

## Correlation of Leucine-Rich- $\alpha$ -2-Glycoprotein-1 (LRG-1) Level in Urine with Cervical Cancer Stage, Histology Type and Histology Grading

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### Abstract

**Objective:** To determine if the level of LRG-1 in urine correlates with cervical cancer stage, histology type and histology grading

**Methods:** This cross-sectional study using ELISA to test urinary LRG-1 of 59 cervical cancer patients. Data were analyzed using Kruskal-Wallis test.

**Result:** From the total of 59 samples, LRG-1 in urine ranged from 0.48 ng/mL to 170.43 ng/mL, with median value 58.42 ng/mL. A median value of 21.42 $\pm$ 52.29 ng/mL was found in the urine at early stage and 115.32 $\pm$ 59.36 ng/mL at advanced stage. Most patients had cervical cancer at advanced stage (69.4%), squamous cell carcinoma (66.1%), and grade cannot be assessed (45.8%). Median LRG-1 levels were highest in squamous cell carcinoma (66.42 $\pm$ 60.89 ng/mL) and poorly differentiated (127.74 $\pm$ 54.13 ng/mL). LRG-1 levels were significantly correlated with cervical cancer stage (p-value=0.045) but not histological type (p-value=0.940) or histopathological grade (p-value=0.488).

**Conclusion:** The more advanced the cervical cancer stage, the more elevated urinary LRG-1 levels. LRG-1 contributes to angiogenesis and antiapoptotic processes in cancer. Further studies are required to identify and evaluate LRG-1 in urine as an important biomarker for making clinical decisions and developing potential treatments.

**Key words:** Cervical cancer; Histology type; Histology grade; LRG-1; Stage

## Hubungan Kadar Protein *Leucine-Rich- $\alpha$ -2-Glycoprotein-1* (LRG-1) Urine dengan Stadium, Tipe Histologis, dan Derajat Diferensiasi Kanker Serviks

### Abstrak

**Tujuan:** Untuk mengetahui hubungan kadar LRG-1 dalam urine dengan stadium, tipe histologis, dan derajat diferensiasi kanker serviks.

**Metode:** Studi ini menggunakan desain penelitian *cross-sectional* pada 59 perempuan yang telah didiagnosis kanker serviks, kemudian diperiksa kadar protein LRG-1 dalam urine dengan metode ELISA. Uji statistik menggunakan Kruskal Wallis.

**Hasil:** Dari total 59 sampel didapatkan kadar LRG-1 dalam urine terendah 0,48 ng/mL dan tertinggi 170,43 ng/mL, nilai median 58,42 ng/mL. Nilai median pada stadium awal 21,42 $\pm$ 52,29 ng/mL dan stadium lanjut 115,32 $\pm$ 59,36 ng/mL. Lebih banyak penderita mengalami kanker serviks pada stadium lanjut (69,4%), tipe histopatologis *Squamous Cell Carcinoma* (66,1%), derajat diferensiasi tidak dapat ditentukan (45,8%). Median tertinggi kadar LRG-1 pada tipe *Squamous Cell Carcinoma* (66,42 $\pm$ 60,89 ng/mL), dan derajat diferensiasi yang buruk (127,74 $\pm$ 54,13 ng/mL). Terdapat hubungan yang signifikan antara kadar LRG-1 dan stadium kanker serviks (nilai p = 0,045), tetapi tidak dengan tipe histologis (nilai p=0,940) dan derajat diferensiasi (nilai p=0,488).

**Kesimpulan:** Semakin tinggi stadium maka semakin tinggi kadar protein LRG-1 dalam urine. LRG-1 berperan dalam proses angiogenesis dan antiapoptosis pada kanker. Diperlukan penelitian lebih lanjut agar identifikasi dan evaluasi biomarker LRG-1 urine dapat menjadi penanda penting yang membantu dalam pengambilan keputusan klinis, serta pengembangan terapi.

**Kata Kunci:** Derajat diferensiasi, Kanker serviks, LRG-1, Stadium, Tipe histologis

## Introduction

Cervical cancer is a concerning health problem for women across the globe. Approximately 569,847 new cases of cervical cancer are estimated to occur globally in 2018, resulting in 311,365 cancer-related deaths. According to age-standardized rates (ASRs), cervical cancer is the fourth most common cancer worldwide, causing 13 cases per 100,000 and 6.9 deaths per 100,000. In Indonesia, there were 32,469 new cases and 18,279 deaths in 2018, making cervical cancer the second highest of the ten most common cancers.<sup>1</sup> The South Sulawesi Health Office reported 732 cervical cancer cases in South Sulawesi in 2017.<sup>2</sup> In addition, the Oncology Division reported 122 new cases in its registration data for 2018 to 2019. Cervical cancer severely threatens women's well-being, placing a tremendous financial burden on affected individuals and their families and increasing the health budget's consumption.<sup>2</sup>

Human Papilloma Virus (HPV) is the main etiological factor of cervical cancer. It affects almost everyone who engages in sexual activity during their lifetime; however, most HPV infections are self-curable within two years of contracting them. There are 2 types of HPV: low-risk and high-risk HPV. The low-risk HPV causes condyloma acuminata, while 15 genotypes of high-risk HPV, especially the subtypes 16 and 18, are oncogenic and can cause precancerous lesions that may progress to cervical cancer if left untreated.<sup>3</sup> The risk factors of HPV infection are young age at the first-time sexual intercourse, multiple sexual partners, and having a sexual partner who has multiple partners.<sup>3</sup>

Mostly, women who had been infected by the high-risk HPV type did not progress into cervical cancer. Human Papilloma Virus infection serves as the beginning of the carcinogenic process in cervical cancer. However, there are some conditions, called

the co-factors, of HPV infection that make the infection become persistent and progress into a precancerous lesion and invasive cervical cancer. The co-factors may come from the HPV itself, such as the HPV type, simultaneous infection of numerous oncogenic HPV types, and high viral load. Some host factors also serve as the co-factors, including high parity and immunological status. Numerous external factors may serve as co-factors, namely smoking, co-infection with other sexually transmitted diseases, and long-term use of oral contraceptive.<sup>3</sup>

In terms of reducing the morbidity and mortality of cervical cancer, prevention of cervical cancer is mandatory. Cervical cancer prevention consists of several phases. Primary prevention, including education and the HPV vaccination program, is performed to minimize or reduce carcinogenic exposure and prevent the initiation and promotion of carcinogenesis.

Secondary prevention involves finding cases as early as possible so that healing is more likely. In this stage, cervical precancerous lesions are screened and detected using various methods (e.g., Pap smears or conventional Pap tests, Pap thin prep tests, Acetic Acid Visual Inspection (IVA), Lugoliodin Visual Inspection (VILI), colposcopy, and HPV DNA testing). As a gold standard for cervical cancer diagnosis, a cervical biopsy is conducted if there are indications following the screening.

The last phase is tertiary prevention. In this phase, if there is any case detected from the screening or early detection, treatments were given to prevent clinical complications, early deaths, and palliative care.<sup>4</sup>

The Pap smear or pap test has been used for over 20 years.<sup>4</sup> In implementing this examination, many obstacles were encountered, including during sampling, fixing after sampling, delivering the samples, and the absence of an anatomical pathologist, leading to a delayed diagnosis. A DNA test

for HPV is another screening option currently being developed to detect the genetic material (DNA or messenger RNA) of high-risk HPV. Some studies indicate that urine tests can be used as a non-invasive alternative for detecting HPV infection, which could lead to increased cervical cancer screening compliance and less loss to follow-up.<sup>4,5</sup>

In another study, 60 types of proteins were found to be more abundant in urine in cervical cancer patients, including leucine-rich  $\alpha$ -2-glycoprotein-1 (LRG-1) and Multimerin-1 (MMRN1). Conversely, the concentration of 73 proteins decreased, including Protein S100-A8 (S100A8), Serpin B3 (SERPINB3) and CD44. Based on Receiver Operating Characteristics (ROC) analysis, both LRG1 and SERPINB3 could detect cervical cancer, respectively, with 100% sensitivity and 87.5% specificity. In addition, urine samples can be used to diagnose cervical cancer by combining these five proteins. Cervical cancer patients had 2.72 times the amount of LRG-1 in their urine as those without. Chokchaichamnankit et al. claim this is the first time this protein has been linked to cervical cancer.<sup>6</sup>

A study in China found that lung cancer patients' urine exosomes and lung tissue had higher levels of LRG-1.<sup>7</sup> Analyzing LRG-1 in urine is promising since it is easy, non-invasive, and accurate.<sup>7</sup> Thus, LRG-1 may be a candidate biomarker for non-invasive diagnosis using urine samples.<sup>7</sup>

Leucine-rich  $\alpha$ -2-glycoprotein-1 is a plasma glycoprotein that consists of 312 amino acids long and molecular weight between 34 to 36 kD. The normal plasma concentration of LRG-1 is around 21-50  $\mu\text{g}/\text{mL}$ .<sup>8</sup> Studies reported that the normal concentration of LRG-1 in urine equals its plasma concentration, which is 55.5  $\mu\text{g}/\text{mL}$ .<sup>8</sup> Several studies enlightened the potential role of LRG-1 in cellular adhesion, granulocyte differentiation, protein interactions, and cellular migration.<sup>7</sup>

The latest study confirmed that the LRG-1 involves in the Transforming-Growth Factor- $\beta$  (TGF- $\beta$ ) signaling pathway and possesses a significant role in maintaining cellular life sustainability and avoiding programmed-cellular death (apoptosis).<sup>9</sup> It has been shown that LRG-1 plays a crucial role in cancer development. Research had shown that the increase of LRG-1 level is linked to various types of cancer and may be considered a diagnostic marker for cancer.<sup>10</sup>

Information and data on the measurement of urinary LRG-1 levels in cervical cancer patients remain scarce. Thus, it might be a potential biomarker to be evaluated in the future. This study was performed to evaluate the correlation of urinary LRG-1 level with cervical cancer staging, histological type and histopathological grading in cervical cancer patients.

## Method

This is a cross-sectional study involving 59 cervical cancer patients. Fifty-nine women diagnosed with cervical cancer at the Wahidin Sudirohusodo Central General Hospital (RSUP) and the network hospital affiliated with the Hasanuddin University Faculty of Medicine were involved in the study. A consecutive sampling method was used to collect samples from December 2021 to June 2022. An Enzyme-Linked Immunosorbent Assay (ELISA) test was performed by Prodia Jakarta Laboratory on the urine sample to determine LRG-1 levels. A biopsy-confirmed cervical cancer diagnosis and consent to participate in this study were the inclusion criteria for this study. Exclusion criteria included (1) previous chemotherapy or radiotherapy; (2) had been diagnosed with other cancer besides cervical cancer (double primary tumor); (3) metastatic cancer from other location (secondary tumor); (4) relapse tumor; and (5) had a history of autoimmune disease or HIV/AIDS. Kruskal Wallis and

two-way ANOVA were used to analyze the data.

### Results

Table 1 showed that samples were mostly in the advanced stage (69.49%), with stage IIB being the most common stage (30.50%). In the early-stage group (30.51%), the most common stage was IB1 (11.90%). From the histological types, the most prevalent type was squamous cell carcinoma (66.1%). Grade cannot be assessed was the most frequent histopathological grading found (45.8%). The median level of LRG-1 in urine was  $58.82 \pm 60.13$  ng/mL.

**Table 1 Characteristics of Research Subjects**

Variable (N = 59)	n (%)
<b>Age</b>	<b>44 ±10,8</b>
<b>Parity</b>	
Primiparity	9 (15,3)
Multiparity	50 (84,7)
<b>Number of Marriages</b>	
One	41 (69,5)
Two	17 (28,8)
Three	1 (1,7)
<b>Early Stage</b>	<b>18 (30,51)</b>
Stage IA1	1 (1,7)
Stage IA2	0
Stage IB1	7 (11,9)
Stage IB2	5 (8,5)
Stage IB3	4 (6,8)
Stage IIA1	1 (1,7)
Stage IIA2	0
<b>Advanced Stage</b>	<b>41 (69,49)</b>
Stage IIB	18 (30,5)
Stage IIIA	2 (3,4)
Stage IIIB	17 (28,8)
Stage IIIC1	2 (3,4)
Stage IIIC2	0
Stage IVA	2 (3,4)
Stage IVB	0

#### Histological Type

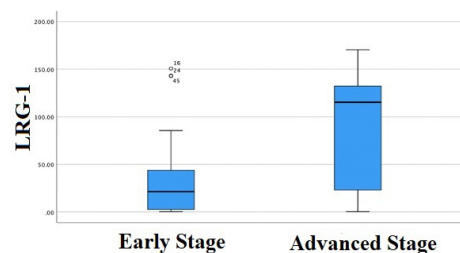
Adenocarcinoma	18 (30.5)
Adenosquamous Carcinoma	1 (1.7)
Clear Cell Adenocarcinoma	1 (1.7)
Squamous Cell Carcinoma	39 (66.1)

#### Histopathological Grading

Well Differentiated	15 (25.4)
Moderately Differentiated	10 (16.9)
Poorly or Undifferentiated	7 (11.9)
Grade Cannot be Assessed	27 (45.8)

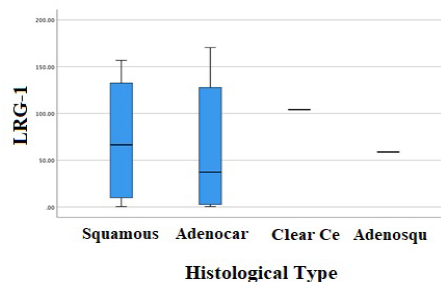
#### LRG-1 Urine Level

Median	23.18
Standard Deviation	60.13
Min	0.48
Max	170.43



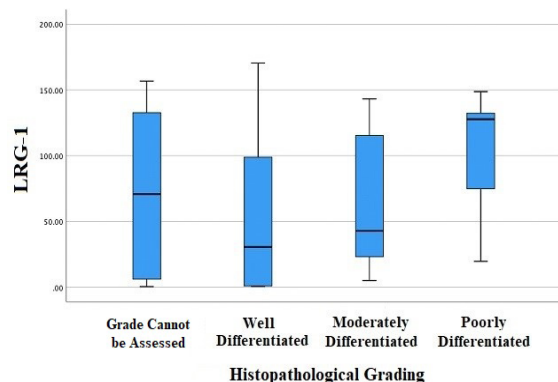
**Figure 1 Levels of LRG-1 in Urine Based on the Cervical Cancer Staging**

The distribution of LRG-1 levels in the urine based on cervical cancer staging was depicted in Figure 1. It was shown that the median level of LRG-1 in urine was lower in early-stage cervical cancer ( $21.42 \pm 52.29$  ng/mL) compared to advanced-stage cervical cancer ( $115.32 \pm 59.36$  ng/mL).



**Figure 2 Levels of LRG-1 in Urine Based on Histological Type of Cervical Cancer**

As shown in Figure 2, adenocarcinoma (37.21±62.45 ng/mL) had a lower median protein level of LRG-1 than squamous cell carcinoma (66.42±60.89 ng/mL). Adenosquamous carcinoma and clear cell adenocarcinoma had only one sample each, and LRG-1 values were 104.01 ng/mL and 58.82 ng/mL, respectively.



**Figure 3 Levels of LRG-1 in Urine Based on Histopathological Grading of Cervical Cancer**

Based on Figure 3, the median level of urinary LRG-1 in the poorly differentiated (127.74±54.13 ng/mL) was greater than the moderately differentiated (42.85±50.00 ng/mL) and well-differentiated (30.55±62.05 ng/mL).

LRG-1 level in urine was significantly correlated ( $p=0.045$ ) with the cervical cancer stage as shown in table 2.

The two-way ANOVA test was used to analyze the effects between cancer staging and histological type, cancer staging and histopathological grading, and histological type and histopathological grading with the

level of LRG-1 in urine. There was a significant correlation between the LRG-1 level in urine with cervical cancer staging, while the histological type and histopathological grading of cervical cancer were not able to show any significant correlation with LRG-1 level in urine. There was no significant interaction between cervical cancer staging and histological type with LRG-1 level in urine, nor does cervical cancer staging and histopathological grading as well as the histological type and histopathological grading of cervical cancer ( $p\text{-value}>0.05$ ).

### Discussion

Most samples were in stage II and III cervical cancers with each of the groups consisting of 20 (33.9%) samples. The stage I groups consist of 17(28.8%) samples. These findings were in accordance with national data from Indonesian Gynecologic Oncology (INASGO) in 2021 which reported the incidence of stage II cervical cancer was 495 cases (32.2%) and the incidence of stage II cervical cancer was 778 cases (50.5%).<sup>11</sup>

A cervical cancer diagnosis is mostly at a late stage due to the asymptomatic nature of early-stage cancer. In contrast, advanced cervical cancer may manifest symptoms, such as abnormal vaginal bleeding, postcoital bleeding, vaginal discharge, low-back pain, and dyspareunia.<sup>12</sup> A lack of cancer screening facilities for asymptomatic women, the culture in society along with the lack of public education to perform the screening also contributes to the delayed diagnosis of

**Table 2 Correlation of LRG-1 Level in Urine with The Staging, Histological Type and Histopathological Grading of Cervical Cancer**

Variable	N	Mean	Min	Max	p-Value
LRG-1 Level in Urine	59	70.67	0.48	170.43	
Staging	59	2.12	1	4	0.045
Histological Type	59	1.39	1	4	0.940
Histopathological Grading	59	1.95	1	4	0.488

Kruskal-Wallis Test

cervical cancer.<sup>13</sup> A delay in diagnosis and treatment can significantly affect a patient's prognosis. It is, therefore, essential to detect and treat cervical cancer at an early stage so that it does not progress to an advanced stage.

In this study, higher levels of LRG-1 in the urine were associated with a higher stage of cervical cancer. Similarly, Chokchaichamnankit et al. (2019) found higher urine levels of LRG-1 in cervical cancer stages IIB, III, and IV than those in the control group.<sup>6</sup> This study supports the theory that angiogenesis and antiapoptotic in cancer are attributed to LRG-1. Upon binding to ALK1, endoglin, and TBR1, LRG-1 exerts its proangiogenic effect by diverting TGF- $\beta$  signals towards ALK1. Furthermore, LRG-1 competes with Apaf-1 for binding to cytochrome c, preventing apoptosis.<sup>6,9,14</sup>

The bigger the tumor, the more protein it secretes; therefore, the LRG-1 levels correlate positively with the size of the tumour.<sup>15,16</sup> Aside from tumor cells, endothelial and myeloid cells also produce LRG-1. Studies in vitro have found that LRG-1 increased tumor cell viability, proliferation, and invasiveness.<sup>15,16</sup> A vital role for LRG-1 in tumor growth has been confirmed in vivo; ablation of LRG-1 slows tumor growth, whereas overexpression promotes it.<sup>17</sup>

The angiogenic process plays a very significant role to support tumor growth and distant metastasis.<sup>18</sup> The LRG-1 arranges the formation of pathological angiogenesis and increases the expression of several proangiogenic factors, including TGF- $\beta$ , VEGF-A, and Angiopoietin-1 in endothelial cells and cancer cells.<sup>19</sup> Another study found that the administration of LRG-1 gene inhibitors successfully suppressed the tumor progression in mice and prolong its survival.<sup>20</sup>

Another study showed that there was an increase in LRG-1 expression in colorectal cancer and pancreatic cancer tissue compared to the adjacent normal tissue. The LRG-1 contributes to inducing migration and

invasion of cancer cells. The LRG-1 also promotes the expression of VEGF-A in cancer cells and contributes to tumor angiogenesis. A high expression of LRG-1 can be a predictor for poor prognosis and an advanced-stage tumor.<sup>19</sup>

In this study, there was no significant correlation between the level of LRG-1 in urine and the histological type of cervical cancer. We found that squamous cell carcinoma has the lowest level of LRG-1 and adenocarcinoma group has the highest level of LRG-1. Adenocarcinoma gives a worsen prognosis compared to squamous cell carcinoma.<sup>21</sup> Similar findings from different studies showed that the adenocarcinoma type had worsened disease-free survival, overall survival, and response to the radiotherapy compared to the squamous cell carcinoma.<sup>22,23</sup>

Numerous studies had shown that HPV infection upregulates epidermal growth factor receptors (EGFR). The LRG-1 can bind with the EGFR and forms a complex that activates interferon-related developmental regulator 1 (IFRD1) which suppresses the production of nuclear factor kappa B (NF- $\kappa$ B) and the production of proinflammatory factors as well as antiviral cytokines. Those proinflammatory factors and antiviral cytokines are essential against HPV infection.<sup>24</sup>

Malignant characteristics, including proliferation and abnormal cell migration, are the foundation of cervical cancer growth and progression. A study conducted by Jemmerson found that cancer cells secrete high levels of LRG-1, which promotes cell proliferation, while low levels of LRG-1 suppress cell proliferation.<sup>25</sup> Several factors should be considered during the sampling process, such as the timing, procedure, and stability of the urine sample in the delivery process. The involvement of those factors could interfere with the results. Since there have been no studies that correlate LRG-1 levels in urine with cervical cancer histological type, this study can serve as the

basis to determine the LRG-1 cut-off value for making clinical decisions and developing potential treatments.

It was found in this study that LRG-1 levels in urine were not correlated with cervical cancer histopathological grading. Nevertheless, LRG-1 levels in urine were higher in poorly differentiated cells than in well-differentiated cells. Previous studies found that LRG-1 induces tumor cell proliferation, migration, and invasion by redirecting TGF- $\beta$  signals to the ALK1 axis.<sup>14,19</sup> Moreover, LRG-1 directly affects tumor cells and increases their viability, proliferation, and invasiveness.<sup>15,16</sup> The histopathological grading is considered one of the prognostic factors, where poorly differentiated cancers result in worsen prognosis compared to well-differentiated cancers.<sup>22</sup>

It is hypothesized that the reasons for insignificant findings in this study may be due to some samples with histopathological grading cannot be assessed (GX) or were not written in the cervical biopsy results. The timing, procedure, and stability of the samples upon the delivery process to the laboratory are also contributing to the results. Since there have been no studies that correlate LRG-1 levels in urine with cervical cancer histopathological grading, this study can serve as the basis to determine the LRG-1 cut-off value for making clinical decisions and developing potential treatments.

## Conclusion

There was a significant correlation between the level of LRG-1 in urine and cervical cancer staging. The higher the LRG-1 level in urine, the more advanced the cervical cancer stage. However, further study is needed to identify and evaluate the LRG-1 biomarker in urine for making clinical decisions and developing potential treatments.

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