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Virulence gene expression of *Pseudomonas aeruginosa* isolated from children with nephrotic syndrome

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Abstract

Background: *Pseudomonas aeruginosa* is Gram-negative, and considered as the most common opportunistic pathogens which lead to high rate of mortality and morbidity when subject suffered from compromised immune systems. **Objectives:** This study was to estimate the *lasI and lasR* genes expression of *P. aeruginosa* isolated from children with nephrotic syndrome. **Methods:** Nineteen isolates from urine samples of children with nephropathy syndrome are included in this study and confirmed by VITEK test as a *Pseudomonas aeruginosa* strains. **Results and Conclusions:** The result showed that the *LasI and LasR* genes showed a high level of gene expression in strains that grown with ceftriaxone than the strains that grown alone, but the gene *Las*I had a higher level of expression than the *Las*R gene, also a regression analysis were done to reveal the effect of *Las*I on the *Las*R gene which showed a positive effect which mean any increment in the gene expression level of *Las*I gene will combined with an increment in the *Las*R gene, (R= 0.899 and P= 0.001).

Keywords: Nephrotic syndrome, *Las*I and *Las*R genes, Gene expression.

1. Background

Nephrotic syndrome (NS) is a condition that exclusively affects the kidney and is usually characterized by the erasure of foot podocyte processes without glomerular deposition or inflammatory lesions [1]. There are several acute complications in children with NS, some of which can be extreme and fatal; as infections, venous thromboembolism (VTE) and

AKI, the epidemiology and fate of AKI remain unclear when clinical reflections of infection and VTE in nephrotic children are clear[2].

Nephrotic children are more vulnerable to mortality without treatment, mainly due to bacterial infections. 40 percent of children died before era of the corticosteroid and antibiotic , and 50 percent of these deaths were due to infection[3]. It has recently been shown that at least 50 percent of pediatric onset NS behaviors are triggered by infection of the viral upper respiratory tract; this could be attributed more to the non-specific host response to infection than to the virus itself or its antibody response[4].

Pseudomonas aeruginosa is Gram-negative bacteria, facultative anaerobic rods, non-fermentative, non-sporulation, motile by one polar flagellum[5]. In recent years, infections caused by this bacterium have become one of the most serious issues, with death rates ranging from 18 % to 61 % [6]. Amplification by PCR for Pseudomonas aeruginosa have been used greatly to detect it in clinical samples by polymerase chain reaction (PCR) in recent decades[7]. This amplification were done for multiple genes as a marker to detect this bacterium since 1992, when PCR detection of *P. aeruginosa* was first reported [5].

To recognize this bacterium, multiple genes have been identified as PCR targets. Subsequently, multiple studies have shown that these genes may not have full bacterial detection as both the sensitive or specificity of this detection [8].

According to studies, for the production of *P. aeruginosa* infection, quorum sensing QS is important and the QS genes are exclusive and retained for each bacterial species [9]. The *las*I and *las*R genes are the bacterium's quorum sensing essential (QS) genes. The previous studies proven that some clinical isolates possess quorum sensing system deficiencies[10].

2. Objectives

This study was to estimate the *lasI and lasR* genes expression of *P. aeruginosa* isolated from children with nephrotic syndrome.

3. Methods

Nineteen isolates from urine samples of children with nephropathy syndrome are included in this study and confirmed by VITEK test as a *Pseudomonas aeruginosa* strains. Twenty

three samples have been excluded as they showed other isolates. Overnight cultures were prepared in a nutrient-rich LB broth under 37 °C with shaking conditions at 180 rpm. Then each sample grown on two plates the first plate contain only nutrient agar and the second one contain the agar and ceftriaxone. The RNA were extracted and the RNA were converted by using PrimeScript™ RT Master Mix kit. 8 µl of the eluted RNA were added to a new sterile 0.2 ml tube and then 2µ of PrimeScript™ RT Master Mix were added and then incubated for 15 min at 37 C temperature.

The prepared cDNA then the gene expression were evaluated by using RT-PCR. In order to amplify the specific regions of the *las*I gene; forward primer, 5"GTTTTCGGTTGCTGGCGAAT"3 and the reverse primer was, 3"GAAACGGCTGAGTTCCCAGA"5. And to amplify the gene *las*R the forward primer was 5" TGTTGCCTAAGGACAGCCAG"3 and reverse primers was "3CTGCTTTCGCGTCTGGTAGA5". Another set of primers were also used to target the *gyr*B gene in order calculate the ΔΔCt and the folding according to the method described by (Livak and Schmittgen, 2001); forward primer, 5"CGAGCGTGGCTACATCTACA3" and the reverse primer 3"TGGTCGTCCTTGATGTACTGC5". The thermal cycling program were as follow, enzyme activation 95 C for 7 min, followed by 40 cycles of two steps the first one was denaturation 95 C for 20 sec and second step of annealing for 20 sec (55 C) and extension for 20 sec. and thermocycling Specific primer sequences used for PCR amplification.

4. Results

Both *las*I and *las*R genes showed a high level of gene expression in strains that grown with ceftriaxone than the strains that grown alone, but the gene *las*I had a higher level of expression than the *las*R gene, as seen in Figure (1).

The results showed a significant correlation between the *las*I and *las*R. A regression analysis were done to reveal the effect of *lasI on the lasR* gene which showed a positive effect which mean any increment in the gene expression level of *las*I gene will combined with an increment in the *las*R gene, (R= 0.899 and P= 0.001).

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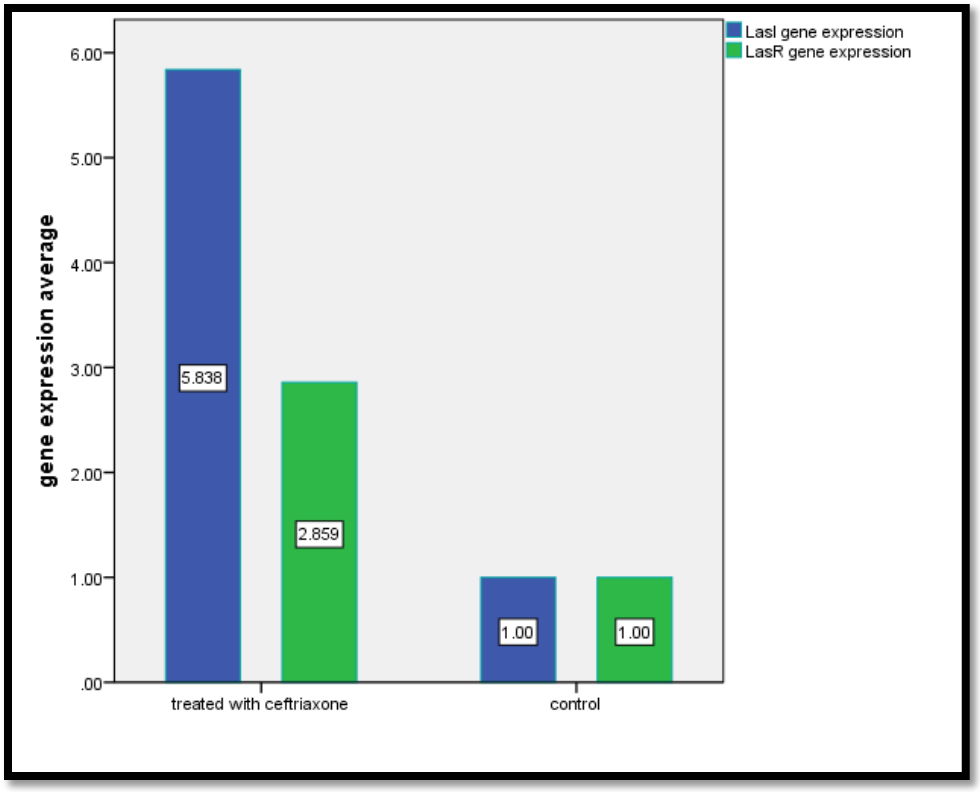

Figure (1): Gene expression the *LasR* and *LasI* genes with and without ceftriaxone.

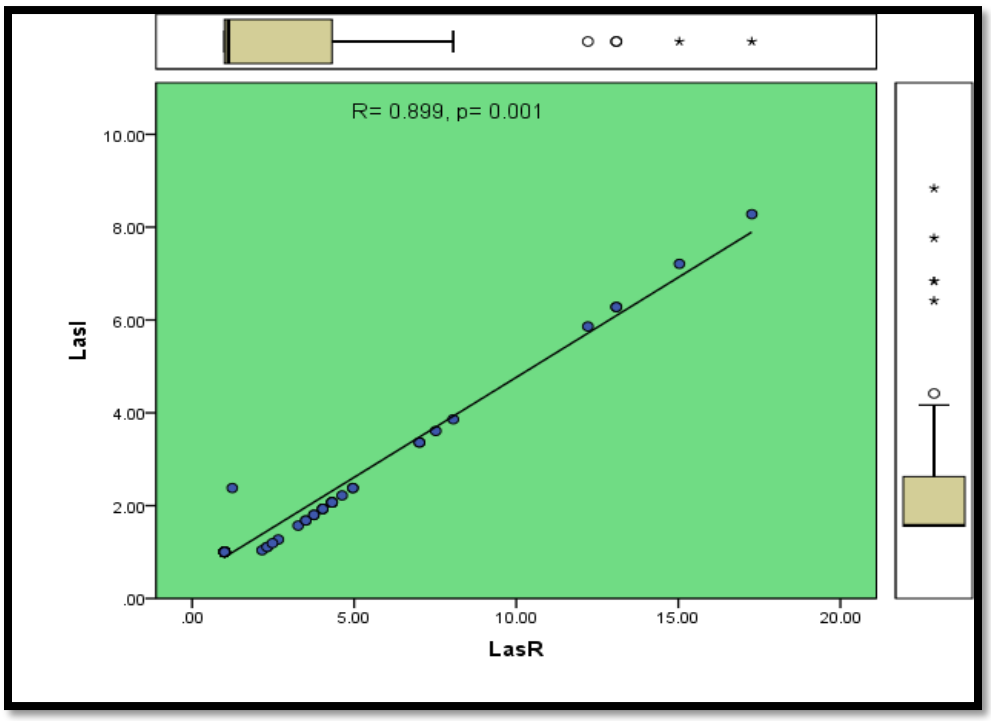

Figure (2): Correlation between the *las*I *and las*R genes.

5. Discussion

In order to strategically organize the expression of virulence factors and biofilm formation, chemical social networking system *were used by Pseudomonas aeruginosa* referred as quorum sensing (QS). The host cells are weakened by virulence, the host immune system is compromised, and bacterial cells are protected from antibiotic attacks. Anti-QS agents could thus be used to treat P. aeruginosa infections as a novel anti-infective therapy. [11,12, 13].

This study showed the *las*I *and las*R genes expression of p. aeruginosa a high level of gene expression in strains that grown with ceftriaxone than the strains that grown alone , this agreement with study conducted by Kumar *et al.,* showed ceftriaxone (CT) reveal both anti-QS and anti-virulence mechanisms against *P. aeruginosa* PAO1 [14].

Both transcriptional regulator Las R and *las*I referred to as the QS system which lead to manufacture the AI 3-oxo-dodecanoyl homoserine lactone [15]. *las*R has long been known to be required for the activation of rhlR gene expression at the start of the QS response [16]. During Pseudomonas aeruginosa infections, *las*R mutants are also chosen [17]. These mutants are called cheaters since they benefit of the common goods (secreted virulnce factors) that are generated from the QS proficient part of the infecting population [18]. According to the findings of some previous studies [19,20,21], the QS cannot be optimally blocked by *las*R, which can be an alternative for the treatment of infections caused by *P. aeruginosa*, as in the case of antibiotics resistance [15].

In the study by Hemati *et al.*, found the frequency of QS genes was high among these isolates, according to PCR results[22]. Cabrol et al. found that the *las*R gene was present in all 66 isolates of *P. aeuroginosa* [23].

Finally the study conducted by Naseri *et al.,* showed case is an infant with congenital nephrotic syndrome ,blood culture revealed *Pseudomonas* aeruginosa infection found low serum gamma globulin levels and deficiency of complement factors B and D predispose cases to infection[24] . Staphylococci and coliforms proved by another study to be cause septic episodes in infants with CNS [25,26].

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