Carcinoma of Unknown Primary Origin: Application of Immunohistochemistry

Maral Mokhtari (✉ maral_mokhtari@yahoo.com)

Shiraz University of Medical Sciences

Domaz Safavi

Shiraz Medical School: Shiraz University of Medical Sciences

Neda soleimani

Shiraz Medical School: Shiraz University of Medical Sciences

Ahmad Monabati

Shiraz Medical School: Shiraz University of Medical Sciences

Akbar Safaei

Shiraz Medical School: Shiraz University of Medical Sciences

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Abstract

Background: The primary origin of some carcinomas may be obscured to the clinicians but it is crucial to identify the origin of these carcinomas because it affects the prognosis and treatment, especially for the novel targeted therapies. Immunohistochemistry (IHC) may be useful for determination of the primary origins. In this study, we conducted a retrospective survey to evaluate the frequency and accuracy of each IHC marker used to identify the origin of these carcinomas.

Method: Archival review of pathology department revealed 307 cases of carcinoma of known primary origin (CUP) between 2015 and 2020 who were available. Demographic information, site of biopsy, clinical and pathologic diagnosis, and IHC results were collected.

Results: The cases consisted of 157 (51.15%) male and 150 (48.85%) female patients. The patients age range was 14-92 years and 106 cases (34.5%) were dead. In 27% of cases, the primary origin remained still unknown. The pathological and clinical diagnostic agreement was 59%. CK7+/CK20- pattern of expression was the most common pattern in CUPs (55.3%) followed by CK7-/CK20- (19%), CK7+/CK20+ (15%), CK7-/CK20+ (10.7%), respectively.

Conclusion: IHC study may aid in the diagnosis of CUP. However, some cases will still remain unknown origin after IHC study, so more diagnostic methods or gene expression studies may be needed for definite diagnosis.

1. Introduction:

Carcinomas of unknown primary origin (CUP) is a term that is used when a patient is diagnosed with cancer (metastatic), but the primary site cannot be found. It is estimated that the incidence of CUP in all patients diagnosed with cancer is about 4% (1). CUP commonly manifests as metastasis to lymph nodes, lung, liver, bone, brain, solid organs, and peritoneal and pleural cavities (2, 3). About 75–85% of cases present with disseminated involvement (4). It is highly important to identify the origin of the CUP because it affects the prognosis and treatment, especially in novel targeted therapies (1).

Immunohistochemistry (IHC) is the gold standard method in the diagnosis of CUP. In addition, in some settings, gene expression-based tests are used with an accuracy similar to IHC for tumor classification ($\geq 75\%$) (5, 6, 7). Since the tumor cells are morphologically similar to the tissue they are originated from, they have the same tissue-specific genes in both primary and metastatic cancers (8). We can use limited IHC markers, which is the most appropriate one because usually biopsy specimens of CUP are small and it is difficult to have many cut sections from that tissue (7). Accurate classification of most of the undifferentiated carcinomas as well as careful preservation of tissues for molecular or other ancillary tests could be possible with effective and also systematic use of appropriate IHC panels (9). Generally, two antibody classes are used in CUP work-ups: (A) Antibody to keratin (B) Antibody to organ-restricted markers (1).

Although subclassification of carcinomas by high and low molecular weight keratins is useful, it is largely replaced by two more powerful discriminators, which are CK7 and CK20 (1). There are four different patterns of expression including CK7+/CK20+, CK7+/CK20-, CK7-/CK20+, and CK7-/CK20- (9). Although these two important markers are used as the first step for the evaluation of carcinomas, in fact, they have limited capability in the definite diagnosis and should be supported by organ-restricted markers (1). Also these two markers should be interpreted with caution, since there are some overlaps between the patterns (10, 11).

Organ-specific makers of carcinomas are subdivided into two classes: (A) Cytoplasmic and/or membranous. Considering the fraction of tumor cells, which are positive for these markers, we can determine the state of differentiation of the tumor. (B) Nuclear transcription factors which are positive in all tumor cells and cannot determine the differentiation of the tumor (1).

In this study, we conducted a retrospective survey to evaluate the frequency and accuracy of each IHC marker used to identify the origin of CUP.

2. Materials And Methods:

In this cross-sectional retrospective study conducted from 2015–2020, archives of the pathology and immunohistochemistry department of Shahid Faghihi hospital, affiliated to Shiraz University of Medical Sciences were searched for cases of “carcinoma of unknown primary origin”. Our center is one of the main referral centers in the Southwest of Iran. The data of 595 patients were obtained, their H&E and IHC slides were retrieved, and reviewed. The ethics committee of Shiraz University of Medical Sciences approved the study. 595 cases were found, among them complete data of 307 cases were available. The patients’ data were retrieved from their medical records or in cases with missing information the patients or their relatives were contacted. Demographic information, including age, gender, and health status, biopsy site, pathological and final clinical diagnosis were recorded. Frequency of cytokeratin expression and other organ specific immunohistochemical markers for different tumor origin and also the agreement between pathological diagnosis and final clinical diagnosis were analyzed using kappa correlation by SPSS software version 20. P value less than 0.05 was considered as statistically significant. It should be mentioned that final clinical diagnosis was based on pathological results and other work ups such as radiological findings and upper and/or lower gastrointestinal tract endoscopy. None of our deceased patients underwent autopsy.

For pathological diagnosis usual pattern of CK7/CK20 expression and organ specific markers according to published data were used.

All the IHC studies performed using antibodies from Master Diagnostica (Spain), Thermo Fisher Scientific, US and Biocare Medical, US with manual method (Details are summarized in Table 1). Selection of antibodies were based on CK7/CK20 pattern and also patient’s age, sex and clinical suspicion, so antibody panels were different in individual cases.

Table 1
List of Immunohistochemical antibodies used in the study

Antibody	clone	Dilution	Company
CK7	BC1	Ready to use	Master diagnostic, Spain
CK20	Ks20.8	Ready to use	Master diagnostic, Spain
GATA3	L50-823	Ready to use	Master diagnostic, Spain
ER	1D5	Ready to use	Master diagnostic, Spain
GCDFP15	D6	Ready to use	Master diagnostic, Spain
TTF-1	8G7G3/1	Ready to use	Master diagnostic, Spain
Napsin-A	TMU-Ad 02	Ready to use	Master diagnostic, Spain
P63	4A4	Ready to use	Master diagnostic, Spain
CK5/6	CK5/6.007	Ready to use	Master diagnostic, Spain
CK19	Ks19.1	Ready to use	Master diagnostic, Spain
CD10	56C6	Ready to use	Master diagnostic, Spain
vimentin	V9	Ready to use	Master diagnostic, Spain
PAX8	BC12	Ready to use	Master diagnostic, Spain
WT-1	BC.6F-H2	Ready to use	Master diagnostic, Spain
CDX2	EP25	Ready to use	Master diagnostic, Spain
SATB2	EP281	Ready to use	Biocare Medical, US
Villin	CWWB1	Ready to use	Thermo Fisher Scientific, US
Thyroglobulin	2H11 + 6E1	Ready to use	Master diagnostic, Spain
calcitonin	N/A	Ready to use	Master diagnostic, Spain
P16	BC42	Ready to use	Biocare Medical, US
Glypican-3	1G12	Ready to use	Master diagnostic, Spain
Arginase	EP261	Ready to use	Master diagnostic, Spain
Hepar-1	OCH1E5	Ready to use	Master diagnostic, Spain
PSA	EP109	Ready to use	Master diagnostic, Spain
P504	13H4	Ready to use	Master diagnostic, Spain
Cytokeratin HMW	34βE12	Ready to use	Master diagnostic, Spain

3. Results:

Among 595 cases of CUP which found during this period, 307 cases were available who enrolled in this study. They consisted of 157 (51.15%) male and 150 (48.85%) female patients. The patients' age range was 14–92 years, 220(71.6%) aged 50 or more than 50 y/o and 87(28.3%) were less than 50 y/o. 106 cases (34.5%) were dead.

The most common site of CUP presentation was the liver. Table 2 shows the frequency of different organs as the first site of tumor presentation.

Table 2
frequencies of different organs involved by CUP as the first presentation

Site of presentation	Number(%)	Site of presentation	Number(%)
Liver	79(25.7)	Soft tissue	3(1)
Bone	45(14.6)	Skin	2(0.7)
Lymph node	43(14)	Upper GI tract	2(0.7)
Peritoneal cavity	30(9.8)	Uterine cervix	2(0.7)
Lung	23(7.5)	Uterus	2(0.7)
Bone marrow	16(5.2)	Kidney	1(0.3)
Ovary	14(4.6)	Esophagus	1(0.3)
CNS	13(4.2)	Lower GI	1(0.3)
Neck mass	11(3.6)	Pancreas	1(0.3)
Pleural cavity	8(2.6)	Thyroid	1(0.3)
Mediastinum	5(1.6)	Prostate	1(0.3)
Bladder	3(1)	Total	307(100)

In 27% of cases, the primary origin could not be determined. The frequency of original organs of metastatic cases are mentioned in Fig. 1.

99.4% of CUP cases were positive for cytokeratin(CK) and only one case had negative CK. CK7/CK20 was checked in 262 cases and we evaluated different patterns of CK7/CK20 expressions in various clinical diagnoses of CUP patients as shown in Fig. 2.

CK7+/CK20-(55.3%) pattern of expression was the most common pattern in CUPs followed by CK7-/CK20- (19%), CK7+/CK20+(15%), CK7-/CK20+(10.7%), respectively.

1) CK7+/CK20- pattern CUPs:

In our study, 55.3% of all CUP cases had CK7+/CK20- pattern of expression, which was the most common pattern in this setting. In this category, unknown origin(24.9%), lung(22.77%), breast(17.24%), pancreatobiliary system(11.72%), ovary(6.9%), thyroid(4.13%), uterus(3.44%), upper GI(2.75%), lower GI(1.37%), urinary bladder(1.37%), prostate(1.37%), liver(0.68%), kidney(0.68%), SCC(0.68%) had the highest frequency, respectively.

2) CK7-/CK20- pattern CUPs:

This pattern was the second common pattern in our study that is estimated 19% of our CUP cases. In this category, Prostate(28%), Unknown origin(26%), Kidney(14%), Lung(10%), Breast(8%), Upper GI(4%), Liver(4%), Nasopharynx and SCC(6%) had the highest frequency, respectively.

3) CK7+/CK20 + pattern CUPs:

This pattern was found as the third common pattern that accounted for 15% of our cases. In this category, Unknown origin (38.5%), Pancreatobiliary system (23.07%), Upper GI(10.25%), Lower GI(7.69%), urinary bladder(10.25%), Lung(5.12%), Ovary(5.12%) had the highest frequency, respectively.

4) CK7-/CK20 + pattern CUPs:

This was the least common category, which included 10.7% of our cases. In this category, Lower GI(60.73%), Unknown origin(21.42%), Appendix(7.14%), Upper GI(7.14%), Urinary Bladder(3.57%) had the highest frequency, respectively.

Table 3 shows the frequency of Organ-specific IHC markers in different clinical diagnosis.

Table 3
The frequency of IHC markers in different clinical diagnosis. *: total number of case which were checked for the marker

The frequency of the markers in different clinical diagnosis. : total number of case which were checked for the marker																							
Breast		Kidney		Cervix		Ovary		Uterus		Lung		Bladder		Prostate		Lower GI		Upper GI		Liver			
Total:32		Total:10		Total:3		Total:17		Total:6		Total:44		Total:7		Total:19		Total:23		Total:15		Total:4			
ER		CD10		P16		WT1		PAX8		TTF1		GATA3		PSA		CDX2		CDX2		Heppar1			
*No	+	No	+(%)	No	+	No	+	No	+	No	+	No	+(%)	No	+	No	+	No	+	No	+		
(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)		
26	73.1	26	73.1	2	100	11	72.2	4	100	41	73.2	6	50	4	50	22	95.5	14	50	2	0		
GATA3		Vimentin		P63		PAX8		ER		Napsin		P63		P504		Villin		Villin		Arginase			
No		+		No		+(%)		No		+		No		+(%)		No		+		No		+	
(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)	
27	88.9	27	88.9	3	33.3	13	76.9	6	50	21	76.2	5	80	18	83.3	3	100	1	100	1	0		
GCDFP15		PAX8		CK5/6		ER		ER&PAX8		PAX8		HMWK		PSA&P504		SATB2		SATB2		Heppar1&Arginase			
No		+		No		+(%)		No		+		No		+(%)		No		+		No		+	
(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)	
7	57.1	7	57.1	3	66.7	11	54.5	4	50	16	0	2	50	4	50	2	100	1	0	1	0		
ER&GATA3		RCC		P16&P63		WT1&PAX8				TTF1&Napsin		GATA3&p63				CDX2&SATB2							
No		+		No		+(%)		No		+		No		+(%)		No		+					
(%)		(%)		(%)		(%)				(%)		(%)				(%)		(%)					
21	69.9	21	69.9	2	50	8	62.5			21	71.4	4	50			2	100						
		CD10&Vimentin		P16&CK5/6		WT1&ER										CDX2&Villin							
		No		+		No		+(%)		No		+				No		+					
		(%)		(%)		(%)				(%)		(%)				(%)		(%)					
		5		80		2		100		9		66.7				3		100					
		CD10&PAX8																					
		No		+(%)																			
		(%)		(%)																			
		6		66.7																			

In this study, the pathological and clinical diagnostic agreement was 59%.

4. Discussion:

This retrospective study included all cases of CUP at Shahid Faghihi hospital from 2015 to 2020 which were available. Among total 307 cases, the final diagnosis for primary origin for 27(8.7%) cases remained unknown despite surveys and ancillary and diagnostic modalities. In a previous study by Brown et al., metastatic tumors were evaluated and the origin of 14% of cases remained indeterminate (12).

Gastrointestinal(GI) tract origin:

In this study, all patterns of CK7/CK20 expression were seen in GI origin cases with the CK7+/CK20- patients were the most prevalent pattern in Upper GI and CK7-/CK20 + in lower GI origins, respectively.

CDX2 was positive in 95.5% of cases of lower GI and 50% of upper GI origin carcinomas. Previous studies showed that CDX2 could be positive in more than 90% of colorectal cancer (1, 13, 14, 15, 16). However, Su et al. and Bayrak et al. showed that CDX2 is not a good predictor of the lower GI origin because it could be positive in all GI tract origin cases (17, 18). CDX2 positivity in different studies ranged from 18% to more than 70% in gastric cancers (17, 15, 22, 24) and Vang et al. showed CDX2 expression in 74 % of metastatic gastric cancers to the ovary (25). Special AT-rich sequence-binding protein 2(SATB2) was positive in our lower GI cases. This marker also showed high sensitivity and specificity for colorectal origin (1, 14, 16) and Ma et al. demonstrated SATB2 as a novel marker with 85% expression in both primary and metastatic signet ring colorectal origins (19). But it is not a good marker for diagnosis of upper GI origin (13, 19, 26). Villin was positive in our upper and lower GI origin malignancies, Previous studies showed a high positive rate range from 75% to more than 90%

in colorectal carcinomas (especially in well differentiated cases) but lower rates in upper GI tumors (1, 14, 20, 21, 22). Although it is noteworthy to mention that villin expression is not limited to GI malignancies. Werling et al. study stated that Villin was positive in 40% of pancreatic carcinomas, 78% of esophageal carcinomas, and 64% of mucinous ovarian adenocarcinomas(21).

It seems that using CDX2, Villin, and SATB2 is helpful for determining the lower GI origin. And combination of Villin and CDX2 in cases with suspicious upper GI origin will increase diagnostic yield.

Liver:

We had 4 cases with clinical diagnosis of liver as the primary source without accurate pathologic diagnosis. They were 66.7% CK7-/CK20- and 33.3% CK7+/CK20-.

Hepatocyte Paraffin 1(Hep Par1) and Arginase were checked and were negative.

In Kandalaft et al. study, Hep Par1 was positive in 70–100% of cases (1). Also, another experiment by Yan et al. showed 96 % sensitivity for arginase1 even for poorly differentiated hepatocellular carcinomas(27). Radwan et al. showed arginase1 and Hep Par1 expression of 84% and 70%, respectively, and they believed that in high-grade tumors, sensitivity and specificity of arginase1 were more than hepar1 (28). Also, a similar study by Koehne de Gonzales et al. estimated the sensitivity and specificity of Hep Par1 as 70% and 84%, and for arginase1 84% and 96%, respectively (29). No hepatic-specific markers were expressed in our cases and they may be better to be considered as undetermined origin.

Breast:

In our study. CK7+/CK20- pattern was seen in 86.2% and CK7-/CK20- pattern in 13.8% of breast origin cases.

Estrogen receptor(ER), was in 73.1%, GATA3 was in 88.9% and Gross cystic disease fluid protein 15(GCDFP15) was positive in 57.1% breast origin malignancies. Previous studies stated that GATA3 has more sensitivity for diagnosis of breast cancer compared to GCDP15 (1, 30, 31, 32, 33, 34, 35, 36). The positive rate for GCDP15 is still lower in metastatic cases (33)

In O'Connell et al. research, ER and GCDP15 expression in metastatic breast cancer was calculated to be 72% and 78%, respectively (30). Kandalaft et al. study recommended GCDP15, GATA3, and mammaglobin panel (1), but in our study it seem that combination of ER and GATA3 can be helpful in diagnosis of breast origin in a high proportion of our cases.

Kidney:

In this study, the most prevalent CK7/CK20 pattern in kidney origin cases were CK7-/CK20- followed by CK7+/CK20- pattern.

For determining kidney origin we used CD10, vimentin and PAX8 panel which showed positivity in more than 70% of renal malignancies.

In previous studies by PAX8 had more than 70% positivity in kidney carcinomas (37, 38). and the intensity of its expression is higher in the metastatic cases (39). Tong et al. (40) and Tacha et al. (37) showed that PAX8 is a specific marker for both primary and metastatic RCC. Liu et al. stated that CD10 is positive in 91% of clear cell RCC and vimentin is 100% sensitive for clear cell RCCs (41). Sharma et al. believed that PAX8 and RCC markers are 100% sensitive in metastasis with kidney origin (42). In our study combination of vimentin, PAX8, and CD10 was successful to identify kidney origin in acceptable number of patients.

Uterine cervix:

CK7+/CK20- was the most prevalent pattern seen in our uterine cervix origin cases. All cases were poorly differentiated; thus, types of cervical cancers (SCC and adenocarcinoma) could not be determined on histomorphology. Combination of P16, P63 and CK5/6 were used in our study. It has been stated that these markers have good sensitivity and specificity for diagnosis of SCC (43, 44, 45, 46, 47, 48).

Wang et al. showed strong P16 expression in 98% of metastatic cervix SCC to lung (49).

Ovary:

In our study, 83.3% of ovarian origin cancers were CK7+/CK20- and 16.7% were CK7+/CK20+.

In CK7+/CK 20 pattern carcinomas, Wilms tumor (WT)1 was positive in 72.7%, PAX8 was positive in 76.9% and ER was positive in 54.5% However one study by Tacha et al. revealed more than 90% positivity for WT1 and another study showed 70–99% for PAX8(37, 38). Also Ordoñez et al showed ER expression in 85–90% of ovarian cancers (50).

WT1 and PAX8 combination were positive in about 66.7% of our patients. Since serous carcinoma of the ovary is the most common ovarian epithelial neoplasm with a high risk of dissemination we can use WT1 and PAX8 and ER as a good panel with high sensitivity for diagnosis of ovarian origin carcinoma especially in CK7+/CK20- pattern. In CK7+/CK20 + pattern mucinous carcinoma should be considered so other IHC markers such as CDX2 will be helpful.

Uterus:

All of uterine origin cases were CK7+/CK20-. PAX8 and ER marker was positive in 100% and 50% of cases, respectively.

PAX8 expression according to Tacha and Ozcan et al. was seen in 84–93% of endometrial carcinomas (37, 38)., and in Yemelyanova et al. study, PAX8 was reported as a useful marker to determine uterine origin of adenocarcinomas in extrauterine sites (51). Thus, all previous studies are concordant with our study

for the PAX8 marker. In addition in Alkushi et al. study and the Kounelis survey, ER expression were seen in 73% and 54% of endometrial carcinomas, respectively (52, 53).

Lung:

In this study, 82.5% lung origin tumors were CK7+/CK20-, 12.5% CK7-/CK20-, and 5% were CK7+/CK20+.

Thyroid transcription factor (TTF)1 was positive in 73.2%. Which is in concordant to previous studies. The positivity of TTF has been reported from 70–100% in various lung malignancies (9, 45, 54–59). And lower frequency in poorly differentiated adenocarcinomas (9).

In our study, Napsin A was positive in 76.2% According to other studies, Napsin A is positive in more than 80% of patients (60, 59). It has been stated that this marker is more sensitive than TTF1 in the diagnosis of lung carcinoma (57). In our study, PAX8 was negative in lung origin carcinomas which is helpful to differentiate these tumors from thyroid origin tumors which both are TTF-1 positive. Ye et al., also stated that PAX8 was negative in their lung adenocarcinomas (59).

We recommend using both TTF1 and Napsin A as a panel with acceptable sensitivity to detect lung adenocarcinoma.

Thyroid:

Thyroid origin were CK7+/CK20-.

Follicular cell origin thyroid carcinoma:

Among 6 follicular cell origin cases, 83.3% were positive for TTF1. Thyroglobulin (TG) and PAX8 were positive in 100% of cases.

C cell type origin thyroid carcinoma:

Both medullary carcinomas cases found to co-express TTF1 and calcitonin and TG was negative.

Our study was concordant with previous studies, i.e. high sensitivity for TTF-1 and PAX8 was documented in thyroid origin cases. The positivity was more frequent in well differentiated thyroid follicular origin tumors (papillary carcinoma and follicular carcinomas) compared to medullary thyroid carcinomas and anaplastic carcinomas (11, 61–66).

However, Zhang et al. results is not consistent with other studies and showed PAX8 positivity in 31.2% of PTC and 40% of FTC cases (67).

TG expression showed variable results in thyroid follicular epithelial malignancies and in metastatic sites range from 60–100% in different studies (61, 62, 68).

So it seems that combination of TTF1, TG, and PAX8 as a panel of markers with 100% sensitivity for follicular cell origin cases. Positive TTF1 and calcitonin with negative TG are also a good predictor for C cell origin.

Prostate:

In our Prostate origin tumors 87.5% were CK7-/CK20- and 12.5% were CK7-/CK20+.

P504 and prostate specific antigen (PSA) were positive in 83.3% and 50% cases respectively.

Co expression of PSA and P504 were seen in 50% of patients. In previous studies PSA expression showed a high sensitivity and specificity for diagnosis of prostate carcinoma (69–70) but its utility will be less in metastatic sites (71).

According to Mai and Renshaw et al. studies, the possibility of PSA expression is higher in well-differentiated prostatic cancers in comparison with high-grade types (72, 73). Regarding p504 expression, 80–100% sensitivity has been reported for this marker in diagnosis of prostate cancers (74–79).

Bladder:

In our study, 57.2% showed CK7+/CK20+, 28.5% CK7+/CK20-, and 14.3% CK7-/CK20 + pattern, respectively.

GATA3 and high molecular weight keratin (HMWK) were positive in 50% and P63 expressed in 80% of cases.

Previous studies showed GATA3 expression is seen in 70–90% of urothelial carcinomas (80–83). Overall, HMWK is positive in 65–97% of urothelial tumor cases (84, 85, 86, 87), In Kunju et al. p63 was positive in 92% of urothelial carcinomas (85). Thus using a panel consisting of P63, HMWK, and GATA3 will have a high sensitivity for detection of bladder origin.

Pancreatobiliary system:

In pancreatobiliary origin tumors 65.4% were CK7+/CK20- and 34.6% were CK7+/CK20+. CK19 was positive in all patients and GATA3 was positive in 12.5%.

As in our study, Hornick et al. showed that CK19 is expressed in 100% of metastatic pancreatic adenocarcinomas to the liver (88) that is coordinate with a study by Zapata et al., which shows a sensitivity of 100% for CK19 marker in pancreatic adenocarcinoma (89).

In a study by Miettinen et al., pancreatic ductal carcinoma was one of the most GATA3 positive non-mammary cancers with 37% sensitivity (90) and Clark et al. showed GATA3 positivity in 10% of pancreatic cancers (36). Although no specific marker is available for detection of pancreaticobiliary cancer, using CK19 may be helpful for the diagnosis of pancreaticobiliary origin cases.

In our study, immunohistochemical diagnosis and final diagnosis agreement in CUP cases was 59%. In a study by Deyoung et al., IHC and final diagnosis concordance was seen in 67% of cases (91). Also, previous studies by Gambel et al., Brown et al., Dennis et al., and Park et al. showed that IHC predicted the primary origin of metastatic tumors in 64%, 66%, 67%, and 65% of cases, respectively, that are approximately in the same range (92, 12, 93, 94). Also, in another previous study by Weiss et al., in 69% of cases, the origin site was diagnosed by an IHC study (5). The lower successful rate of finding primary origin of carcinomas in our study compared to other studies may be caused by incomplete clinical studies and imaging before patients death. Also, new IHC markers are updating and emerging day by day and there is not any definite standard IHC panel for determination of each carcinoma origin. In our study a uniform and homogenous panels were not used for each individuals and this limitation affects the precise sensitivity of each marker.

Another issue is that the expression of cytoplasmic IHC markers are usually dependent on the grade of tumor differentiation. In metastatic tumors, in many cases, the loss of tumor cell differentiation will occur and many tissue-specific cytoplasmic markers will be lost, but nuclear markers have the least changes. In this study by using IHC panels upper and lower GI, breast, kidney, prostate, ovary, lung and thyroid origin carcinoma was more readily diagnosed compared to Cervix and pancreatobiliary and liver origin tumors

5. Conclusion:

IHC study may aid in the diagnosis of CUP. However, some cases will still remain unknown origin after IHC study, so more diagnostic methods or gene expression studies may be needed for definite diagnosis.

Declarations:

- Ethics approval and consent to participate: Ethics committee of Shiraz University of Medical Sciences approved the study
- Consent for publication: The authors give their consent for publication
- Availability of data and materials: All the data are available.
- Competing interests: The author declare no competing interest.
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Figures

Figure 1

The frequency of original organs of metastatic cases

Figure 2

Different patterns of CK7/CK20 expression in various clinical diagnoses of CUPs