ORIGINAL ARTICLE

UDC: 616.5-006.81-07
DOI: 10.2298/VSP150227042S

Validity of ultrasound-guided aspiration needle biopsy in the diagnosis of micrometastases in sentinel lymph nodes in patients with cutaneous melanoma

Validnost ultrazvučno vođene aspiracije tankom iglom u dijagnostici mikrometastaza u limfnim čvorovima stražarima kod obolelih od melanoma kože

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Abstract

Background/Aim. Cutaneous melanoma is one of the most aggressive solid cancers, that develops local, regional and distant metastases. The presence of metastases in lymph nodes is in correlation with Breslow tumor thickness. According to various researches, in melanoma with more than 4 mm Breslow thickness, lymph node micrometastases can be found in 60–70% of cases. Sentinel lymph nodes biopsy is a diagnostic procedure for lymph node micrometastasis detection, which is necessary for disease staging. In recent studies, ultrasound-guided fine needle aspiration with cytology (US FNAC) of the sentinel lymph node was used as less invasive procedure, but is not accepted as the standard procedure. The goal of this work was to define sensitivity, specification and precision of the ultrasound-guided fine needle aspiration method in comparison with standard sentinel lymph node biopsy. **Methods.** After obtaining the Ethics Committee's permission, from 2012 to 2014 a total of 60 patients with cutaneous melanoma were enrolled, and divided into three groups: group I with thin melanoma, group II with intermediate thickness melanoma and group III with thick melanoma. The presence of micrometastases in sentinel regional lymph nodes was analyzed by US FNAC. The results obtained were compared to sentinel lymph nodes biopsy (SLNB) results. The golden standard for calculating the specific, sensitive and precise characteristics of the method of US FNAC of sentinel lymph nodes was histopathologic lymph node examination of sentinel lymph nodes acquired through biopsy. **Results.** Detection rate of US FNAC was 0% in the group I, 5% in the group II and 30% in the group III. SLNB detection rates were: 10% in the group I, 15% in the group II, and 45% in the group III. In melanoma thicker than 4 mm, 15% of the patients were false negative by US FNAC. The sensitivity of US FNAC for all the patients was 50%: in the group I, 0%; in the group II, 33.3%; and in the group III, 66.6%. The method specificity for all examined patients was 100% and accuracy 88%: group I, 90%; group II, 90%; group III, 85%. The FNAC and SLNB micrometastasis detection rate was significantly higher in melanoma with Breslow thickness > 4 mm (group 3) in comparison to thin and intermediate thickness tumors. **Conclusion.** The method of ultrasound-guided fine needle aspiration of sentinel lymph nodes, according to its sensitivity, has a place in the diagnostics of micrometastasis in regional lymph nodes only in thick melanoma, but not in thin and intermediary thickness melanoma. The results must be confirmed in a larger number of patients. If this observation could be confirmed, it would rationalize treatment of patients with thick melanoma, decrease the number of operations and shorten the time to make the diagnosis.

Key words:
endoscopic ultrasound-guided fine needle aspiration; neoplasm, micrometastasis; sentinel lymph node biopsy; skin; melanoma; diagnosis; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Kožni melanom je jedan od najagresivnijih solidnih malignih tumora, koji se širi lokalno, regionalno i udaljeno metastazira. Prisustvo metastaza u limfnim čvorovima u vezi je sa debljinom tumora prema Breslow-u. Prema različitim istraživanjima, kod melanoma debljih od 4 mm prema Breslow-u, mikrometastaze u limfnim čvorovima mogu se naći kod 60–70% slučajeva. Biopsija

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limfnog čvora stražara je dijagnostička procedura za otkrivanje mikrometastaza u limfnim čvorovima, što je neophodno za određivanje stadijuma bolesti. U poslednjim istraživanjima, ultrazvučno vođena aspiracija tankom iglom (UZ ATI) sa citološkim nalazom limfnog čvora stražara korišćena je kao manje invazivna procedura, ali nije prihvaćena kao standardna peocedura. Cilj ovog rada bio je da se definiše senzitivnost, specifičnost i tačnost metode ultrazvučnog vođenja aspiracije tankom iglom u odnosu na standardnu biopsiju limfnog čvora stražara. **Metode.** Nakon dobijanja dozvole Etičkog komiteta u periodu od 2012. do 2014. godine, 60 bolesnika sa kožnim melanomom grupisano je u tri grupe: grupa I sa tankim melanomom, grupa II sa intermedijarnom debljinom melanoma i grupa III sa debelim melanomom. Prisustvo mikrometastaza u regionalnim limfnim čvorovima stražarima analizirano je UZ ATI sa citološkom analizom. Dobijeni rezultati su poređeni sa rezultatima biopsija limfnog čvora stražara. (BLCS) Zlatni standard za izračunavanje specifičnosti, senzitivnosti i tačnosti metoda UZ ATI limfnog čvora stražara je bio patohistološki nalaz limfnih čvorova dobijenih biopsijom limfnog čvora stražara. **Rezultati.** Dobijeni rezultati UZ ATI bili su 0% u grupi I, 5% u gupi II i 30% u grupi III. Dobijeni rezultati BLČS bili su: 10% u grupi I, 15% u grupi II i 45% u grupi III. Kod melanoma debljih od 4 mm, 15% bolesnika imalo je lažno negativni rezultat UZ ATI. Senzitivnost UZ ATI za sve bolesnike bila je 50%: u grupi I 0%; grupi II 33,3%; u grupi III 66,6%. Specifičnost metode za sve bolesnike bila je 100%, a tačnost 88%: grupa I 90%, grupa II 90%; grupa III 85%. Otkrivanje mikrometastaza UZ ATI i BLČS bilo je značajno veće kod melanoma debljine veće od 4 mm prema Breslow-u (grupa III) u poređenju sa tankim i intermedijarnim tumorima. **Zaključak.** Metod UZ ATI limfnih čvorova stražara prema senzitivnosti ima mesto u dijagnostici mikrometastaza u regionalnim limfnim čvorovima samo kod debelih melanoma, ali ne i kod tankih i intermedijarnih melanoma. Rezultati moraju biti potvrđeni na većem broju bolesnika. Ako se ovo posmatranje potvrdi, može se racionalizovati lečenje bolesnika sa debelim melanomom, smanjujući im broj operacija i skraćujući im vreme dijagnostike.

Ključne reči:
endoskopska, ultrazvukom-vođena, aspiracija tankom iglom; neoplazma, mikrometastaza; limfni čvorovi, stražarski, biopsija; koža; melanom; dijagnoza; senzitivnost i specifičnost.

Introduction

Melanoma, malignant tumor of melanocytes, is one of the most aggressive solid malignant tumors. It develops local, regional and distant metastases. The presence of metastases in lymph nodes is in correlation with Breslow tumor thickness. According to various researches, in melanoma with more than 4 mm Breslow thickness, regional lymph node micrometastases can be found in in 60–70% of cases [1].

Regional lymph node metastases in melanoma are diagnosed by ultrasound examination, biopsy of sentinel lymph nodes in order to detect micrometastases and fine needle aspiration for macrometastases. Ultrasound examination of regional lymph nodes gives data on the presence of macrometastases. The precision of this exam can be enhanced by using color Doppler sonography and power mode [2]. By analysis of ultrasound characteristics of the lymph node, the lymph node length to width ratio, echogenic characteristics of lymph node, arborisation, lymph node vascularization type and resistance index, the sensitivity of the exam of 100% and the precision of 93.3% are obtained [3]. This exam gives secure ultrasound detection of macrometastasis in lymph nodes of 2 mm or bigger. It was also used in recent studies to analyze sentinel lymph node after lymphoscintigrpahy, but with various results [4–7].

Sentinel lymph nodes biopsy (SLNB) is a diagnostic procedure for detection of micrometastases necessary for disease staging. The method of intraoperative marking of lymph nodes and biopsy of sentinel lymph nodes, which were developed by Morton et al. in 1991, represents a more rational and effective way of diagnosing of patients with clinically negative regional lymph nodes [8, 9]. Using this procedure the extent of metastases in regional lymph nodes can be estimated with a high degree of reliability, through biopsy of one, sometimes two or three sentinel lymph nodes [10, 11]. The methodology is based on the theory that the first node among the regional lymph nodes is also the place of the first metastasis. The sentinel lymph node status is predictive of further non-sentinel lymph node metastases. If a sentinel lymph node is negative, it can be expected with 99% accuracy that other regional lymph nodes are also without metastases. The possibility of the presence of skipping metastases (when the nearest lymph node region is skipped and metastases are found in the next one) is 2%. If sentinel lymph node biopsy is positive, the patient is diagnosed as stage III disease according to the American Joint Committy of Cancer (AJCC) classification [12]. In patients with positive sentinel lymph node, complete regional lymph node dissection is indicated [13].

Sentinel lymph node is marked with a radioactive marker [technetium (Tc 99) nano colloid], which is applied around the primary tumor excision scar 24 hours before the procedure. After the placement of the radio marker, gamma camera is used for determination of the lymph node region. During this procedure, the patient is positioned in the same way as he will be positioned during the actual operation: for axilla, arms above head; for inguinal region – shin in flexion, thigh in abduction; for retroauricular salivary gland and neck – head turned to the opposite side from the place of marked sentinel lymph node.

In addition to radioactive tracer, lymph nodes can be marked with 1% methylene blue which is injected 1–2 hours before the procedure (1–2 mL). According to various researches, this double marking of the sentinel lymph node raises the accuracy of the method to 93–99.7% depending on the center where the procedure is done [14, 15]. Possible complications after the sentinel lymph node biopsy are: edema, hematoma, infection, wound dechiscention; lymphocela, and also all complications due to general anesthesia [16].

The significance of this method is both diagnostic and prognostic. This method prolongs disease free survival and in intermediate thickness melanoma, overall survival, too [17–26]. Success of this method depends on a good collaboration of nuclear medicine specialist, surgeon and pathologist.

Ultrasound-guided fine needle aspiration (US FNA) is used as a standard procedure for histopathological confirmation of melanoma macrometastases. This method was also used for evaluation of sentinel lymph node, with various success [27]. The procedure, monitored with ultrasound, is done by moving the needle through the lymphatic node and, simultaneously, aspirating the specimen [27]. According to some authors, in 65% of cases, micrometastases can be found using this method, and hence this matheod could replace the standard SLNB [28–31]. Other authors think that this procedure is not reliable because it is impossible to visualize by ultrasonography sentinel lymph nodes with a diameter less than 4.5 mm and perform the fine needle aspiration. Also, it is possible that the distance between the cortex and medulla of the sentinel lymph node is too small making the ultrasound-guided fine needle biopsy hard to perform [32].

If this method can be proven to be of similar specificity and sensitivity as SLNB, it would be possible to reduce the number of operations and shorten the time for disease staging. The method of sentinel lymph node ultrasound-guided fine needle biopsy would be less aggressive and much cheaper than sentinel lymph node biopsy. Thus, the aim of our work was to determine sensitivity, specificity and accuracy of ultrasound-guided fine needle biopsy method in comparison to standard SLNB.

Methods

After obtaining the Ethics Commity's permission, this prospective study, performed from 2012 to 2014, enrolled 60 patients with histopathologically confirmed cutaneous melanoma with indication for SLNB. They were divided into three groups according to Breslow thickness: group I, 20 patients with melanoma of less than 1 mm Breslow thickness, and the presence of mitoses and/or ulcerations and/or Clark IV and/or regression more than 25%; group II, 20 patients with intermediate thickness melanoma, 1–4 mm; group III, 20 patients with Breslow thickness of more than 4 mm. Analysis included patients with only one positive sentinel lymph node and in only one lymphatic region. Initial staging consisted of palpation, regional lymph node ultrasound, abdominal and pelvic ultrasound chest radiography and laboratory analyses. Sentinel lymph node was determined in all the patients by the method of lymphoscintigraphy using gamma camera, Adac vertex, after subdermal placing of Technetium 99 marked with nanocolloid. Two hours prior to the operation, 1% methylene blue was also injected intradermaly around the excision biopsy scar.

Sentinel lymph nodes were identified by ultrasound examination of the patients from all the three groups, using the ultrasound device type TOSHIBA APLIO X6SA-790A with multi-frequent cannula, frequency rate 6-12 MHz. All lymph nodes were examined in B-mode real time, by pulse color Doppler, in power mode. Then, ultrasound-guided fine needle aspiration of sentinel lymph nodes was done with 27G (0.4 × 20 mm) and 25G (0.5 × 25 mm) needles, dependent of the distance between sentinel lymph node and the surface of the skin (Figure 1). The appearance of hematoma and punctuation in extracted sentinel lymph node was used as evidence of good aspiration (Figure 2). The specimens were stained with May-Grünwald-Giemsa and analysed. After fine needle aspiration, sentinel lymph node biopsy was performed in all the patients. Intraoperative sentinel lymph nodes detection was done by Gamma camera type Europrobe. Histopathological analysis of lymph nodes was done with hematoxylin and eosin staining (HE) and S-100 and HMB 45 imunohistochemistry as per protocol.

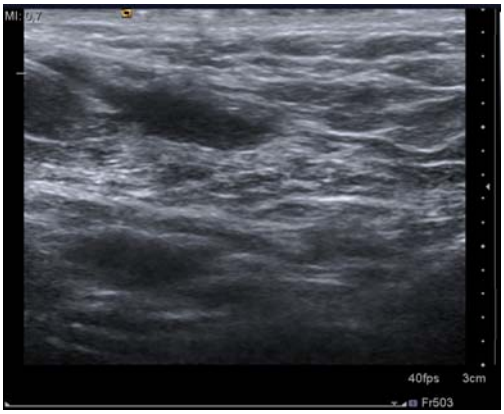

Fig. 1 – Ultrasound image of the needle in sentinel lymph node (LN).

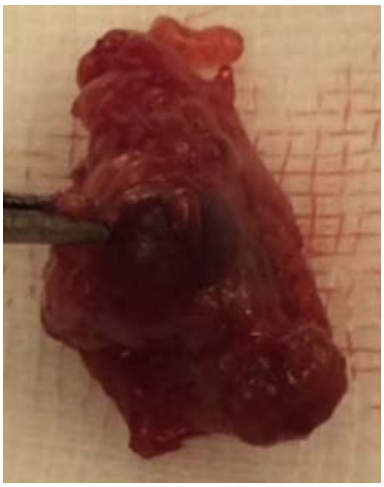

Fig. 2 – Needle mark and hematoma in a removed sentinel lymph node (LN).

Sensitivity was calculated as a quotient between really positive findings and the sum of really positive findings and false negative findings. Specificity was calculated as a quotient between really negative findings and the sum of really negative findings and false positive findings. Accuracy or prediction of positive outcome was calculated as a quotient between total number of positive findings and total number of negative findings and the total number of patients.

Descriptive statistic parameters (mean, standard deviation, spread, median and frequency of appearance of some parameters), and variance of analysis were used in the first direction and finished with Tukey test or non-parameter Kruskal-Wallis test for independent parameters which was finished with Mann-Whitney *U*-test. The presence of statistic significance between frequencies distribution in the particular gro-

up was checked by χ^2 test. Minimal statistic significance was set at the standard level of $p < 0.05$. For statistical analysis commercial statistic software SPSS, version 18 (USA) was used.

Results

Demographical data of all the patients are presented in Table 1. In all the three groups, the average age was almost the same, in the group I 50.25 years; in the group II, 55.45 years and in the group III 52.15 years. There were more male than female patients – in the group I, 11 males; in the group II, 15 males while in the group III the number of male and female patients was equal, 10.

Table 2 shows the distribution of detection rates of cytological analysis made by sentinel lymph node US FNAC method and histopathological analysis made by the sentinel lymph node biopsy method in relation to the tumor thickness.

In the group with melanoma thicker than 4 mm there were 30% of patients with the diagnosis of micrometastasis in SLN by cytological analysis after US FNAC, which was significantly more frequent than in thin and intermediary melanoma ($p < 0.05$). In the group with melanoma thicker than 4 mm there were 45% of patients with the presence of micrometastases in SLN histopathological analysis after SLNB, also significantly more frequent than in thin and intermediate thickness tumors ($p < 0.05$). So, 15% of the patients were falsely negative in US FNAC method.

Table 3 shows that there is a statistically significant difference in sensitivity between SLNB and US FNAC in all the patients and all the patient groups, in favor of SLNB. There was no statistically significant difference in specificity between these two methods. There is a statistically significant difference of $p = 0.017$ in the accuracy in all the patients, while this difference is absent in the patient groups, probably due to the small number of the patients.

Table 4 shows comparative analysis of surfaces of sen-

Table 1

Demographic data of the patients enrolled in the study

Melanoma thickness (mm)	Sex, n (%)			Age (years)		
	male	female	total	x̄	SD	median
< 1	11 (30.6)	9 (37.5)	20 (33.3)	50.05	14.96	52.00
1–4	15 (41.7)	5 (20.8)	20 (33.3)	55.45	15.85	57.00
> 4	10 (27.8)	10 (41.7)	20 (33.3)	52.15	15.64	51.00
Total	36 (100.0)	24 (100.0)	60 (100.0)			
Comparison	Distribution comparison $\chi^2 = 2.91$; $p = 0.23$			Group comparison (ANOVA) F = 0.61; $p = 0.54$		

SD – standard deviation.

Table 2

Detection rates of ultrasound-guided fine needle aspitration cytological analysis (US FNAC) and sentinel limph node biopsy (SLNB) histopathological analysis

Melanoma thickness (mm)	Cytology, n (%)			Histopathology, n (%)		
	negative	positive	total	negative	positive	total
<1	20 (0.0)	0 (0.0)	20 (33.3)	18 (90)	2 (10)	20 (33.3)
1–4	19 (95.0)	1 (5.0)	20 (33.3)	17 (85)	3 (15)	20 (33.3)
> 4	14 (70.0)	6 (30.0)	20 (33.3)	11 (55)	9 (45)	20 (33.3)
Total	53 (88.3)	7 (11.6)	60 (100.0)	46 (76.6)	14 (23.3)	60 (100.0)
Distribution comparison	$\chi^2 = 10.02$; $p = 0.007$			$\chi^2 = 8.01$; $p = 0.018$		

Table 3

Sensistivity, specificity and accuracy of ultrasound-guided fine needle aspitration cytology (US FNAC) in comparison to sentinel limph node biopsy (SLNB) histopatology

Parameters	Procedure	Patient groups regarding melanoma thickness			
		All (n = 60)	< 1 mm (n = 20)	1–4 mm (n = 20)	> 4 mm (n = 20)
Sensitivity	SLNB	1.0	1.0	1.0	1.0
	US FNAC	0.50	-	0.33	0.66
Comparison (*t*-test proportion)		z = 6.11 $p < 0.001$	-	z = 4.15 $p < 0.001$	z = 2.44 $p = 0.015$
Specificity	SLNB	1.0	1.0	1.0	1.0
	US FNAC	1.0	1.0	1.0	1.0
Comparison (*t*-test proportion)		*ns*	*ns*	*ns*	*ns*
Accuracy	SLNB	1.0	1.0	1.0	1.0
	US FNAC	0.88	0.90	0.90	0.85
Comparison (*t*-test proportion)		z = 2.38 $p = 0,017$	z = 0.72 *ns*	z = 0.72 *ns*	z = 1.20 *ns*

Table 4

Surface of sentinel limph node (SLN) and cytological analysis

Surface of SLN (mm^2)	Melanoma thickness (mm)			Cytological analysis	
	< 1	1–4	> 4	positive	negative
mean	73.85	75.75	53.00	254.17	109.06
SD	194.39	224.64	106.95	333.97	101.62
median	109.71	77.92	93.00	179.34	78.00
	Group comparison (Kruskal-Wallis test) $\chi^2 = 8.07$; $p = 0.018$ 1:2 – $p < 0.01$; 1:3 – *ns*; 2:3 – *ns*			Mann-Whitney test z = 1.69; $p = 0.091$	

SD – standard deviation; *ns* – no significance.

tinel lymph node in patients with or without SLN micrometastases according to cytological analysis.

There was no statistical significance between means of surfaces of affected lymph nodes in the patients with positive cytological findings compared to the patients with negative cytological findings ($p = 0.091$).

Discussion

From a surgical point of view, advantage is always given to methods that are more reliable and safer for the patient, with fewer complications during the procedure, and which are simpler to perform and economically more feasible. Due to the above mentioned, one of our goals was to determine the sensitivity, specificity and accuracy of sentinel US FNAC method. The method accuracy was found to be 88%: in the group I, 90%; in the group II, 90%; and in the group III, 85%. The method specificity for all examined patients was 100% in all the groups.

In this part special emphasis should be put on the segment which is the most important in clinical environment – the sensitivity of the method, which gives us data about successfully diagnosed micrometastases in a sentinel lymph node.

We have hypothesized, based on previous studies of that the sensitivity of this method would be similar to the sensitivity of sentinel lymph node biopsy, which was 93–97.3% [15]. However, that was not shown in this study (Table 3). In our study, the method sensitivity for all the patients was 50%, in the group I, 0%; in the group II, 33.3%; and in the group III, 66.6%. The sensitivity of US FNAC was found to be of inferior sensitivity in comparison to SLNB, especially in thin and intermediate thickness melanoma, and thus it cannot be recommended [6, 33] (Table 2). The method shows some validity only in melanoma with Breslow thickness of more than 4 mm, and it could find its use within this group, but this must be confirmed in a larger number of patients. Since the specificity is similar to SLNB, if the results of preoperative FNAC are positive, and micrometastasis is found, regional lymph node dissection could be done immediately. However, if it is negative, sentinel lymph node biopsy must be performed, since 15% of US FNAC cytology was falsely negative in our patient series. Thus, micrometastases found using US FNAC could decrease the number of SLNB, which is a more precise but also a more complex method. Based on this pilot study, 30% of patients with Breslow thickness of more than 4 mm could be diagnosed with micrometastases with US FNAC, skip SLN biopsy and beswitched to regional lymph node dissection. However, these results must be confirmed in a larger number of patients. As expected, and found in previous studies, the positivity rate of both US FNAC detected and SLNB detected micrometastases was higher in tumors with Breslow thickness of > 4 mm in comparison to thin and intermediate thickness melanoma ($p < 0.01$). The possibility that the tumor burden (diameter and location of micrometastatis) within a sentinel lymph node is in correlation with detection rate of the US FNAC must be explored in future studies.

The sensitivity of this method was not dependent of the patient's sex or the surface of the sentinel lymph node (Table 4). The biggest registered affected surface of sentinel lymph nodes was in the group with intermediate thickness melanoma, which was 194.39 mm. Despite that, the number of positive cytology findings and sensitivity of this method was greatest in the group with melanoma thicker than 4 mm (Table 2). In the patients with positive cytological findings of sentinel lymph nodes, the average surface was 254.17 mm compared to 109.6 mm in the patients whose cytological findings were negative. This was not a statistically significant difference. That also showed that sentinel lymph node area did not interfere with the method sensitivity (Table 3).

Voit et al. [28], a group of German dermatologists, are the most cited authors regarding US FNAC method. According to their Berlin ultrasound morphological criteria (loss of central echo of lymph node, balloon shape of lymph node, periphery vascularization of lymph node), four groups of sentinel lymph nodes were formed in their study: benign, probably benign, probably malignant, and malignant. The sensitivity of the US FNAC method in the first two groups was 56%, while in the third and fourth groups it was 82% [34]. Our criteria for analysis of the lymph nodes in the regional lymph basins before US FNAC were stricter than Berlin ultrasound morphologic criteria. We also added the resistance index and arborization of lymph nodes. Our findings can be compared with the total sensitivity of the methods employed

by Voit et al. [35] for the total number of patients, which was 59% [34], compared to our 50%. In the second study of Voit et al. [36] the sensitivity of the US FNAC method in melanoma thicker than 4 mm was 76%. In this study, the sensitivity of US FNAC in melanoma thicker than 4 mm was 66.6%.

Conclusion

The ultrasound-guided fine needle aspiration method with the sensitivity of 66.6% in thick melanomas (Breslow > 4mm), the accuracy of 88% and the specificity of 100% has a place in the diagnosis of micrometastases in regional lymph nodes only in this patient group. However, these findings should be confirmed in a larger sample of patients. If the results from this pilot study could be reproduced in larger number of patients, it could be possible to rationalize the treatment of patients with thick melanoma, decrease the number of operations and shorten the time to diagnosis. In thin and intermediate thickness melanoma, however, this method is of no value, and should not be further explored, since it was 0% in thin melanoma and 33.3%, in intermediate thickness melanoma.

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Received on February 27, 2015.
Revised on June 21, 2015.
Accepted on June 25, 2015.
Online First March, 2016.