other genetic tests, such as chromosomal microarray analysis and NGS, offer high diagnostic accuracy, they are associated with higher costs, longer turnaround times, and the need for specialized laboratory setups. In our study, we specifically evaluated the cost per test for the *SMN1*/2 gene using ddPCR, which amounted to 600 Indian Rupees per test, with a turnaround time of 8 h. ddPCR exhibits robustness and reliability in challenging sample types due to its reduced susceptibility to PCR inhibitors.[23] Moreover, partitioning samples into thousands of individual droplets in ddPCR minimizes variations in amplification efficiency, resulting in enhanced precision and sensitivity. Interpreting test results is also straightforward. A notable limitation of ddPCR is its inability to detect carriers of SMA with a 2 + 0 genotype, which is consistent with the gold standard, MLPA. However, advancements in techniques such as optical genome mapping and long-read sequencing offer potential solutions to overcome these limitations, although at higher costs and with the need for sophisticated laboratory setups.

PSA outcomes emphasize the economic viability of ddPCR-based SMA diagnosis. The method demonstrates a high probability of being cost-effective from a health sector perspective, even at very low WTP levels. This is particularly relevant in moderate-to-high transmission settings, where the efficient allocation of healthcare resources is crucial. Furthermore, the PSA results underscore the robustness of ddPCR across various economic scenarios. The ICERs remain favorable across different WTP thresholds, reinforcing the potential of ddPCR to be a sustainable and scalable diagnostic tool for SMA. This comprehensive analysis provides strong evidence for policymakers to consider integrating digital PCR into routine clinical practice for SMA diagnosis, potentially leading to improved patient outcomes and optimized healthcare expenditure.

Our findings offer valuable insights into the feasibility and efficiency of ddPCR in SMA screening protocols, guiding the optimization of screening strategies in clinical practice.

The findings support the notion that ddPCR is more cost-effective and efficient for diagnosing SMA than MLPA. This can result in significant cost savings and more accurate diagnostic outcomes, which are critical for effective disease management and treatment planning. Integrating ddPCR into clinical practice could transform the landscape of SMA diagnosis, offering a practical and economically advantageous alternative to existing methods.

Data and availability

It can be made available from the corresponding author. However, all data are included in the article and the Supplementary File 1.

CONCLUSION

The study showed strong concordance in *SMN1* and *SMN2* copy numbers across seven exons using both ddPCR and MLPA. ddPCR platform exhibited promising potential as a diagnostic tool for SMA, showcasing several advantages over MLPA. These advantages include simplicity, promptness, time-saving attributes, and cost-effectiveness. The findings underscore the viability of ddPCR as a valuable alternative to routine methods, such as MLPA, in diagnosing SMA, suggesting its potential to enhance diagnostic accuracy and efficiency while reducing associated costs. PSA indicates that ddPCR is a significantly cost-effective method for diagnosing SMA compared to the standard MLPA method.

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Ethical approval: This research received ethical approval from the All India Institute of Medical Sciences Ethics Committee, Jodhpur, with approval number AIIMS/IEC/2022/4008. This study adheres to the ethical principles outlined in the Declaration of Helsinki.
Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.
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