Adaptive LASSO Logistic Regression applied on gene expression of prostate Cancer

Amir Hossein Hashemian (1,4)
Maryam Ghobadi Asl (2)
Soodeh Shahsavari (3)
Mansour Rezaei (4)
Hadi Raeisi Shahraki (5)

(1) Research Center for Environmental Determinants of Health (RCEDH), Kermanshah University of Medical Sciences, Kermanshah, Iran.
(2) Department of Biostatistics and Epidemiology, School of Public Health, Kermanshah University of Medical Sciences, Kermanshah, Iran.
(3) Department of Biostatistics and Epidemiology, School paramedics, Kermanshah University of Medical Sciences, Kermanshah, Iran.
(4) Department of Biostatistics and Epidemiology, School of Public Health, Kermanshah University of Medical Sciences, Kermanshah, Iran.
(5)Department of Biostatistics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:
Maryam Ghobadi Asl
Department of Biostatistics and Epidemiology, School of Public Health,
Kermanshah University of Medical Sciences,
Kermanshah, Iran
Email: ghobadiasl92@gmail.com

Abstract

Introduction: The high number of prostate cancer patients has signified the importance of identifying its risk factors. The aim of the present study was to employ stratification method and penalized logistic regression with the adaptive LASSO for selecting appropriate and important genes in prostate cancer.

Materials and Methods: Microarray data used in this study include a prostate cancer gene expression dataset with [HG_U95B] Affymetrix Human Genome U95B Array platform which consisted of 12,620 genes and 167 subjects, among whom 76 subjects were unaffected and the rest were affected with cancer. Using adaptive Lasso regression, important genes in prostate cancer were stratified and the results were analyzed using ROC analysis and gene ontology annotation. To modify and conduct primary measures on the dataset, SPSS software version 22 was used. To fit the models and draw the diagrams, R software version 3.3.1 and penalty specific packages were used.

Findings: According to this research, the obtained adaptive Lasso regression accuracy and confidence interval (CI) were 0.99 and 0.97-0.99, respectively. Considering criteria such as area under the curve and gene ontology annotation, it can be argued that adaptive Lasso regression was fairly effective in stratification and selection of appropriate genes in prostate cancer.

Conclusion: Based on the results of this study, it can be said that in gene expression data, where there are both linear and large scale data, techniques such as adaptive Lasso can be useful in diagnosis of effective genes.

Key words: Regression with adaptive LASSO, Prostate cancer, Gene expression, Stratification, Gene Ontology annotation.

Introduction

Prostate cancer is the most common and most dangerous cancer among men, the fifth most common cancer worldwide, and the second most common cancer among men(1). In 2012, 1.1 million men were diagnosed with prostate cancer and 307,000 of them died(2). This cancer is the most common cancer among men in 84 countries; it mostly affects developed countries and is rising in developing countries. Recent incidence rates of prostate cancer in developed countries and developing countries are 19% and 5%, respectively(3). Autopsy studies indicated that about one-third of over 50 men have microscopic evidence of prostate cancer; however, most of these cancers are so slow growing that they never risk the lives of the affected individuals. Thus, most of men die of prostate cancer rather than other types of cancer(4).

Although prostate cancer may occur at any age, 80% of men diagnosed with this type of cancer are ≥65 years of age and it rarely occurs in men under 50 years. Only 2% of prostate cancer patients are <50 years of age. The average age of diagnosis of prostate cancer is 68 years and 63% of cases are diagnosed after 65. About 1 out of 9 men will be diagnosed with prostate cancer in his lifetime. In recent years, annual rate of prostate cancer has increased by 4% in the United States. Prostate cancer is responsible for 11% of deaths from cancer among American men(5, 6).
Doctors rarely know why a man develops prostate cancer and another does not, but statistical studies show a set of causes for incidence of prostate cancer in men, the most important of which are age, genetics, inflammation, infection, genetic predisposition, dietary factors,

sexually transmitted diseases, lack of vitamin D, vasectomy, smoking, fat diet, obesity, some medicines- e.g. daily use of medications such as anti-inflammatory drugs, prostate specific changes, and genome-specific changes(6, 7).

Unfortunately, most prostate cancers are asymptomatic and have no significant symptoms or problems for months or even years. In general, to identify warning symptoms for prostate cancer, we should be familiar with blood in urine, burning while urinating, slow urinary stream, urinary incontinence, urinary retention, abdominal pain, general symptoms of cancer, acute erectile dysfunction, and intense pain during intercourse. These symptoms may also be seen in benign enlargement of the prostate. The main difference between these two is that in benign enlargement of prostate, symptoms appear progressively, but prostate cancer may start quite suddenly(7).

High number of prostate cancer patients has signified the importance of identifying its risk factors. With the onset of microarray technology in 1995, measures were taken to stratify cancers based on genotypic characteristics(8). Several studies have shown that only a small subset of genes have significant association with the investigated diseases and according to cancer stratification, many genes are proved to be non-related. These genes may represent 'noise' to data and reduce stratification accuracy. In addition, these genes may cause more fitting to model and leave adverse effects on stratifications and due to the presence of the importance of such issues, it was necessary to introduce methods for effective gene selection given the situation and improve the accuracy of prediction. Several statistical methods have been introduced for cancer stratification among which logistic regression is regarded as a powerful method for gene differentiation; however, this method is neither appropriate nor applicable for stratification of large scale data and thus repetitive methods such as Newton-Raphson cannot be applied. Recently, penalized methods have been used for stratification of large-scale cancer data. In order to estimate coefficients of genes and select appropriate genes, Penalized Logistic Regression has been successful in stratification of large-scale cancer data(9). Therefore, the current research was conducted to employ effective stratification method and Adaptive LASSO Logistic Prognosis for selecting appropriate and important genes in prostate cancer.

Material and Methods

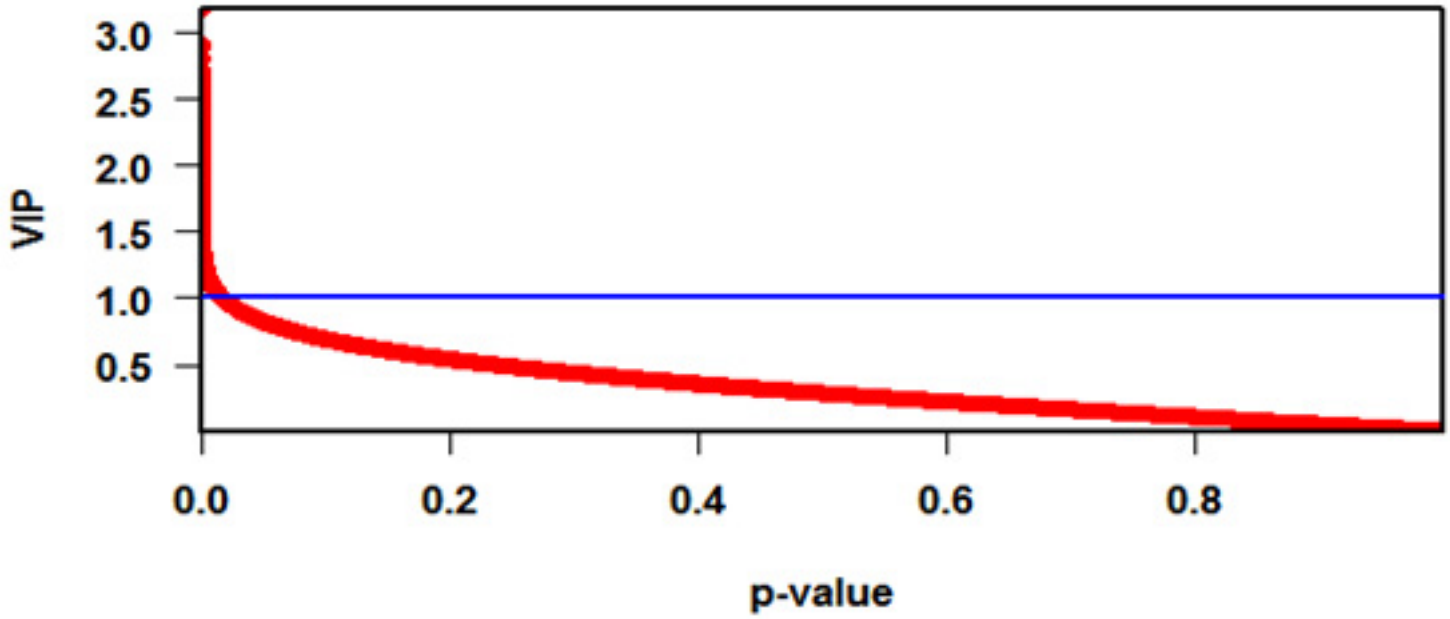
This is a cross-sectional study aimed at measuring the application of logistic regression using Adaptive LASSO in studying the association between different genes and prostate cancer. Required information were downloaded from ncbi.nlm.nih.gov website, GEO gene expression data sets. Here, a prostate cancer gene expression dataset with [HG_U95B] Affymetrix Human Genome U95B Array platform was used. A total of 167 subjects participated among whom 76 subjects were unaffected and the rest were affected. Also, 12,620 genes were analyzed. Thus, the prostate cancer gene expression dataset dimension was 12,620 x 167.

Since data obtained from the matrix of microarray technology for prostate cancer data are raw data and require pre-processing to be used, various statistical methods, called normalization, were performed to remove or minimize some of the unwanted changes brought about by biases and laboratory or technical inconsistencies. Then, they were imputed for missing values and filtering methods were used to remove large-scale data effects. Quantiles were used in this method; values less than the first quarter and larger than the third quarter were excluded. Using filtering method, the number of examined genes was reduced to 3,145. To select the most important and significant features used in stratification, the next step was to select important variables which were allowed to participate in stratification. In order to be able to identify significant genes, data scale should be reduced. PLS method was used for reducing data dimensions. PLS model was conducted because of having large scale and correlated data. PLS-DA regression was used for gene expression. Adaptive LASSO method was employed for stratification of important genes in prostate cancer. To modify and conduct primary measures on the dataset, SPSS software version 22 was used. To fit the models and draw the diagrams R software version 3.3.1 and penalty specific packages were used. Finally, the results were analyzed using ROC analysis and gene ontology annotation.

Findings

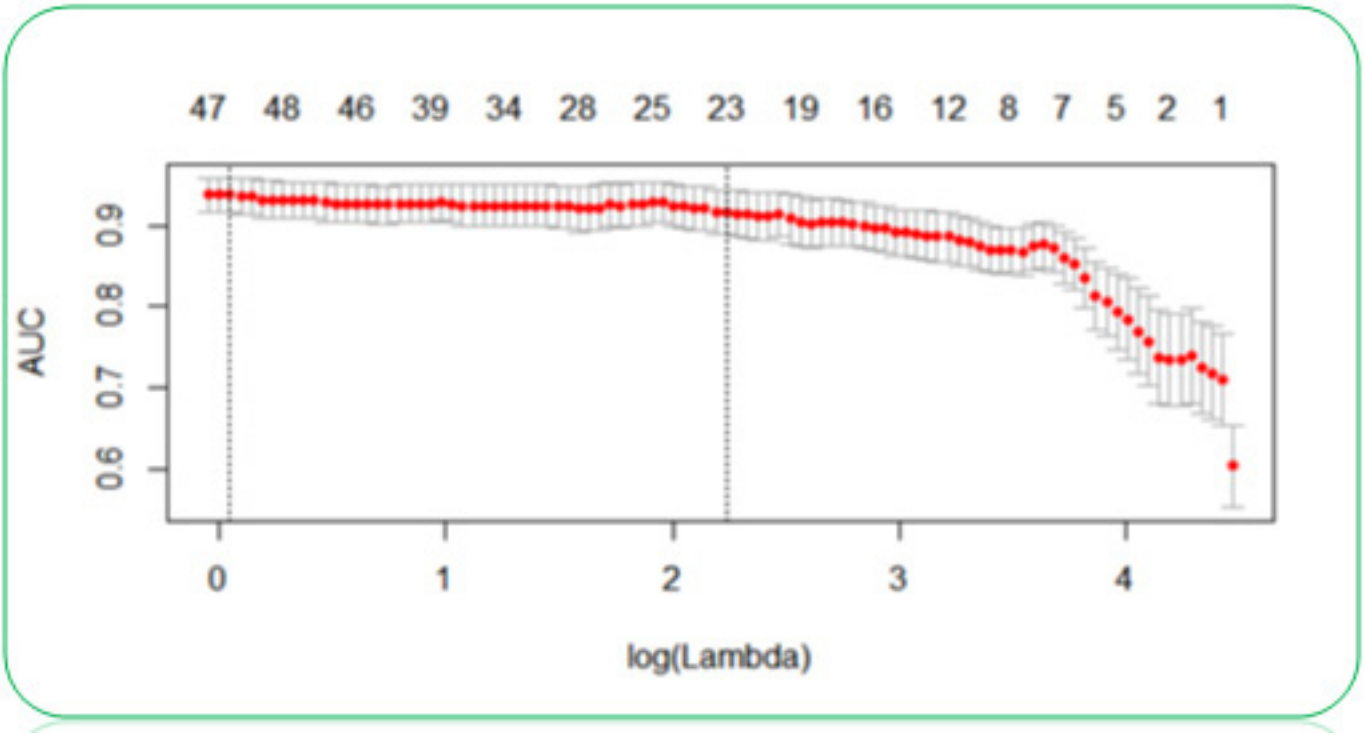
PLS-DA model fitting for selecting important variables in stratification analysis
After normalization, the goal was to select important variables in stratification. Thus, according to PLS-DA model, variables with p-value <0.01 were included (n=1700) (p-value calculation for Variable Importance in the Projection index). Figure 1 shows variables' significance based on their P-values.

Figure 1: The importance of studied genes using PLS-DA model fitting



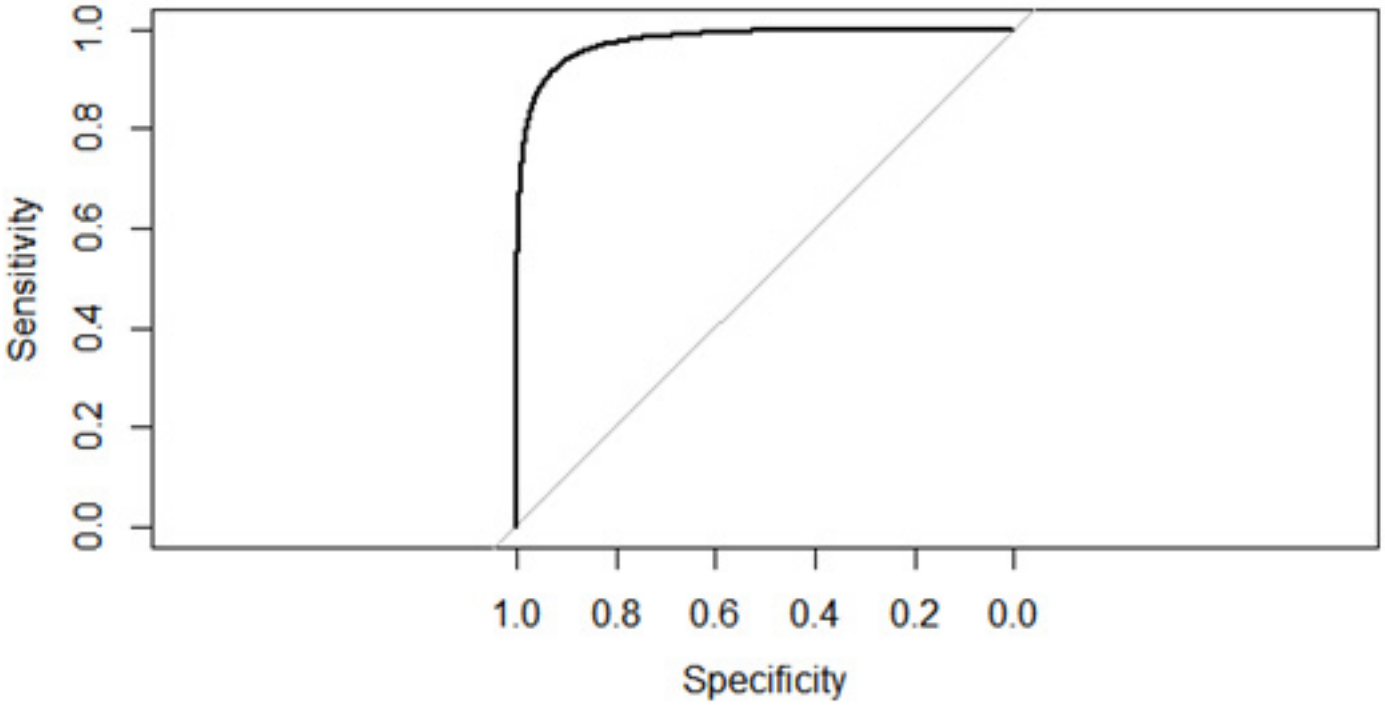
The Application of Lasso Adaptive Regression Model for the studied data Regularization Parameter Estimation
To find the best regularization parameter, 47 different modes are regulated for 47 Lasso adaptive regressions with estimated regularization parameter so as to find the best for the best value (Figure 2).

Figure 2: Regularization parameter estimation in 47 different modes



Then, adaptive Lasso regression model with estimated regularization parameter was carried out on the study dataset based on which the variables were stratified. Accuracy of area under the ROC curve was 0.99 (95% CI:0.97-0.99) (Figure 3).

Figure 3: ROC curve obtained from stratification based on adaptive Lasso regression model with estimated regularization parameter



Biovalidation of data from stratification of prostate cancer gene expression data

In order to properly measure predicted models for stratification of prostrate cancer gene expression data using Gene Ontology (GO), it was determined that, based on a specific GO interpretation in different models, whether the set of genes obtained from stratification are significant or not. The most significant GO terms used to describe the identified genes at P-value<0.05 are shown. Stratification rate of the studied models were between 76 to 89% (Table 1).

Table 1: GO analysis of prostate cancer gene expression data

Cellular function	No. of GO terms	Significant term (%)
Biological process	7116	89.3
Molecular function		86.5
Cellular component		76.2

Discussion

Medical studies in recent years, and especially after the publication of information about sequencing the human genome project, have experienced a new field that opens promising ways for researchers to identify and treat incurable diseases, such as cancer. Yet, unlike medical data collected in earlier studies where researchers have been dealing with a small number of variables, the new field of cellular and molecular Medicine is faced with a very high volume of information which is being produced by laboratory-based methods and techniques.

One of the most efficient methods to extract genetic information is microarray method which has given researchers very high potential for extracting huge volumes of information instantaneously and according to arbitrary conditions. It is one of the most controversial scientific topics in the field of computational methods, management methods, and analysis of this type of data. Given the researchers' goal of using microarray techniques in data mining, which is understanding the function of genes in cell activities, computational methods related to this area, known as stratification methods, are also more important and one of the most important questions which have been raised on the use of these methods was to identify the method or methods with proper function of gene stratification. Adaptive Lasso regression was evaluated in different conditions.

The best value estimated for regularization parameter in 47 different modes was 3.79, with 99% accuracy and CI between 97%-99% for area under the ROC curve which was a very suitable model with more acceptable performance than other values estimated for regularization parameter. Tibshirani investigated the effect of 8 variables on prostate specific antigen using data from the prostate cancer in analysis of Lasso model performance and its comparison with least squares method and the best subset. Results showed equality of the best subset and Lasso and both

introduced 3 variables as influential ones(10). He also used penalized model, for the first time in 1997, for survival analysis and variable selection in Cox model. Using Lasso method, he studied the effect of 6 variables on survival time of patients with lung cancer(11).

Li and Fan (2001) introduced 3 features for a good penalty model, i.e. unbiasedness, sparsity and continuity. As Lasso model may lead to over-estimation with big penalty coefficient, it is not unbiased in all conditions. Techniques, such as ridge regression, lack sparsity features, as well, because it has not the potential of excluding insignificant variables from the model. Also, criteria such as Akaike criterion (for variable selection) or the best subset method lack the third feature because of instability in variable selection. Yet, given these 3 features, Fan proposed a penalty function called Smoothly Clipped Absolute Deviation (SCAD). The major difference between SCAD and Lasso is that the former considers fixed penalties for coefficients bigger than aλ. Considering 7.3 as an optimum value for "a" resulted in SCAD method notation solely with λ subscript(12).

Efron et al. (2004) introduced least angle regression. Inspired by forward selection, in each step of this method, only one variable can be entered. The advantage of Least Angle Regression (LARS) is that, with a simple modification and no need for complex mathematical algorithms, all estimates may be calculated using Lasso(13).

Zou and Hastie (2005) used elastic net and blood cancer data to identify genetic factors affecting cancer and predict its types (I or II) by these genes. They used blood cancer data of 7,129 genes selected by t1000 gene statistics which had the highest significance level. Sample sizes of training data and test were 38 and 34, respectively(14).
Huang et al. (2008) compared Lasso and iterative Lasso (adaptive Lasso with reverse weight of Lasso coefficients) using breast cancer data. At first, they selected 500 genes with the largest absolute values of correlation coefficients and obtained missed values using median values. Then, Leave-one-out cross validation (LOOCV) method was used to calculate prediction error. The obtained results suggested more sparsity of the model proposed by iterative Lasso so that it selected only 22 genes while Lasso selected 42 genes with the same function(15).

Raeisi Shahraki et al. (2016) used Lasso and adaptive Lasso to identify genes affecting bladder cancer. In this study, with sample size of 48, expression of 22 different genes in peripheral blood of people with bladder cancer was compared with control group using logistic regression, Lasso, and adaptive Lasso so as to identify genes which can increase or decrease the risk of bladder cancer. The first notable point was that Lasso and adaptive Lasso methods could be fitted to data, despite high correlation between some variables, by considering all 22 variables; however, logistic regression was not able to converge even by taking one third of variables into account. By controlling the multicollinearity, adaptive Lasso method was well fitted to data and estimated coefficients with a very high accuracy and low error. The model proposed by this method was a reliable model with many other optimal features such as reliability, compatibility, predictability, sparsity, and ideal flexibility(16).

Due to massive amounts of gene expression data, which is an important characteristic of this study, results obtained from various studies showed that all the previous research has been conducted on a limited number of genes. Since 1996, introduction of microarray technology in simultaneous expression of thousands of genes revolutionized analysis of genes so as not to limit gene analysis to few number of genes. Considering that microarray knowledge is used in this study, various models are predicted and compared, final results are evaluated using ROC analysis and validation of gene ontology, and the most appropriate stratification method is carried out to identify significant genes, it is not so much similar to previous studies.

Conclusion

Based on the results of this study, in gene expression data, where there are both linear and large scale data, techniques such as adaptive Lasso provide higher performance in stratification aid diagnosis of effective genes.

Acknowledgment:
This research is derived from a thesis approved by deputy of research at Kermanshah University of Medical Science numbered 95072.

References

1. Grubb 3rd R, Kibel A. Prostate cancer: screening, diagnosis and management in 2007. Missouri medicine. 2006;104(5):408-13; quiz 13-4.
2. Organization WH. Global status report on noncommunicable diseases 2014: World Health Organization; 2014.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA: a cancer journal for clinicians. 2005;55(2):74-108.
4. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet□Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015;65(2):87-108.
5. Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nature genetics. 2000;24(3):227-35.
6. Ruijter E, van de Kaa C, Miller G, Ruiter D, Debruyne F, Schalken J. Molecular genetics and epidemiology of prostate carcinoma. Endocrine reviews. 1999;20(1):22-45.
7. Carter H. partin AW. Diagnosis and staging of prostate cancer. Campbell-Walsh Urology 8th ed Sydney: Elsevier Health Sciences. 2002.
8. Meyer C, Davis S. It's alive: The coming convergence of information, biology, and business: Crown Business; 2003.

9. Carter HB, Partin AW. Diagnosis and staging of prostate cancer. Campbell's urology. 2002;3:2519-37.
10. Tibshirani R. Regression shrinkage and selection via the lasso. Journal of the Royal Statistical Society Series B (Methodological). 1996:267-88.
11. Tibshirani R. The lasso method for variable selection in the Cox model. Statistics in medicine. 1997;16(4):385-95.
12. Fan J, Li R. Variable selection via nonconcave penalized likelihood and its oracle properties. Journal of the American statistical Association. 2001;96(456):1348-60.
13. Efron B, Hastie T, Johnstone I, Tibshirani R. Least angle regression. The Annals of statistics. 2004;32(2):407-99.
14. Zou H, Hastie T. Regularization and variable selection via the elastic net. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 2005;67(2):301-20.
15. Huang J, Ma S, Zhang C-H. The iterated lasso for high-dimensional logistic regression. The University of Iowa, Department of Statistics and Actuarial Sciences. 2008.
16. Shahraki HR, Jaberipoor M, Zare N, Hosseini A. The Role of 22 Genes Expression in Bladder Cancer by Adaptive LASSO. 2016.