Comparison of Automated and Conventional IHC Visual Scoring Analysis for MHC Class I and Tapasin Expression in Cervical Carcinoma

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Background: *Cervical cancer (CXCA) is the second most common cancer among women in Thailand and worldwide. Immune evasion caused by down-regulation of host immune responsive genes, such as MHC class I and loss of antigen processing machinery (APM), presents a capability leading to cancer development. Immunohistochemical staining (IHC) is regarded as a common technique for protein marker detection in clinical laboratories. At present, IHC automation has been launched to facilitate the speed and feasibility to replace conventional IHC. However, evaluation of its use is still limited.*
Objective: *This study aimed to evaluate IHC scoring by automated visual analysis compared to conventional IHC analysis.*
Material and Method: *The paraffin-embedded tissues of 96 invasive CXCA were processed using a tissue microarray (TMA) platform followed by automated IHC staining of the anti-MHC class I (heavy chain, β2M) and an APM-Tapasin expression. Conventional IHC and automated slide scanning with scoring visual analysis were compared.*
Results: *The results showed significant association between conventional and automated IHC evaluation (p-value >0.05, Chi-square) for MHC class I and Tapasin stated in percentage of positive cancer cells, whereas intensity was found (p-value <0.05, Chi-square) with moderate agreement (p-value <0.001, kappa) 0.434-0.615 and 0.353-0.554, respectively. After calculated values, the results showed significant association between conventional and automated IHC evaluation (p-value >0.05, Chi-square) for MHC class I and Tapasin with the highest agreement level (p-value <0.001, kappa) of summation 0.595-0.755 and multiply scoring 0.633-0.689, respectively.*
Conclusion and Discussion: *The automation software for IHC scoring and interpretation can be used for the determination of MHC class I and Tapasin in CXCA. In addition, an antigen presentation pattern must be included to allow an accurate result for MHC class I in clinical use. An appropriate sample size and design of staging coverage as well as clinical prognosis outcomes of progression should be used in further investigation.*

Keywords: *Automated immunohistochemistry, Automated visual analysis, Automated scoring, Cervical cancer, MHC class I, Tapasin*

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Recent clinical scoring procedures rely on a pathologist's visual examination and are based on two characteristics: percentage of positive tumor cells staining and intensity. The evaluated scoring of positive staining involves intrinsic subjectivity because criteria are visually judged or have limitations depending on the skill training. This technique is also restricted because the scores for the two categories remain as separate functions and cannot be combined for analysis and comparison[1]. Automated immunohistochemistry (IHC) scanning and imaging visualization have more potential to overcome these limitations. The automated IHC analyses are precise in ranges of staining that appear weak to the eye[2] and produce continuous data[3]. Furthermore, they can provide pathologists with support for visual scoring, as computer-aided IHC analysis substantially improves both intra- and inter-observer agreement[4]. The automation has the ability

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of commercially available software algorithms (Genie Histology Pattern Recognition software suite including Genie Training v1 and Genie Classifier v1, and Color Deconvolution v9, Aperio Technologies, Vista, CA, USA) to replicate results obtained solely through visual inspection by pathologists or investigators, especially in the evaluation of tumor cell staining[5].

Cervical cancer (CXCA) is the second most common female malignant cancer in Thailand and the world[6-8]. Host immune response is a crucial factor during the development of CXCA. Major histocompatibility class I (MHC I) consisting of heavy chain and β_2-microglobulin (β2m) and antigen processing machinery (APM) such as Tapasin expression are genes necessary for cellular-mediated immunity functions[9,10]. In this study, tissue microarray (TMA) as platform slides for the clinical sample of patients were performed and followed by automated IHC staining with antibody directed against MHC class I and Tapasin. The automation of IHC scanning and imaging visual analysis were evaluated and compared to conventional visualization by training investigators and/or pathologists.

Material and Method

Clinical samples

The patients were selected and provided with informed consent forms EC 009/2012 for tissue sampling and analysis at the Ubon Ratchathani Cancer Hospital. All patients represented as invasive cervical carcinoma from FIGO staging I-IV resulting tumor cell types of squamous cell carcinoma (SCCA) and adenocarcinoma (ADC). The paraffin-embedded tissues from all patients were set for tissue microarray (TMA) depending on the location of the tumors and were stained with standard hematoxylin and eosin (H&E) to identify the regions of the tumor cells followed by automated immunohistochemistry (IHC) staining, slide scanning, and scoring visualization.

TMA, antibodies and automated IHC staining

The TMA slides provided duplicate 0.4 mm core samples from 96 cases of invasive cervical carcinoma. The monoclonal antibody (mAb), which recognizes a framework determinant expressed on β2m (clone ab15976; Abcam), the mAb HC recognizing HLA class I heavy chains (clone ab15976; Bio-active), and the mAb Tapasin (Bio-active clone sc-80647), were used. All procedures were performed automatically in VENTANA Bench Mark ULTRA (Roche, Germany). The automated IHC staining with the optimal dilution of primary antibodies including anti-heavy chain, β2m, and Tapasin were 1:1,000, 1:1,000 and 1:100, respectively. Tonsils were used for positive control tissue assessment.

Conventional IHC evaluation

The evaluation criteria were determined for the percentage of positive cells staining and positive cells intensity by three independent investigators. According to the enumerated criteria of positive cells staining, lesions were scored as positive when the percentage of stained positive cells in the entire lesion was >75% (grading as 3^+; strongly positive), between 25-75% (grading as 2^+; heterogeneous), and <25% (grading as 1^+; trace), no staining (grading as 0; negative), respectively. The enumerated criteria of positive cells intensity was also scored as grading 3^+ (strong intensity), 2^+ (moderate intensity), 1^+ (low intensity), and 0 (no intensity positive cells). Finally, the scoring was evaluated using a combination of staining and intensity resulting in summation and multiple scoring as modified from a previous study[11]. Interpretation of the scoring was categorized as loss of expression (including total loss and/or partial loss) and normal expression. The scorings depending on both summation and multiply were classified into three groups of scores, 0-2, 3-4, and 5-6 or 5-9, in relation to total loss of expression, partial loss of expression and normal expression, respectively.

Automated slide scanning and scoring visual analysis

Stained whole slide tissue sections were digitally imaged using the Aperio ScanScope XT (Aperio Technologies, Vista, CA) using a 20 x 0.75NA PlanApo objective for complete 200x magnification using a Basler tri-linear array camera technology (0.50 μm/pixel). Scan time at 20x magnification ranged from 2 to 6 min, depending on the size of the tissue sections. Slide details regarding patient information and pathologist scoring were blinded. Digital images were recorded within the Aperio Spectrum Database to be analyzed. Positive and negative controls, as described above, were scanned and analyzed each batch of slides. The automated visualization was performed by quantitative scoring algorithms customized for automated IHC staining, using commercially-available templates from Aperio Technologies. The Aperio product, a combination of the Genie® Histology Pattern Recognition tool, was used to select tumor regions of interest, and either the Membrane Quantification v_9 cellular analysis tool or the Nuclear Quantification

v_9.1 cellular analysis tool was used[5]. These adjustments for the nuclear stain included segmentation, nuclear curvature threshold, intensity thresholds, size, roundness, compactness, and elongation. These modifications for membrane staining included segmentation intensity thresholds, cell size, nuclear size, cell radius, roundness, compactness, and elongation.

Statistical analysis

The results from three conventional visual investigators as goal standard procedure were compared to the automated image analysis procedure. Statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Chicago, IL). A *p*-value <0.05 was considered statistically significant. The scores were compared using KAPPA analysis and Chi-square test for the association.

Results

Evaluations of conventional IHC visual analysis on MHC class I and tapasin

The evaluations of final grading of positive tumor cell staining and percentage of their intensity were determined. The IHC staining was examined for expression of MHC class I including heavy chain and β2m, and an APM-Tapasin followed by the optimal dilution of each antibody using TMA platform. An example of MHC class I expression via the IHC staining is shown in Fig. 1A-F. The quality of the tumor cell staining was determined according to percentages of positive staining and their intensity. Lymphocyte and red blood cells were used for internal positive control (IPC) and internal negative control (INC) respectively as shown in Fig. 1B. Different levels of invasive CXCA using conventional IHC visual analysis consisting of grading 3^+, 2^+ and 1^+ or 0 representing for normal, partial loss and total loss of expression, respectively, are shown in Fig. 1C-F. Localization of MHC class I expression patterns was presented for membranous and cytoplasmic patterns as shown in Fig. 1C and Fig. 1D, respectively.

Evaluations of automated IHC visual analysis of MHC class I and tapasin

The evaluations of IHC automated visual procedure were analyzed. The results after staining with the specific antibodies for MHC class I including heavy chain and β2m, and an APM-Tapasin protein expression were determined. The visualization showed deter-

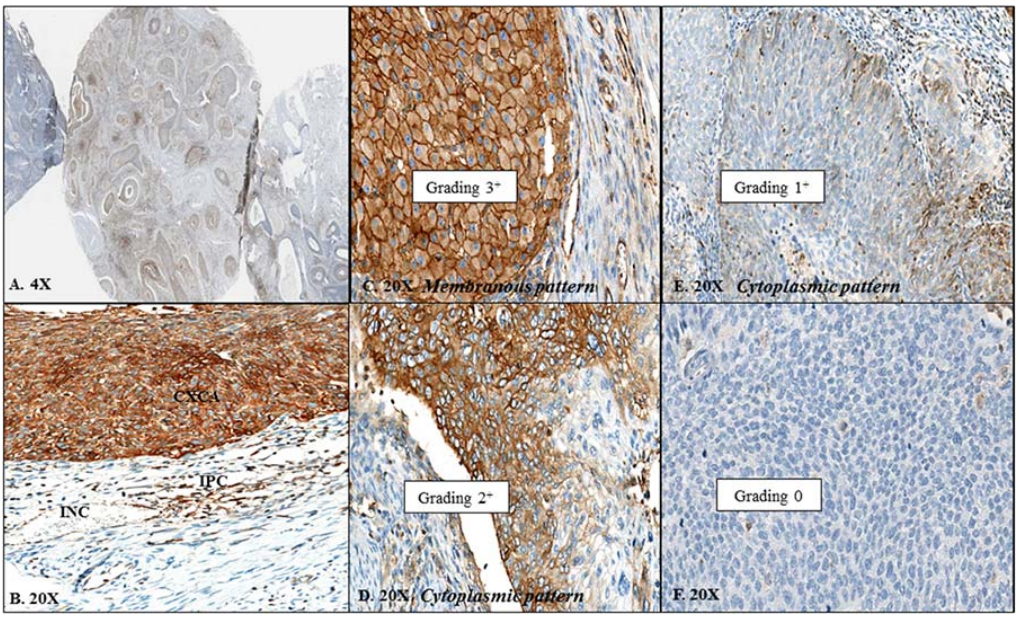

Fig. 1 Evaluation of IHC conventional method stained with anti-MHC class I heavy chain representation from TMA platform was examined (A). Increased resolution showed positive cancer cell staining (CXCA), lymphocytes as an internal positive control (IPC) and red blood cells as internal negative control (INC) (B). Levels of IHC showed grading 3^+ (C), 2^+ (D), 1^+ (E) and 0 (F) as normal, partial loss, and total loss of expression respectively. The localized staining was also found as membranous and cytoplasmic patterns.

mination and interpretation of a combination between percentages of positive staining and their intensity. An example analysis of MHC class I by heavy chains shows different levels of automated scanning and imaging analysis (Fig. 2). The different levels of invasive CXCA using automated IHC visual analysis consisting of grading 3^+, 2^+ and 1^+ or 0 as representing normal, partial loss, and total loss of expression, respectively, were presented. The final grading in percentages of the positive staining and their intensity evaluations showed the highest grading and the middle grading as 3^+ and 2^+, representing normal and partial loss of expression, respectively.

Comparison between conventional and automated IHC evaluation

Grading score analysis significant association in percentage staining and intensity

The association analyses between conventional visualization and automated IHC visual procedure according to percentages of positive tumor cell staining (P) and their intensity (I) were determined as shown in Table 1. The grade difference from the three investigators showed the highest consensus percentage of each protein expression in the same grade and followed by one grade difference. The total agreement showed the highest percentage of positive staining for anti-β2m, anti-heavy chain, and anti-Tapasin as 100, 98.246 and 97.368, respectively, with their intensity equal to 100. The results showed significant association between conventional and automated IHC evaluation (*p*-value >0.05, Chi-square) for MHC class I and Tapasin in percentage of positive cancer cells, whereas intensity was found (*p*-value <0.05, Chi-square) with moderate agreement (*p*-value <0.001, kappa) 0.434-0.615 and 0.353-0.554, respectively.

Interpretation scoring significant association in summation and multiplication

The association analyses between the

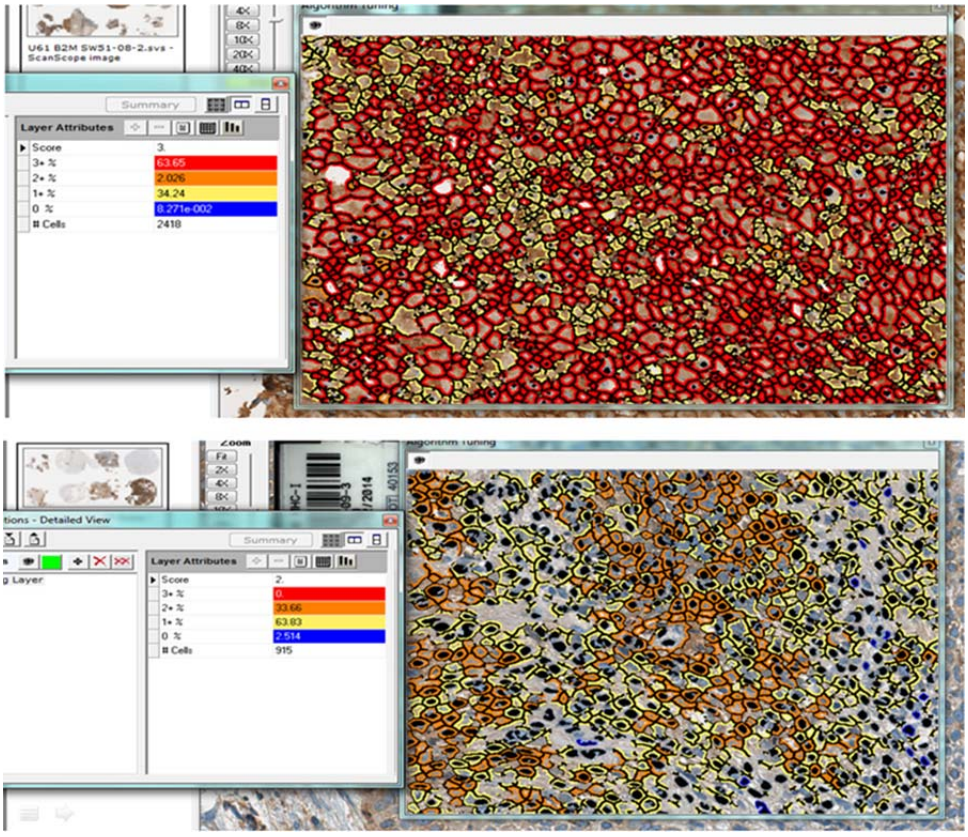

Fig. 2 Evaluations of IHC automated visual analysis stained with anti-MHC class I heavy chain representation were examined. Examples of different levels were determined with combinations between percentages of positive cancer cell staining and their intensity. The upper picture shows grading 3^+ whereas the lower picture shows 2^+.

conventional visualization and automated IHC visual procedures according to percentages of summation (S) and multiplication (M) were determined as shown in Table 2. The degrees of difference from the three investigators showed the highest consensus percentage of each protein expression in the same grade and followed by a different grade. The total agreement of both summation and multiplication scoring showed the percentages for anti-Tapasin higher than anti-heavy chain and anti-β2m respectively. After calculated values, the results showed significant association between conventional and automated IHC evaluation (*p*-value >0.05, Chi-square) for MHC class I and Tapasin with the highest agreement level (*p*-value <0.001, kappa) of summation 0.595-0.755 and multiply scoring 0.633-0.689, respectively.

Discussion

The results from the automated IHC staining were more important to use and be crucial platform to reduce false positive and negative results. The study evaluated the manual or conventional IHC visual interpretation of MHC class I expression composed of heavy chain and β2m, and showed that localization of MHC class I expression patterns resulting for protein expression presented as membranous and cytoplasmic patterns. The findings were similar to a previous study that proposed an MHC class I loss of expression in various cancers[12,13], especially in CXCA[14]. The results noted that a loss of MHC class I expression may be related to advanced invasive CXCA or poor prognosis outcome of progression in an advanced stage, rather than an early stage of invasive CXCA. Nevertheless, this study did not show prognostic factors associated with their expression nor the clinical outcome of progression. This study's examination found that the β2m showed higher expression than heavy chain. The results supported that β2m is not only a compartment of MHC class I molecule but also exists in other molecules such as MICA associated with NK cells or CD1 resulting for antigen presentation of lipid[15,16]. This study also evaluated an automated technique for visual scoring analysis the IHC staining on TMA slides. The evaluation was similar to previously defined measures of scoring IHC slides such as the Allred score and the HSCORE[1,5,16]. This study's findings noted that the analyses of automated IHC visual method on MHC class I and Tapasin expression is feasible to score considers. The researchers proposed that this method can be used for further routine applications and showed automated scoring gives internally consistent results within a given tissue sample.

The present study compared the automated IHC visual analysis with conventional IHC scoring-standard procedure, and found that multiplication scoring gave more association with the automated IHC visual analysis than summation scoring (data not shown). This is similar to the study of Rizzardi et al of a comparison between manual and automated grading[5]. The present study found that the automation was a more sensitive method, involved less time, high-throughput and gave reliable results, and its findings were similar to various previous reports that proposed that automation should be suitable for further IHC evaluation[17-19]. However, the present study's results did not show any association between MHC class I and Tapasin expression with the clinical outcomes of invasive CXCA progression. The establishment of significant differences in prognostic ability between the scoring according to MHC class I loss of expression requires further investigation.

Conclusion

The automated IHC comprising of advanced protocols, reliable results, high-throughput, should be used for the further standard, routine analyses. The automation software for IHC visual scoring and interpretation can be used for the determination of MHC class I and Tapasin in CXCA. However, an appropriate sample size and design of staging coverage as well as clinical prognosis outcomes of progression should be used in further investigation.

Funding

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Ethical approval

Ethical clearance was provided by Ubon Ratchathani Cancer Hospital HE No. 09/2012.

Authors' contributions

All the authors contributed to the design of the paper: Mr. Jakkra Sombatwong, Mr. Anun Sakunpong were Bachelor of Sciences students, Dr Parichart Wongsena acted as the pathologist and provided clinical specimens, Dr. Prinya Prasongdee and Assistant Professor Dr. Pawana Panomket were responsible for the laboratory tests and statistical analysis respectively, Associate Professor Dr. Patcharee

Table 1. Association analyses between conventional visual procedures and automated IHC visual analysis. The percentages of positive cancer cell staining and their intensity were stained using specific antibodies for anti-MHC class I (anti-heavy chain and anti-beta-2-microglobulin) and anti-Tapasin

Types of antibody	Differentiation between conventional and automated visual evaluation		Total agreement (P, I)	Kappa analysis		Chi -square
	Degree of difference	Percentage of positive staining (P) and (I) intensity		*p*-value (P, I)	Kappa value (P, I)	*p*-value (P, I)
Anti-heavy chain	Same grade	69.298, 69.298	98.246, 100	<0.001, <0.001*	0.535, 0.455	0.094, <0.001*
	1 grade difference	30.702, 30.702				
	2 grade difference	0, 0				
Anti-beta 2 microglobulin	Same grade	70.175, 70.175	100, 100	<0.001, <0.001*	0.434, 0.353	0.529, <0.001*
	1 grade difference	29.825, 29.825				
	2 grade difference	0, 0				
Anti-tapasin	Same grade	78.070, 78.070	97.368, 100	<0.001, <0.001*	0.615, 0.554	0.129, <0.010*
	1 grade difference	21.930, 21.930				
	2 grade difference	0, 0				

* Statistically significant (*p*-value <0.05). a, acceptable criteria = no more than 1 grade difference = same grade

Table 2. Association analyses between conventional visual procedures and automated IHC visual analysis. The percentages of summation and multiply scoring were determined using specific antibodies for anti-MHC class I (anti-heavy chain and anti-beta-2-microglobulin) and anti-tapasin

Types of antibody	Differentiation between conventional and automated visual evaluation		Total agreement (S, M)	Kappa analysis		Chi-square
	Degree of difference	Percentage of summation (S) and multiply (M) scoring		*p*-value (S, M)	Kappa value (S, M)	*p*-value (S, M)
Anti-heavy chain	Same grade	78.070, 78.825	78.070, 79.825	<0.001, <0.001*	0.657, 0.666	0.760, 0.299
	Different grade	21.930, 20.175				
Anti-beta 2 microglobulin	Same grade	78.070, 76.316	78.070, 76.316	<0.001, <0.001*	0.595, 0.633	0.332, <0.400
	Different grade	21.930, 23.684				
Anti-tapasin	Same grade	86.842, 83.333	86.842, 83.333	<0.001, <0.001*	0.755, 0.689	0.747, <0.213
	Different grade	13.158, 16.667				

* Statistically significant (*p*-value <0.05). a, acceptable criteria = same group

Jearanaikoon and Professor Temduang Limpaiboon acted as the mentors for this project. All authors contributed to the write-up's of the paper led by Assistant Professor Dr Surasak Wanram.

What is already known on this topic?

IHC and manual visual scoring are regarded as common routine technique for protein marker detection in clinical laboratories. Application of automation visual scoring and interpretation for molecular detection is still limited.

What this study adds?

We proposed to use automated visual scoring for routine work in further molecular, clinical pathologic assessment.

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Potential conflicts of interest

None.

References

1. Choudhury KR, Yagle KJ, Swanson PE, Krohn KA, Rajendran JG. A robust automated measure of average antibody staining in immunohisto-chemistry images. J Histochem Cytochem 2010; 58: 95-107.
2. Rimm DL. What brown cannot do for you. Nat Biotechnol 2006; 24: 914-6.
3. Rimm DL, Giltnane JM, Moeder C, Harigopal M, Chung GG, Camp RL, et al. Bimodal population or pathologist artifact? J Clin Oncol 2007; 25: 2487-8.
4. Gavrielides MA, Gallas BD, Lenz P, Badano A, Hewitt SM. Observer variability in the interpretation of HER2/neu immunohistochemical expression with unaided and computer-aided digital microscopy. Arch Pathol Lab Med 2011; 135: 233-42.
5. Rizzardi AE, Johnson AT, Vogel RI, Pambuccian SE, Henriksen J, Skubitz AP, et al. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. Diagn Pathol 2012; 7: 42.
6. Sriamporn S, Snijders PJ, Pientong C, Pisani P, Ekalaksananan T, Meijer CJ, et al. Human papillomavirus and cervical cancer from a prospective study in Khon Kaen, Northeast Thailand. Int J Gynecol Cancer 2006; 16: 266-9.
7. Wongsena M, Suebsamran P, Panomket P, Tirat S, Wongsena P, Wanram S. Prognostic factors of human papillomavirus genotypes of invasive cervical carcinoma: an analytical cross-sectional study in lower north-east Thailand. J Med Assoc Thai 2014; 97 (Suppl 4): S12-9.
8. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-917.
9. Hulpke S, Baldauf C, Tampe R. Molecular architecture of the MHC I peptide-loading complex: one tapasin molecule is essential and sufficient for antigen processing. FASEB J 2012; 26: 5071-80.
10. Porter KM, Hermann C, Traherne JA, Boyle LH. TAPBPR isoforms exhibit altered association with MHC class I. Immunology 2014; 142: 289-99.
11. Hsieh CH, Hsu YJ, Chang CC, Liu HC, Chuang KL, Chuang CK, et al. Total HLA class I loss in a sarcomatoid renal carcinoma cell line caused by the coexistence of distinct mutations in the two encoding beta2-microglobulin genes. Cancer Immunol Immunother 2009; 58: 395-408.
12. Tanaka K, Tsuchikawa T, Miyamoto M, Maki T, Ichinokawa M, Kubota KC, et al. Down-regulation of Human Leukocyte Antigen class I heavy chain in tumors is associated with a poor prognosis in advanced esophageal cancer patients. Int J Oncol 2012; 40: 965-74.
13. Ogino T, Shigyo H, Ishii H, Katayama A, Miyokawa N, Harabuchi Y, et al. HLA class I antigen down-regulation in primary laryngeal squamous cell carcinoma lesions as a poor prognostic marker. Cancer Res 2006; 66: 9281-9.
14. Mehta AM, Jordanova ES, Kenter GG, Ferrone S, Fleuren GJ. Association of antigen processing machinery and HLA class I defects with clinicopathological outcome in cervical carcinoma. Cancer Immunol Immunother 2008; 57: 197-206.
15. Hassan I, Ahmad F. Structural diversity of class I MHC-like molecules and its implications in binding specificities. Adv Protein Chem Struct Biol 2011;

83: 223-70.
16. Kambayashi T, Kraft-Leavy JR, Dauner JG, Sullivan BA, Laur O, Jensen PE. The nonclassical MHC class I molecule Qa-1 forms unstable peptide complexes. J Immunol 2004; 172: 1661-9.
17. Arihiro K, Umemura S, Kurosumi M, Moriya T, Oyama T, Yamashita H, et al. Comparison of evaluations for hormone receptors in breast carcinoma using two manual and three automated immunohistochemical assays. Am J Clin Pathol 2007; 127: 356-65.
18. Fatima N, Cohen C, Lawson D, Siddiqui MT. Automated and manual human papilloma virus in situ hybridization and p16 immunohistochemistry: comparison in metastatic oropharyngeal carcinoma. Acta Cytol 2013; 57: 633-40.
19. Laurinaviciene A, Plancoulaine B, Baltrusaityte I, Meskauskas R, Besusparis J, Lesciute-Krilaviciene D, et al. Digital immunohistochemistry platform for the staining variation monitoring based on integration of image and statistical analyses with laboratory information system. Diagn Pathol 2014; 9 (Suppl 1): S10.

การเปรียบเทียบผลวิเคราะห์คะแนนด้วยเทคนิคอิมมูโนฮิสโตเคมิสทรีแบบวิธีดั้งเดิมและเครื่องอัตโนมัติในการแสดงออกของ MHC class I และ Tapasin ของผู้ป่วยมะเร็งปากมดลูก

สุรศักดิ์ แว่นรัมย์, จักรา สมบัติวงศ์, อนันต์ สกุลพงษ์, ปริญญา ประสงค์ดี, ภาวนา พนมเขต, ปาริชาติ วงศ์เสนา, เต็มดวง ลิ้มไพบูลย์, พัชรี เจียรนัยกูร

ภูมิหลัง: โรคมะเร็งปากมดลูกเป็นมะเร็งที่พบบ่อยเป็นอันดับสองของสตรีไทยและทั่วโลก การหลบหลีกภูมิคุ้มกันของร่างกายโดยพบความบกพร่อง และลดการแสดงออกของ MHC class I ในการนำเสนอไวรัสแอนติเจนบนผิวเซลล์ทำให้มีการพัฒนาเป็นมะเร็งปากมดลูก เทคนิคอิมมูโนฮิสโตเคมิสตรีเป็นที่รู้ว่าใช้สำหรับย้อมการตรวจวัดตัวบ่งชี้โปรตีนในห้องปฏิบัติการทางคลินิก ในปัจจุบันได้มีการพัฒนาเครื่องวิเคราะห์อัตโนมัติมาใช้ในห้องปฏิบัติการเพื่อความสะดวกและเพิ่มความรวดเร็วซึ่งสามารถนำมาใช้แทนวิธีดั้งเดิมได้ อย่างไรก็ตามการประเมินการใช้ยังพบข้อจำกัดอยู่

วัตถุประสงค์: ประเมินความสอดคล้องของผลการอ่านจากเครื่องวิเคราะห์อัตโนมัติกับวิธีดั้งเดิม ที่อ่านด้วยตาใต้กล้องจุลทรรศน์ในการตรวจการแสดงออกของโปรตีน MHC class I (Heavy chain และ β2M) และ โปรตีน Tapasin

วัสดุและวิธีการ: ตัวอย่างชิ้นเนื้อผู้ป่วยมะเร็งปากมดลูกจำนวน 96 ตัวอย่าง ผ่านกระบวนการจากรูปแบบของ TMA และย้อมด้วยเครื่องย้อมสไลด์อัตโนมัติ และศึกษาวิเคราะห์เปรียบเทียบระหว่างวิธีอ่านสไลด์ด้วยเครื่องอ่านอัตโนมัติกับวิธีดั้งเดิม

ผลการศึกษา: ผลการประเมินพบความสอดคล้องของการอ่านระหว่างวิธีดั้งเดิมและวิธีอัตโนมัติไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p-value >0.05, Chi-square) ทั้ง MHC class I (heavy chain, β2M) และ Tapasin ในด้านร้อยละของเซลล์มะเร็งที่ติดสี ขณะที่ความเข้มของเซลล์มะเร็งที่ติดสีพบความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p-value <0.05, Chi-square) โดยมีความสอดขล้องระดับปานกลางที่ (p-value <0.001, kappa) 0.434-0.615 และ 0.353-0.554 ตามลำดับ ผลการประเมินเมื่อมีการแปลงค่าพบความสอดคล้องในการอ่านระหว่างวิธีดั้งเดิมและวิธีอัตโนมัติไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ (p-value <0.05, Chi-square) ทั้ง MHC class I (heavy chain, β2M) และ Tapasin โดยค่าคะแนนในผลรวมและผลคูณพบความสอดคล้องเพิ่มขึ้นในระดับสูง (p-value <0.001, kappa) 0.595-0.755 และ 0.633-0.689 ตามลำดับ

สรุป: สามารถใช้เครื่องวิเคราะห์อัตโนมัติในการให้ระดับคะแนนและการแปลผลอ่านการติดสี MHC class I และโปรตีน Tapasin แทนคนได้ นอกจากนี้เครื่องวิเคราะห์อัตโนมัตินี้อาจนำไปเพื่อบ่งชี้การดำเนินโรคมะเร็งปากมดลูกได้ เมื่อใช้ร่วมกับรูปแบบการนำเสนอแอนติเจนและศึกษาเพิ่มเติมโดยใช้จำนวนตัวอย่างที่มีการกระจายตามระยะของโรคที่เหมาะสมและครอบคลุมข้อมูลพยากรณ์โรคของผู้ป่วย