



BJGH

Brazilian Journal
of Global Health

Revista Brasileira
de Saúde Global

Acute pain after laparoscopic cholecystectomy: prevention and anesthetic management in a public health system hospital

Deborah Bruna Gomes Simoni¹, Guilherme Erdmann da Silveira²

¹Medical student at Universidade Santo Amaro

²Advising Professor. Anesthesiologist. Professor at Santo Amaro University

ABSTRACT

OBJECTIVE

To analyze the anesthetic techniques and medications used in the prevention and management of pain in a hospital that is part of the public health system.

METHOD

A retrospective descriptive cross-sectional study based on the analysis of virtual medical records of patients who underwent laparoscopic cholecystectomy at the Hospital Geral do Grajaú/SP in 2022.

RESULTS

The cross-sectional study showed a prevalence of general anesthesia, both balanced and total intravenous, over the combination of local or regional anesthesia. Regarding regular postoperative analgesia, a preference for multimodal analgesia was noted, with the regimen of choice being the combination of a simple analgesic, nonsteroidal anti-inflammatory drug, and weak opioid. Analgesia on demand was prescribed in 75% of cases, and the drug of choice in most cases was a strong opioid. Finally, postoperative pain was observed in only 15 of the records analyzed.

CONCLUSION

Based on the data collected and analyzed in conjunction with the literature, it was possible to conclude that despite some suggested techniques and medications not being widely used (notably regional blocks, NMDA receptor antagonists, and gabapentinoids), the techniques adopted by anesthesiologists at the hospital analyzed proved to be effective for the prevention and management of pain.

KEYWORDS

Postoperative pain; Laparoscopic cholecystectomy; Analgesia; Anesthesia.

Corresponding author:

Deborah Bruna Gomes Simoni

Rua São Benedito, 2367, apt. 33D. Santo Amaro, São Paulo - SP. CEP: 04735-005

E-mail: dbgsimoni@gmail.com

ORCID ID: <https://orcid.org/0000-0003-3041-9438>

Copyright: This is an open-access article distributed under the terms of the Creative Commons.

Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original author and source are credited.

DOI:

INTRODUCTION

Since its first description in 1882, cholecystectomy, or removal of the gallbladder, has become the treatment of choice in cases of acute cholelithiasis.^{1,2} Over the years, the technique has undergone some changes. With the advent of laparoscopic surgery, it has come to be considered the gold standard, to the detriment of open surgery, as it is a less invasive technique, with less trauma and associated morbidity rates.^{1,2,3}

However, laparoscopic cholecystectomy (LHC) is not free from complications and post-surgical discomfort, with pain being one of the most frequent complaints.^{4,5} This is understood as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage"⁶ and can be classified, according to its duration, as acute—for a period less than or equal to 12 (twelve) weeks—or chronic—beyond the interval of 3 (three) months.^{6,7}

Acute pain is defined as a "normal physiological response to mechanical stimuli associated with trauma" and has different components: neuropathic, resulting from damage to the central nervous system; inflammatory, originating from the inflammatory response of the tissue after trauma; nociceptive, generated by the activation of nociceptors by surgical trauma, which is categorized as somatic and visceral.^{8,9,10}

According to the literature, the intensity of acute pain and its inadequate treatment are related to persistent postoperative pain, which in turn is the main iatrogenic cause of chronic pain.^{10,11} Inadequate management of acute pain after CVL is also responsible for neuroendocrine, hemodynamic, respiratory, and gastrointestinal changes, which lead to delayed patient mobilization, increased hospital stay, and consequent increased costs.^{9,12,13}

Since 1992, guidelines have been created and updated to standardize both perioperative prevention and postoperative pain management. However, Apfelbaum et al.¹³ point out in their article that ineffective treatment of pain after CVL is common among anesthesiologists. Thus, given the importance of the topic, it is essential to analyze the anesthetic techniques—regional, general, or local—adopted for performing the procedure, as well as the medications used for pain prevention and management in hospitals that are part of the public health system.

METHODS

A retrospective descriptive cross-sectional analysis was conducted based on the review of virtual medical records of patients who underwent laparoscopic cholecystectomy in 2022 at Grajaú General Hospital in São Paulo, a tertiary healthcare facility.

Data collection took place after obtaining approval from the Ethics Committees of the institutions involved (opinion 59345522.7.0000.0081; opinion 59345522.7.3001.5447), and participants signed the Informed Consent Form sent to the participants. In order to fit the theme, records related to open cholecystectomies were excluded. Also, to delimit the age group studied, records of procedures performed on patients under 18 and over 65 years of age were excluded.

Data were collected on the demographic characteristics of the patients, the type of anesthesia, the drugs used for postoperative analgesia, the prescription of analgesia on demand, and the postoperative pain condition—quantified using the visual analog pain scale at six different moments.

A convenience sampling method was adopted, given the time available for access to medical records. EXCEL software was used for the descriptive statistical analysis of the information collected. The results observed were arranged in tables. They were then presented in a discursive manner.

RESULTS

Initially, 1,208 virtual medical records related to laparoscopic cholecystectomies were identified. Of these, 179 were excluded because they involved patients under 18 or over 65 years of age. Thus, adopting convenience sampling, 120 records were selected for statistical representation of the whole.

Regarding the demographic characteristics of the patients (Table 1), a higher proportion of female patients (55.63%) was

observed in relation to male patients (44.17%). In addition, 62.5% of patients were between 36 and 55 years old, while only 14.17% of patients were 35 years old or younger and 18 years old or older.

Table 1 - Demographic characteristics of patients undergoing laparoscopic cholecystectomy

Demographic characteristics	Total	
	N	%
Gender		
Female	67	55.83
Male	53	44.17
Age		
18-35	17	14.17
36-55	75	62.5
56-64	28	23.33

Source: authors (2025)

The types of anesthesia used (Table 2) were classified as follows: balanced general anesthesia - combination of inhaled and intravenous anesthetics: fentanyl, lidocaine, atracurium, and propofol; total intravenous anesthesia - infusion of anesthetics exclusively by intravenous route: propofol, remifentanyl, and atracurium; general anesthesia associated with local anesthesia - infusion of ropivacaine at the trocar insertion site; general anesthesia associated with regional anesthesia - transverse abdominal plane (TAP) block.

There was a greater adoption of the balanced general modality (86.67%), followed by the total intravenous technique (10%). The application of the combination of general anesthesia with local anesthesia was observed in only three cases. Finally, only in one case was regional anesthesia adopted in combination with general anesthesia.

Table 2 - Types of anesthesia used in laparoscopic cholecystectomies

Types of anesthesia	Total	
	N	%
Balanced general	104	86.67
Total venous	12	10
General + Local	3	2.5
General + TAP Block	1	0.83
Total	120	100

Source: Authors (2025)

The medications used for regular postoperative analgesia (Table 3) were categorized as follows: simple analgesics - dipyrrone and paracetamol; nonsteroidal anti-inflammatory drugs - ketoprofen, sodium diclofenac, ketorolac trometamol; mild opioid - tramadol; strong opioid - morphine.

Table 3 - Postoperative analgesia prescriptions

Analgesia prescriptions	Total	
	N	%
Regular postoperative analgesia	120	10
Simple analgesic	1	0.83
Mild opioid	3	2.5
Strong opioid	1	0.83
Simple analgesic + NSAIDs	11	9.17
Simple analgesic + mild opioid	32	26.67
Combination of simple analgesics	1	0.83
Combination of simple analgesics + NSAIDs	3	2.5
Simple analgesic + NSAID + mild opioid	50	41.67
Two simple analgesics + NSAIDs + mild opioid	18	15
Analgesia on demand	90	10
Strong opioid	83	92.22
Mild opioid	1	1.11
Strong opioid + simple analgesic	2	2.22
Strong opioid + NSAID	2	2.22
Strong opioid + weak opioid	1	2.22
Simple analgesic + NSAID + Strong opioid	1	1.11

NSAID: nonsteroidal anti-inflammatory drugs

Source: authors (2025)

It was observed that, in most of the records (50), the analgesic prescription consisted of a combination of simple analgesic, nonsteroidal anti-inflammatory drug, and mild opioid. In 44 cases, the prescription consisted of a combination of two drugs: simple analgesic and opioid (32); simple analgesic and anti-inflammatory drugs (11); two simple analgesics (1). Only in 5 cases was a single drug prescribed for analgesia: mild opioid (3); strong opioid (1); simple analgesic (1).

As for the prescription of analgesia on demand (Table 3), this was performed in 90 cases, most often consisting of strong opioids (83). Finally, in relation to the remaining prescriptions, the following were noted: strong opioid associated with simple analgesic (2); strong opioid associated with nonsteroidal anti-inflammatory drug (2); combination of strong opioid and mild opioid (1); mild opioid (1); association of strong opioid, simple analgesic, and nonsteroidal anti-inflammatory drug (1).

Postoperative pain was observed in only 15 patients, 14 of whom had undergone balanced general anesthesia and 1 of whom had undergone total intravenous anesthesia (Table 4). In addition, it was noted that patients who underwent general anesthesia associated with local anesthesia or transverse abdominal plane block did not complain of pain.

Table 4 - Relationship between the type of anesthesia used and the presence of pain

Type of anesthesia	Pain	
	Present	Absent
Balanced general	14	90
Total venous	1	11
General + Local	-	3
General + TAP Block	-	1
Total	15	105

Source: Authors (2025)

Regarding the numerical pain score and the time of assessment (Table 5), it was noted that of the fifteen patients who complained of pain, three presented the onset of pain at the first moment of assessment (0 minutes). Of these, one

had mild pain, one had moderate pain, and one had severe pain. Only one of the patients did not experience pain in the following assessments.

At the second assessment (30 minutes), ten patients complained of pain—two who already had pain and eight who began experiencing pain at this time. Of these, seven reported moderate pain and three reported severe pain. Half of the patients had their pain resolved.

At the third assessment (60 minutes), eight patients reported pain—five had already been in pain in the previous minutes. Of these, three complained of mild pain; one, moderate pain; and four, severe pain. Only one patient's pain was resolved.

At the fourth assessment (90 minutes), eight patients reported some degree of pain, with only one of them experiencing pain for the first time at this point. Of these, four reported mild pain, three reported moderate pain, and one reported severe pain. Only one patient continued to experience pain, rated as moderate, at the fifth assessment (120 minutes). Finally, at the time of discharge, three patients again complained of pain, classified as mild.

Table 5 - Pain score and time of assessment, per patient

Patient	Pain score at the time of assessment					
	0 min	30 min	60 min	90 min	120 min	High
1	9	-	-	-	-	-
2	2	4	2	2	-	2
3	3	5	8	9	-	-
4	-	9	2	-	-	-
5	-	7	8	2	-	2
6	-	6	2	2	-	-
7	-	7	-	-	-	-
8	-	8	-	-	-	-
9	-	8	-	-	-	-
10	-	5	-	-	-	-
11	-	6	-	-	-	-
12	-	-	8	1	-	1
13	-	-	8	3	-	-
14	-	-	5	7	7	-
15	-	-	-	7	-	-

Source: Authors (2025)

DISCUSSION

According to the literature, acute cholecystitis is one of the most prevalent diagnoses in emergency rooms, most frequently affecting women of reproductive age, with peak incidence after the fourth decade of life.^{14,15} The demographic characteristics observed in the present study, based on the analysis of medical records, were consistent with this information, showing a higher proportion of female patients (55.63%) and patients between 36 and 55 years of age (62.5%).

Laparoscopic cholecystectomy is the recommended treatment for cases of acute cholecystitis.^{1,2} Regarding the anesthetic techniques used to perform the procedure, it was observed that anesthesiologists prefer balanced general anesthesia (86.67%) and total intravenous anesthesia (10%), rather than general anesthesia combined with local anesthesia (2.5%) or regional anesthesia (0.83%).

According to the literature review conducted by Jesus et al., the use of peripheral blocks associated with general anesthesia in patients undergoing laparoscopic cholecystectomy reduced acute pain scores, proving to be an effective technique as an adjunct to analgesia.¹² Furthermore, Ortiz et al. did not observe statistically significant changes in analgesia when comparing TAP block and the local infiltration technique at the portal sites.¹⁶

Regarding the local anesthetics used, Ar et al. demonstrated that both bupivacaine and levobupivacaine produce similar results in pain control.¹⁷ Salimnia et al.,¹⁸ corroborated the efficacy of the block performed with bupivacaine, but found that the addition of sufentanil to the local anesthetic is ineffective.

However, although the literature indicates the use of general anesthesia in combination with transverse abdominal

plane block or instillation of local anesthetic at the trocar insertion site, it was found that the use of general anesthesia alone—both balanced and total intravenous opioid-based—resulted in reduced postoperative pain in the patients analyzed (15 cases).

Regarding prescriptions for regular postoperative analgesia, it was observed that these were preferably composed of a combination of a simple analgesic, nonsteroidal anti-inflammatory drug, and mild opioid, or by a combination of two drugs—simple analgesic and opioid; simple analgesic and anti-inflammatory drugs; two simple analgesics. As for prescriptions for analgesia on demand, these were made in 90 of the 120 cases analyzed, most often consisting of strong opioids—morphine.

In this regard, Jesus et al.¹² state that there is no consensus in the literature regarding the most appropriate technique, so that, in a context of multimodal analgesia, drugs from different classes can be adopted. In this sense, NSAIDs have proven effective in controlling pain, since the pathophysiology of acute postoperative pain has an inflammatory component due to the release of mediators such as prostaglandins.¹⁹⁻²²

According to Aml et al., the combination of dextropropofol, tramadol and tramadol in a patient-controlled analgesia (PCA) pump resulted in effective pain control with less need for opioids without causing an increase in adverse effects.¹⁹

The application of parecoxib—a selective COX-2 inhibitor—before anesthetic induction was associated with shorter hospital stays, less need for additional analgesics, and pain relief.²⁰ When combined with dexmedetomidine, an improvement in postoperative sedation and cognitive conditions of patients was observed.²¹

The use of ibuprofen before surgery proved effective in controlling pain, both at rest and during activity, and in reducing the use of opioids in the first 24 hours after surgery. Furthermore, it did not present statistically significant changes in side effects.²²

The main representative of N-methyl-D-aspartate (NMDA) receptor antagonists is ketamine, which acts by noncompetitively blocking the NMDA receptor, resulting in an antinociceptive and hypnotic effect.²³⁻²⁵

The use of ketamine until the end of surgery, as a supplement to total intravenous anesthesia with propofol and alfentanil, produced better control of postoperative pain and the demand for analgesics.²³

However, when combined with remifentanyl, this drug was not able to significantly reduce pain scores. In addition, its use was associated with adverse effects such as agitation, hallucinations, diplopia, discomfort, and nausea.²⁵

Gabapentinoids are antiepileptic drugs commonly used in the treatment of neuropathic pain, as they bind to calcium channels, reducing the release of several excitatory neurotransmitters involved in the pathophysiology of pain, including glutamate, norepinephrine, and substance P, among others.²⁷⁻²⁹ In this regard, Sarakatsani et al. found that the administration of pregabalin PO causes a positive analgesic effect, with control of pain and opioid consumption in the postoperative period.²⁶

However, it was observed that in combination with celecoxib, pregabalin did not show satisfactory results in terms of pain and the use of rescue fentanyl. In addition, there was an increase in symptoms such as drowsiness and dizziness.²⁷

The use of gabapentin ensured a reduction in opioid consumption similar to that obtained with pregabalin, in the same regimen. However, patients who received pregabalin had a lower pain score.²⁸

Kotsovolis et al. demonstrated that there is no statistically significant difference in morphine consumption and pain scales between the use of gabapentin, alone or in combination with ketamine, lornoxicam, and ropivacaine.²⁹

Opioids act on the nervous system through mu, delta, and kappa receptors. They can be classified as weak—tramadol and codeine—and strong—oxycodone, fentanyl, sufentanil, morphine, among others. In recent years, the use of oxycodone as a component of multimodal analgesia has become popular.^{30,31}

It has been found that, compared to fentanyl, the application of oxycodone in the final minutes of surgery is more effective in providing immediate pain relief without causing an increase in adverse effects.³⁰ The use of oxycodone in pa-

tient-controlled analgesia (PCA) resulted in analgesia similar to that obtained using fentanyl.³¹

Thus, it can be noted that prescriptions for both regular analgesia and analgesia on demand are consistent with what has been observed in the literature, with an emphasis on the preference for combining drugs from different classes rather than the exclusive use of opioids.

However, although the literature indicates the possibility of using oxycodone as a component of multimodal analgesia, in practice, tramadol is used for regular analgesia and morphine for on-demand analgesia.

Finally, it should be noted that the assessment of pain was carried out by the hospital team in a systematic manner, at five different times, resulting in effective management of pain complaints, with only three patients reporting pain at the time of discharge.

CONCLUSION

Based on the data collected and analyzed in conjunction with the literature, it was possible to conclude that despite some suggested techniques and medications not being widely used (notably regional blocks, NMDA receptor antagonists, and gabapentinoids), the techniques adopted by anesthesiologists at the hospital analyzed proved to be effective for the prevention and management of pain—especially given the limitations inherent to the economic and administrative reality of a hospital that is part of the Unified Health System.

REFERENCES

1. Soper NJ, Stockmann PT, Dunnegan DL, Ashley SW. Laparoscopic Cholecystectomy: The new 'gold standard'. *Arch Surg.* 1992; 127; 917-923.
2. Csikesz NG, Tseng JF, Shah SA. Trends in surgical management for acute cholecystitis. *Surgery* 2008 Aug;144(2):283-9. DOI: 10.1016/j.surg.2008.03.033.
3. Wilson RG, Macintyre IMC. Symptomatic outcome after laparoscopic cholecystectomy. *Br J Surg.* 1993; 80; 439-441. DOI: 10.1002/bjs.1800800410.
4. Kandil TS, Hefnawy EE. Shoulder pain following laparoscopic cholecystectomy: factors affecting the incidence and severity. *J Laparoendosc Adv Surg Tech* 2010; 20. DOI: 10.1089/lap.2010.0112.
5. Bisgaard T, Klarkov B, Rosenberg J, Kehlet H. Characteristics and prediction of early pain after laparoscopic cholecystectomy. *Pain* 2001;90(3):261-269. DOI: 10.1016/S0304-3959(00)00406-1.
6. Merksey H, Boguk NA. A current list with definitions and notes on usage. In: Classification of chronic pain: Descriptions of chronic pain syndromes and definition of pain terms. 2nd ed. Seattle: IASP Press; 1994.
7. Chambers D, Huang C, Matthews G. Pain physiology. In: Basic physiology for anaesthetists. Cambridge: Cambridge University Press; 2018.
8. Carr DB, Goudas LC. Acute pain. *Lancet* 1999; 353; 2051-2058.
9. Agerson AN, Benzon HT. Management of acute and chronic pain. In: Barash PG et al, Fundamentals of clinical anesthesiology. São Paulo: Artmed; 2017.
10. Blichfeldt-Eckhardt MR. From acute to chronic postsurgical pain: The significance of the acute pain response. *Dan Med J* 2018; 65.
11. Blichfeldt-Eckhardt MR, Ørding H, Anderson C, Licht PB. Early visceral pain predicts chronic pain after cholecystectomy. *Pain* 2014; 155; 2400- 2407. DOI: 10.1016/j.pain.2014.09.019.
12. Jesus RR, Leite AM, Leite SS, Vieira MC, Villela NR. Anesthetic therapy for the relief of acute pain after laparoscopic cholecystectomy: systematic review. *Rev Col Bras Cir* 2018; 45; 1885. DOI: 10.1590/0100-6991e-20181885.
13. Apfelbaum JL, Chen C, Mehta SS, Gan TJ. Postoperative pain experience: Results from a national survey suggest postoperative pain continues to be undermanaged. *Anesth Analg* 2003; 97; 534-540. DOI: 10.1213/01-ANE.0000068822.10139E.
14. Jones MW, Weir CB, Ghassenzadeh S. Gallstones (Choleli-

thiasis). StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.

15. Vasconcellos LAS, et al. Acute Cholecystitis: Clinical Aspects and Therapeutic Management Brazilian Journal of Development 2022; 8;10. DOI: 10.34117/bjdv8n10-240.

16. Ortiz J, Suliburk JW, Wu K. Bilateral transversus abdominis plane block does not decrease postoperative pain after laparoscopic cholecystectomy when compared with local anesthetic infiltration of trocar insertion sites. Reg Anesth Pain Med 2012; 37. DOI: 10.1097/AAP.0b013e318244851b.

17. Ar AY, et al. Ultrasound-guided transverse abdominal plane block in patients undergoing laparoscopic cholecystectomy: comparison of the efficacy of bupivacaine and levobupivacaine in postoperative pain control. Brazilian Journal of Anesthesiology 2018; 68. DOI: 10.1016/j.bjan.2018/02.004.

18. Saliminia A, Azimaraghi O, Babayipour S, Ardavan K. Efficacy of transversus abdominis plane block in reduction of postoperative pain in laparoscopic cholecystectomy. Acta Anaesthesiologica Taiwanica 2015. DOI: 10.1016/j.aat.2015.07/003.

19. Aml A, et al., Comparison of postoperative analgesic efficacy of intraoperative single-dose intravenous administration of dexketoprofen trometamol and diclofenac sodium in laparoscopic cholecystectomy. Journal of clinical Anesthesia 2016. DOI: 10.1016/j.jclinane.2016.02.020.

20. Shuying L, et al., Preoperative intravenous parecoxib reduces length of stay on ambulatory laparoscopic cholecystectomy. International Journal of Surgery 2014;12. DOI: 10.1016/j.ijssu.2014.03.013.

21. Du X, Song F, Zhang X, Ma S. Protective efficacy of combined use of parecoxib and dexmedetomidine on postoperative hyperalgesia and early cognitive dysfunction after laparoscopic cholecystectomy for elderly patients. Acta Cirurgica Brasileira 2019; 34. DOI: 10.1590/s0102-86502019009000000005.

22. Ahiskalioglu E, Ahiskalioglu A, Aydin P, Yayik A, Temiz A. Effects of single-dose preemptive intravenous ibuprofen on postoperative opioid consumption and acute pain after laparoscopic cholecystectomy. Medicine 2017;96. DOI: 10.1097/MD00000000000006200.

23. Karcioğlu M, et al., Addition of ketamine to propofol-alfentanil anesthesia may reduce postoperative pain in laparoscopic cholecystectomy. Surg Laparosc Endosc Percutan Tech 2013;23. DOI: 10.1097/SLE.0b013e3182827f09.

24. Mercanoglu EE, et al., Comparison of the effect of ketamine and dexmedetomidine combined with total intravenous anesthesia in laparoscopic cholecystectomy procedures: a prospective randomized controlled study. Hindawi 2022. DOI: 10.1155/2022/1878705.

25. Leal PC, Sakata RK, Salomão R, Sadatsune EJ, Issy AM. Assessment of the effect of ketamine in combination with remifentanyl on postoperative pain. Revista Brasileira de Anesthesiologia 2013;63. DOI: 10.1016/S0034-7094(13)70211-3.

26. Sarakatsianou C, Theodorou E, Georgopoulou S, Stamatou G, Tzovaras G. Effect of pre-emptive pregabalin on pain intensity and postoperative morphine consumption after laparoscopic cholecystectomy. Surg Endos, 2013;27. DOI: 10.1007/s00464-012-2769-3.

27. Gurunathan U, Rapchuk IL, King G, Barnett AG, Fraser JF. The effect of pregabalin and celecoxib on the analgesic requirements after laparoscopic cholecystectomy: a randomized controlled trial. Journal of Anesthesiology 2015. DOI: 10.1007/s00540-015-2078-9.

28. Bekawi MS, Wakeel LME, Taher EMM, Mageed WMA. Clinical study evaluating pregabalin efficacy and tolerability for pain management in patients undergoing laparoscopic cholecystectomy. The Clinical Journal of Pain 2014. DOI: 10.1097/AJP.0000000000000060.

29. Kotsovolis G, Karakoulas K, Grosomanidis V, Tziris N. Comparison between the combination of gabapentin, ketamine, lornoxicam, and local ropivacaine and each of these drugs alone for pain after laparoscopic cholecystectomy: a randomized trial. Pain practice 2014. DOI: 10.1111/papr.12183.

30. Choi YJ, Park SW, Kwon HJ, Choi JM, Lee YM. Efficacy of early intravenous bolus oxycodone or fentanyl in emergence from general anesthesia and postoperative analgesia following laparoscopic cholecystectomy: a randomized trial. Journal of international medical research 2015. DOI: 10.1177/0300060515594194.

31. Hwang BY, et al, Oxycodone vs. fentanyl patient-controlled analgesia after laparoscopic cholecystectomy. Inter-

national journal of medical sciences 2014;11. DOI: 10.7250/ijms.8331.